Research Article

Biomimetic Coating of Modified Titanium Surfaces with Hydroxyapatite Using Simulated Body Fluid

Mohsin Nazir,[1] Ong Pei Ting,[2] Tan See Yee,[2] Saravanan Pushparajan,[1] Dasan Swaminathan,[1] and Muralithran G. Kutty[1]

[1]*Department of Restorative Dentistry, Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[2]*Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia*

Correspondence should be addressed to Mohsin Nazir; mohsin-101@hotmail.com

This study investigated the viability of coating commercially pure titanium (CPTi) surfaces, modified via sandblasting and acid etching, with hydroxyapatite (HA)/tricalcium phosphate coatings using a simulated body fluid (SBF) solution. The samples were immersed in SBF from 3 to 7 days. The morphology and the chemistry of the HA/tricalcium phosphate coating were then analysed. Prior to immersion in SBF, the samples were sandblasted and acid etched to mimic the morphology and roughness of commercially available dental implants. The SBF aided in the formation of crystalline HA/tricalcium phosphate coatings on all the samples. The coatings were uniform and had roughness values higher than the underlying substrate. The highest roughness values for the coatings on the surfaces were obtained at 7 days of immersion in SBF with average S_a values of $2.9 \pm 0.2\,\mu m$. The presence of HA/tricalcium phosphate on the surfaces was confirmed by the Scanning Electron Microscope (SEM), Energy Dispersive Spectrometer (EDS), the X-Ray Diffraction (XRD), and the Fourier Transform Infrared Spectrometer (FTIR) analysis. This study shows that it is possible to obtain an adequate and uniform hydroxyapatite coating on pure titanium substrates in a shorter period of time with characteristics that favour the ultimate goal of implants therapy, that is, osseointegration.

1. Introduction

There has been extensive research on titanium surface modifications in order to improve the time and quality of implant therapy. It has been found that the surface roughness of implants directly influences the speed of healing and the quality of the bone-implant interface [1]. An ideal surface roughness of 1-2 μm is deemed optimal if desirable results are to be accomplished [2]. In recent times, the commonest technique used to achieve surface roughness in dental implants is a combination of grit-blasting and acid etching. This combination produces both microroughness and waviness which significantly increase early endosseous integration, peri-implant bone healing, and the stability of the implant [3].

After attaining a prescribed value for roughness and morphology, an obstacle henceforth is to create titanium surfaces that are biomimetic. The shifting paradigm of implant surface technology makes researchers employ nature as their guide.

The human bone inherently embodies hydroxyapatite (HA) [4]. A significant mineral portion of the bone is composed of these nanometre sized calcium phosphate crystals that measure up to about 5–20 nm in width and 60 nm in length. A cycle of osteoclastic and osteoblastic activity ensures that a certain haemostatic level of HA is maintained at all times in living bone [5]. HA is a bioactive and biocompatible material and has been used rigorously in research and clinical settings in various shapes and forms [6]. HA could influence protein interactions and consequently lead to favourable healing and greater osteoblast adhesion, proliferation, and differentiation [7, 8].

There is extensive literature that deals with the various procedures of acquiring HA coating on different surfaces. Amongst these methods, the wet chemical deposition, thermal plasma spraying, pulse laser deposition, the sol-gel, electrodeposition, and biomimetic deposition are the ones that have been reported [9]. One of the easiest methods amid these

techniques that satisfies the various needs of implant therapy is the biomimetic deposition method in which a simulated body fluid (SBF) solution is made in accordance with the inorganic ingredients of the human blood plasma [10–12].

The fact that SBF creates apatite crystals that resemble human bone on titanium and other surfaces is testified by various literatures [12–15]. The ability of forming this apatite layer often determines the bonding ability of an implant material to adjacent living bone [16]. This bioactive deposition can stimulate bone apposition and the healing process. Hence, early osseointegration of titanium implants can be established [17–21]. When SBF is used to coat titanium surfaces, a wide range of therapeutic or biomimetic agents can be incorporated into the layer that forms on the titanium surface. This opens new doors into medicinally or biomimetically enhancing the implant surface ensuring a steady and controlled release of the surface constituents [4, 22, 23].

There have been various studies to date that use the HA/calcium phosphate to coat titanium surfaces. The authors of this paper are not aware of any research that uses modified sandblasted and acid etched titanium surfaces with the Tas-SBF solution according to the work performed by Jalota et al. [24]. Therefore, the aim of this study is to investigate the viability of coating modified CPTi surfaces with HA/calcium phosphate coating using a Tas-SBF solution.

2. Materials and Methods

2.1. Samples. For this study, 4 commercially pure grade 2 titanium (CPTi) discs from E-Steel Sdn. Bhd. were used. The discs were square in shape and measured 10 mm × 10 mm.

2.2. Surface Standardization and Modification. Silicon carbide paper with 400 grit was used for standardizing and hence gritting the titanium substrates by using a twin variable speed grinding and polishing machine for two minutes at 300 rpm. The gritted titanium substrates were then sandblasted to increase their surface roughness by using a sandblasting machine (Model number SB-8060-KP Techno Finishing Sdn. Bhd.). Grade A brown alumina of mesh 120 and an average abrasive grain size of 125 μm was blasted at a distance of 5 cm perpendicular to the sample surface through an air gun for approximately 3 seconds, at a pressure of 50 psi. The samples were then rinsed in ethanol and distilled water to remove the sandblasted residua and contaminants from the samples surface, followed by air-blasting until they were dried.

An Alicona Infinite Focus Optical 3D Measurement Device G4f was used to capture three-dimensional stereo-images of the implant surface at seven areas on each of the titanium surfaces. The average height parameter (S_a) of the surfaces studied was measured up to millimetre scale using the IFM 2.1.5 software. For every surface, the area of measurement was within the limit of 40 μm × 40 μm in dimension. Seven measurements were taken and an average value and standard deviation of implant surface roughness were calculated henceforth.

The sandblasted CPTi samples were then acid etched by using a mixture of 37% hydrochloric acid (HCl) and 98% sulphuric acid (H_2SO_4) with a volumetric ratio of 1 : 3,

respectively. 5 mL HCl and 15 mL H_2SO_4 were diluted in 100 mL distilled water. This dual acid solution was kept in a water bath of 80°C for 30 minutes and it was ensured that the surface to be etched was facing the acidic solution. The samples were then taken out and cleaned with ethanol and distilled water, followed by air-blasting to dry the titanium surfaces. These acid etched CPTi samples were observed again under the Alicona machine and the surface roughness was measured.

2.3. Addition of the Hydroxyl Group. After acid etching, the samples were treated with sodium hydroxide (NaOH) so that the surfaces could be activated with hydroxyl groups from the alkaline solution. The CPTi discs were immersed inside a 50 mL 5 M NaOH solution for 24 hours at 60°C in an oven, followed by rinsing in distilled water and drying at 40°C [24].

2.4. SBF Coating. Subsequently, a TRIS-buffered 1.5x Tas-SBF solution was prepared based on the work by Jalota et al. [24]. A magnetic stirrer plate (Model name: Favorit) was used in order to mix the solution well for 2 hours at a speed of 700 rpm. All four titanium surfaces were immersed in the Tas-SBF solution and incubated at a temperature of 37°C for a total of 7 days. In the initial 3 days, the Tas-SBF solution was changed for all samples daily at 24-hour intervals. From the third day onwards, the Tas-SBF solution was replenished after every 48 hours till the seventh day.

2.5. Surface Analysis. Lastly, the Alicona (Infinite Focus Optical 3D Measurement Device G4f Metrology) was used to evaluate the surface roughness. The Scanning Electron Microscope (SEM) (Low Vacuum Operating Mode, Model number FEI Quanta 250F) was kept at 10 kV, with a Working Distance (WD) of 10 mm and magnifications of 2000x, 10,000x, and 20,000x, to analyse the surface morphology and thickness. The X-Ray Diffraction (XRD) (Bruker X-Ray Powder Defractometer) and the Fourier Transform Infrared Spectrometer (FTIR Spectrometer) (Model number Nicolet 6700 FTIR, Thermo Scientific.) were used to check the HA/tricalcium phosphate compound presence and elemental composition of the Tas-SBF coated samples on the 3rd, 4th, 5th, 6th, and 7th day, respectively.

3. Results and Discussion

3.1. Results. Analysis of the surface roughness profiles of the samples, using S_a values, showed that for sandblasted and acid etched samples CPTi displayed a higher S_a than an unstandardized CPTi surface. Within the SBF coated samples, there was a general increase in S_a in all surfaces involved. The SBF coatings on the fifth, sixth, and seventh day showed higher S_a values as compared to the third and the fourth day. Figure 1 shows a graphical representation of the S_a values obtained. There was an increase in S_a value for commercially pure titanium from approximately 1.6 ± 0.1 μm at the sandblasting stage to about 2.9 ± 0.2 μm on seventh day of immersion in SBF solution with a decline in S_a value on sixth day.

Figure 2 shows the FTIR spectrum peaks. Analysis with the FTIR spectrum for the SBF coatings on different days of
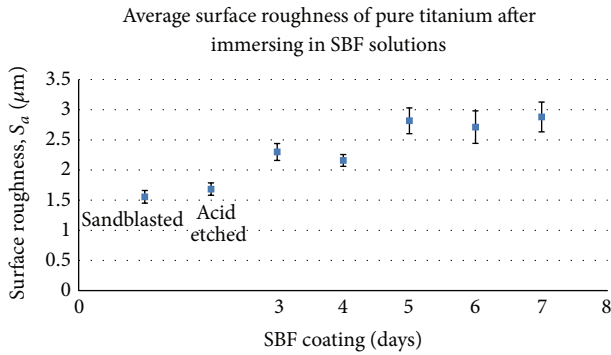
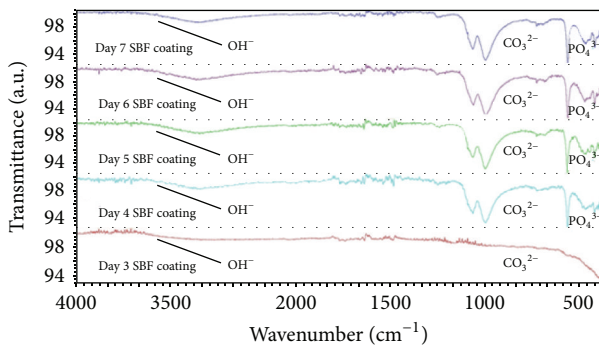Figure 1: Graph showing S_a values of the CPTi samples at various stages and times during the experiment.

Figure 2: Showing the FTIR spectrum peaks for CPTi surfaces. Wavenumber (cm^{-1}).

Figure 3: Showing the EDS of CPTi samples coated with HA/tricalcium phosphate.

immersion of CPTi surfaces showed a gradual elevation in the peak intensity linked with an increase in the number of SBF immersion days. The absorption bands at 1400–1550 cm^{-1} are the peak of CO_3^{2-} group and 560–600 cm^{-1} and 1030–1090 cm^{-1} are the characteristic peaks of the PO_4^{3-} group, whereas bands around 3500 cm^{-1} show the presence of OH^- groups. With the results of the FTIR spectrum, it was not possible to deduce that HA/tricalcium phosphate was present on the sample surfaces. Hence, additional analytical evaluation was required.

The EDS results showed that CPTi gives the typical Ti peak with the presence of Ca, P, and O from the HA/ tricalcium phosphate coating. Figure 3 shows the EDS analysis of CPTi surfaces coated with HA/tricalcium phosphate. All the elements shown are the result of the formation of HA/tricalcium phosphate from the simulated body fluid solution. The Ca/P ratio was 1 : 2, respectively, at the end of the seventh day of Tas-SBF immersion. There were no impurities detected from the sandblasting process or the coating methodology ensuring success of the HA/tricalcium phosphate coating.

The X-Ray Diffraction (XRD) patterns in this study can be viewed in Figure 4. The highest peaks seen in all the samples belonged to substrate metals in the Tas-SBF. XRD beams can penetrate the thin coatings of hydroxyapatite and are diffracted from the underlying metals. For the samples t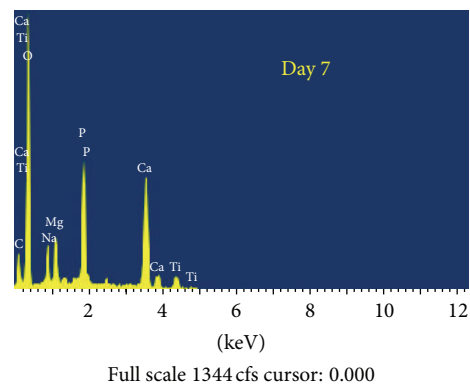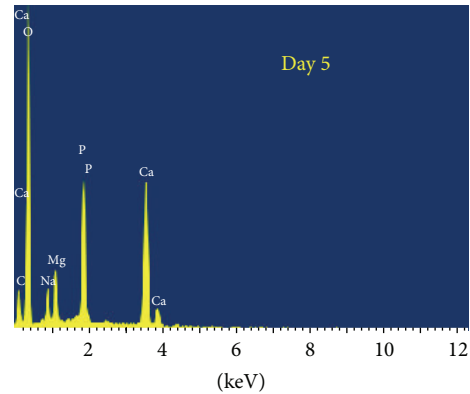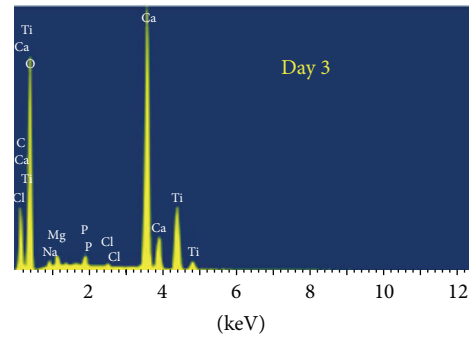hat were immersed in Tas-SBF for three days, the presence of HA/tricalcium phosphate is just visible with a small peak at 2θ angle of 32°. As the coating time was increased, the intensity of the HA/tricalcium phosphate peaks also increased gradually indicating more deposition of the HA/tricalcium phosphate crystals. The only anomaly was seen on the fourth day of the Tas-SBF coated titanium alloy samples where HA/tricalcium phosphate peaks are barely visible. This could be attributed to HA/tricalcium phosphate coating discrepancies or mishandling of the sample.

When the SEM was used to analyse pre- and post-HA coated samples, the acid etched surfaces showed multiple pits and craters. CPTi bore a uniform and well-defined porous surface. In addition to small acid induced indentations, the surfaces displayed large concavities that were induced by the sandblasting process that preceded acid etching. Figure 5
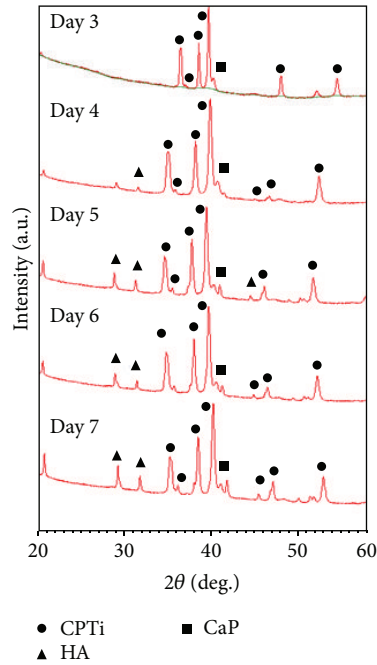
FIGURE 4: Showing the XRD analysis of the surface on various days of the experiment.
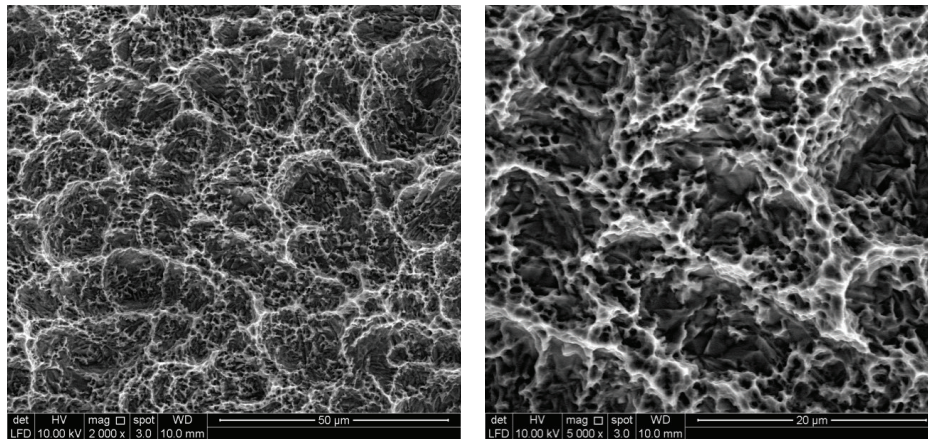

FIGURE 5: Showing the appearance of the acid etched CPTi samples under the SEM at 2000x and 5000x magnification.

shows the SEM micrographs of the acid etched surfaces of CPTi.

Under the SEM, the Tas-SBF coated surfaces possessed a crystalline coating indicated by an intricate crystal assembly that resembled a "flower-like" structure. This is typical of the formation of HA and calcium phosphate crystals that deposit from the Tas-SBF solution. For the CPTi, the crystal size of the coating was bigger on the seventh day of immersion into Tas-SBF as compared to the third, fourth, fifth, and sixth day. Figure 6 shows the SEM micrographs of the HA coating on the third, fourth, fifth, sixth, and seventh day at different magnification.

The thickness of the coated surfaces was also measured for the samples at various days of immersion in the Tas-SBF solution. Figure 7 shows the thickness of the surface coating on the fifth day which was $14.4 \pm 0.2\,\mu$m.

3.2. Discussion. The surface roughness of a dental implant has been shown to have an appreciable effect on the surrounding bone of the implant [25]. It is an important factor that could influence the way osteoblasts attach onto titanium surfaces. Studies indicate that average S_a values of about 1 and $2\,\mu$m optimize osseointegration at the bone-implant interface [26, 27]. So taking this into consideration, sandblasting and acid etching were performed on the surfaces to introduce surface roughness as it increases the surface area for bone contact and hence induces successful implant treatment [28]. It was possible to attain a surface similar to the acid etched surface of
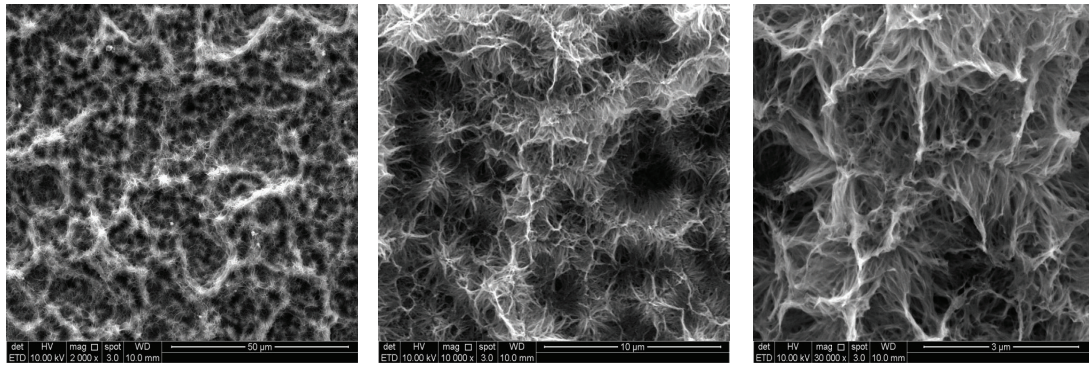
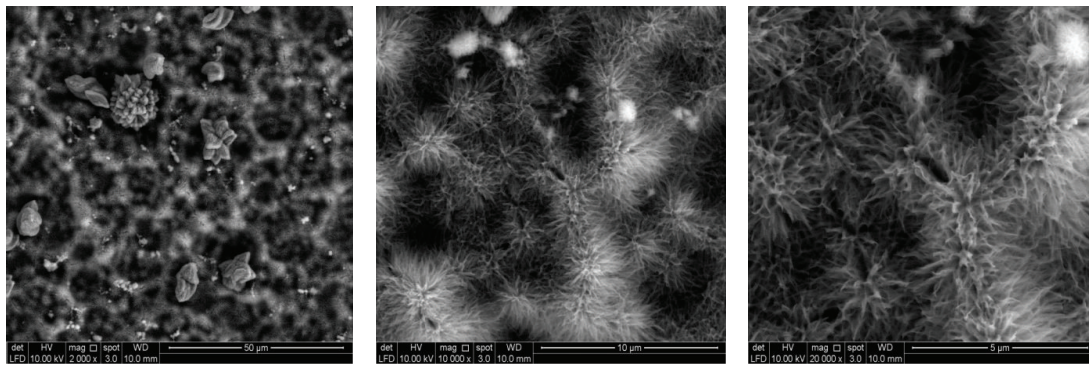Mag.: 2000x   Mag.: 10,000x   Mag.: 30,000x

(a)

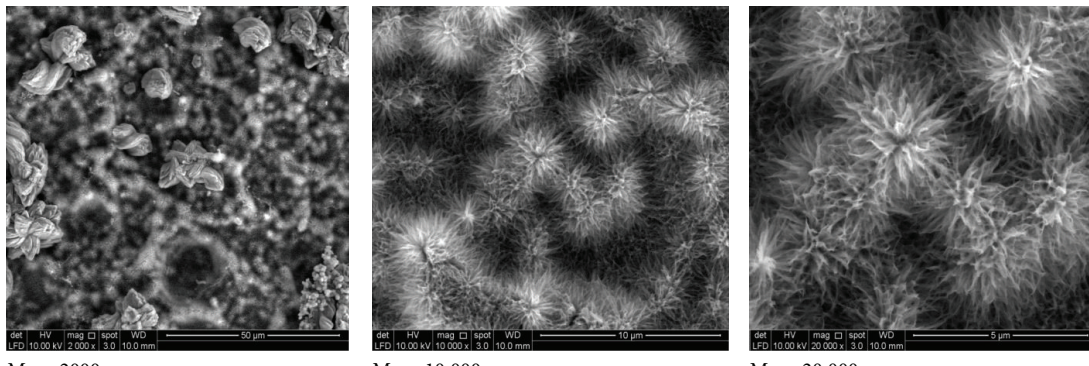Mag.: 2000x   Mag.: 10,000x   Mag.: 20,000x

(b)
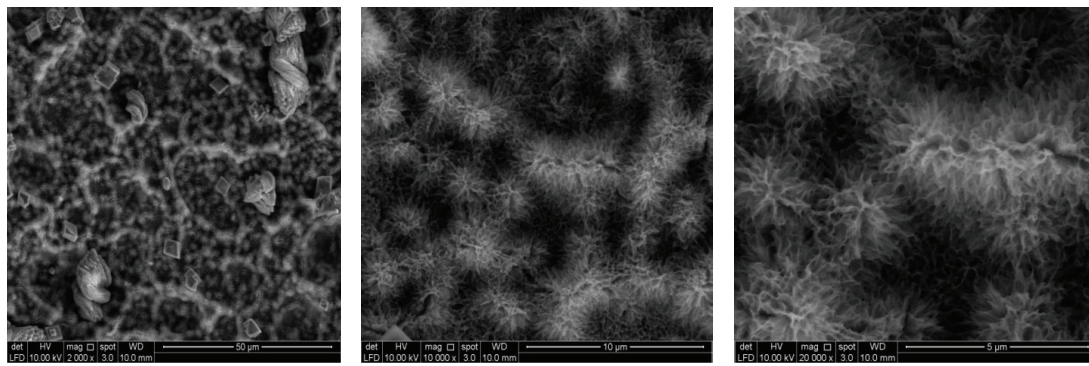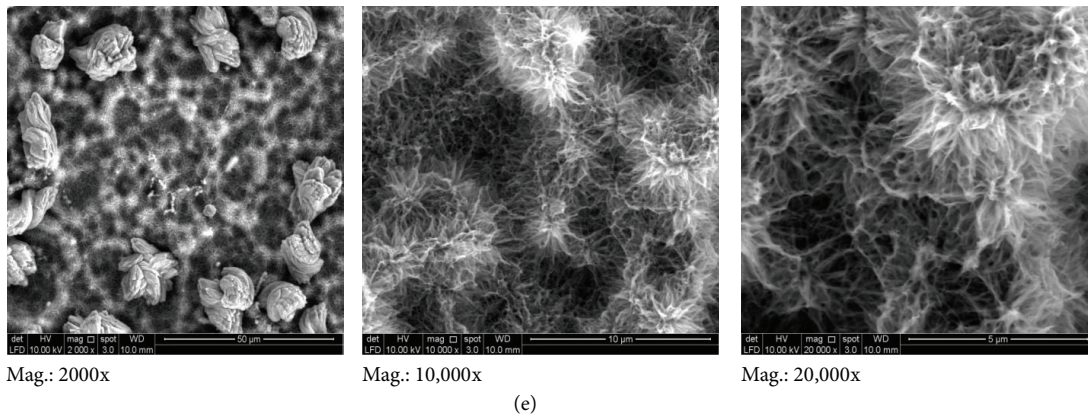
Mag.: 2000x   Mag.: 10,000x   Mag.: 20,000x

(c)

Mag.: 2000x   Mag.: 10,000x   Mag.: 20,000x

(d)

FIGURE 6: Continued.

Mag.: 2000x   Mag.: 10,000x   Mag.: 20,000x

(e)

FIGURE 6: (a)–(e) Showing SEM micrographs of the HA coating on CPTi surfaces on the third, fourth, fifth, sixth, and seventh day, respectively, at different magnifications.
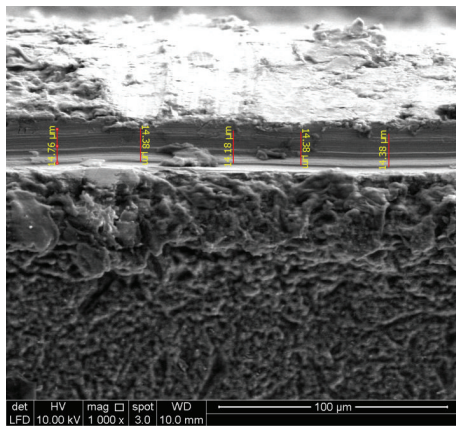

FIGURE 7: Showing $14.4 \pm 0.2\,\mu$m surface layer thickness of the sample on the fifth day of Tas-SBF immersion.

a commercial implant with roughness between 1 and $2\,\mu$m. The process of acid etching was also able to remove the residual impurities from the surface left by the sandblasting process that usually leaves alumina particles embedded in the sample [29]. EDS and XRD analysis of the surfaces did not show a presence of alumina in any sample.

After modifying the surface, latest research suggests the application of various biomimetic coatings that can be beneficial for implant therapy success [30, 31]. This study employed an HA surface coating on the samples because of its proven osteoconductive properties [32]. The biomimetic approach which exploits the advantageous consequences of using Tas-SBF solution to coat HA onto titanium surfaces was used. An HA layer on titanium surfaces using Tas-SBF is capable of achieving crystallinity and morphology similar to those of a bone-like apatite [33]. A favourable aspect of this study coincides with the proposition that increased crystallinity of HA coatings appears to slow resorption of HA [34].

The surface roughness of the Tas-SBF coated samples in this study showed a significant increase after the experiment reached its conclusion. This is important to note because consequentially this layer could improve the surface area

for cell attachment and proliferation. The FTIR spectrum analysis showed peaks for –OH bond which was an indication that the surface contained HA. The XRD data showed peaks indicating the presence of crystalline HA phases as well. It can be concluded from the results that it was possible in this study to obtain a bioactive thin and relatively uniform coatings of HA on both pure titanium and titanium alloy surfaces.

Another key parameter is that the duration of immersion into the Tas-SBF solution has an effect on the surface roughness and also the crystal size of the HA/calcium phosphate coating. Generally, biomimetic coating processes are performed for about seven to fourteen days [35]. There are some treatment methods however that temper with the concentration and constituents of Tas-SBF solutions and can considerably reduce the Tas-SBF immersion time to create a favourable HA/calcium phosphate deposition [36, 37]. This study was conversely able to ascertain that it is possible to reduce the time of Tas-SBF immersion while maintaining the standard Tas-SBF solution concentration. This was done by preparing surfaces that had negligible influence on the composition and the morphology of the coating. Furthermore, this research was able to prove that a more uniform and thinner layer of HA/tricalcium phosphate that was immersed in Tas-SBF for seven days or less may be sufficient for implant surface coating. The biomimetic coating obtained for all the Tas-SBF immersion periods portrayed compositional and structural features that closely resembled human bone.

4. Conclusion

This study was successful in investigating that coating HA/tricalcium phosphate on titanium surfaces with the Tas-SBF solution for 7 days to achieve uniform and thin coatings is a viable option for biofunctionalizing titanium surfaces. Further research needs to be conducted in this sphere of implantology so as to improve implant therapy.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgments

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