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Effect of Argon Plasma Treatment on Surface-Treated Titanium

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Abstract

This work compares the consequence of argon bombardment plasma treatment on sandblasted large-grit, acid-etched (SLA) and hydroxyapatite (HA) treated titanium surface. Titanium disc specimens were distributed into two groups depends on the surface treatment (SLA, HA). The SLA and HA-coated surfaces were further subdivided according to non-plasma and plasma treatments. An argon plasma treatment was done at ambient temperature under vacuum. The specimens (surface treated) were subjected to surface analysis using field-emission scanning electron microscopy, a three-dimensional optical profiler, and X-ray photoelectron spectroscopy (XPS). Furthermore, contact angle examination was performed via the sessile drop technique. Pre-osteoblast cells (MC3T3-E1) were employed here to evaluate the in vitro bioactivity. The obtained results were statistically studied by two-way analysis of variance and Tukey post-hoc tests ($P < 0.05$). The surface morphology and surface topography results revealed no significant differences before and after the plasma treatments. However, a considerable difference was observed in the oxygen concentration via XPS, which significantly controlled the surface hydrophilicity, as confirmed from contact angle measurements. The in vitro results revealed that cell growth on the HA group is better than that on the SLA group, indicating that surface modification significantly affects cell adhesion and cell proliferation.

Keywords: Plasma Treatment; Titanium; Surface-Treated; Hydrophilic; SLA; HA

Introduction

The utilization of titanium-based implant materials in the field of biomedical, orthopedic, and dental implants has drastically increased owing to their excellent biocompatibility, yield strength, satisfactory hardness, and relatively low weight [1-3]. More importantly, the life expectancy of titanium and its alloys is superior among other metallic alloys characteristically utilized in implants [4]. However, the long-term health problems associated with the discharge of V and Al ions in to the interior body have posed major limitations to the use of the most common alloy, Grade 5 titanium (Ti-6Al-4V), for dental implants with excellent fatigue and yield strength [5,6]. Therefore, researchers have explored various surface modification strategies, such as anodic oxidation, chemical treatments, grit blasting, and bioactive ceramic coating [7,8]. Surface roughness can be achieved either by the addition (coating) or subtraction of an implant material. In clinical applications, the combination of chemical treatment and grit blasting, i.e., sandblasted, large-grit, acid-etching treatment, is widely used to enhance osteointegration by creating a hydrophilic implant surface [9].

It has also been discovered that hydroxyapatite (HA) coatings, when used on titanium alloys, not only arrest the discharge of alloying elements, but also improve the osseointegration of the titanium alloy due to increase in the mechanical strength, corrosion resistance, and biocompatibility [10]. HA has been coated on titanium alloys via various approaches such as sol-gel [11], electrolytic [6], physical vapor deposition [12], and plasma thermal spray methods [13]. Among these techniques, plasma spray has proven to have the best chemical control, bio-corrosion resistance, and process efficiency [14]. It is well known that osteointegration properties can be enhanced through surface modification techniques by developing a hydrophilic implant surface. This is because the surface characteristics of the implant aid in the vascularization and growth of bone tissue [15,16]. In the current study, HA was initially coated on a titanium disc by

plasma spray, and then subjected to argon plasma bombardment Sandblasted, large-grit, acid-etched (SLA) treated samples are also post treated with argon plasma treatment. The surface characteristics, in vitro bioactivity, and hydrophilicity of the SLA- and HA-treated titanium surfaces were carefully investigated and compared for with and without plasma treatments.

Materials and Methods

Sample Preparation

The commercial Ti-6Al-4V (Grade 5) disc had a diameter of 15 mm and thickness of approximately 2 mm. The as-received specimens were grounded and polished by 600 mesh silicon carbide paper, and then ultrasonically cleaned for 15 min successively using acetone, ethanol, and distilled water. The polished disc specimens were distributed into 4 sets based on the external treatments. The experimental groups are listed in Table 1.

Table 1: Experimental groups.

Group	Description
SLA	Sandblasted, large-grit, acid-etching on titanium
SLA-P	SLA-treated specimen was further treated with argon plasma
HA	Hydroxyapatite coated on titanium
HA-P	HA-coated specimen was further treated with argon plasma

Sandblasted with Large-Grit, Acid-Etching

Sandblasting was performed using an airborne particle abrasion method (Basic Master, Renfert GmbH, Hilzingen, Germany). The disc specimen was retained upright to the nozzle at a 10 mm space, and its surface was abraded with 110 μm Al_2O_3 particles (Cobra, Renfert, Germany) for 10 s under a pressure of 0.4 MPa. Ultra-sonication treatment is employed to clean all the samples (acetone, ethanol, and DI water) for 15 min. Subsequently, the specimens were treated in an acid solution containing 7% hydrochloric acid (HCl) and 27% sulfuric acid (H_2SO_4) at 110 °C for 5 min.

Hydroxyapatite Coating

The HA coating was deposited on the Grade 5 titanium specimen by non-thermal plasma coating using argon gas (65 sccm), with an applied current output of 220 W under vacuum (RB301, Atte System, Korea).

Argon Plasma Treatment

The SLA surface and HA-coated surface were subjected to argon plasma treatment under vacuum (Pladen, Polybiotech, Korea) for 10 min at an output power of 68 VA with a driving frequency of 50 kHz.

Characterization

The surface microstructures of the samples were detected by high-resolution field emission scanning electron microscopy (FE-SEM, JSM-7500F, JEOL, Tokyo, Japan). Platinum was coated on to the specimen by sputtering process to ease the exterior charge. Moreover, energy dispersive X-ray spectroscopy (EDS, Oxford, UK) was employed to determine the elemental distributions on the surface-treated specimens, in conjunction with FE-SEM. Moreover, a three dimensional (3D) optical surface profiler (Nb-E1000, Nanosystem, Daejon, Korea), was used to measure the surface roughness of the specimens; Nanoview and Nanomap software were used to generate the 3D surface topography images. Elemental composition and bonding properties of every group of samples were analyzed by X-ray photoelectron spectroscopy (XPS, VG Mulrilab 2000, Thermo scientific, UK) The peak area values were normalized and expressed as quantitative ratios. In addition, Phoneix 300 contact angle measurement structure (Surface Electro-Optics, Seoul, Korea) was used to measure the contact angle to determine the wettability of the surface of the specimen via the sessile drop method. Image Pro 300 software was used to analyze the corresponding results.

Cell Culture

Murine pre-osteoblast cells (MC3T3-E1) were cultured in α-MEM medium (Gibco) culture media were appended with 10% fetal bovine serum and 1% antibiotic. A humidified atmosphere with 5% CO_2 at 37 °C was maintained to culture the cells.

Cell Attachment

Seeding of MC3T3-E1 cells on the surface of the specimens was done at a density of 5×10^4 cells/mL in a 24-well plate, respectively. After 1 and 3 days of culture, the unattached cells were washed with phosphate buffered saline (PBS). Glutaraldehyde (2.5%) was used to fix the attached cells at room temperature for 2hrs. The resultant solution was sucked out, and the specimens were washed using PBS and ethanol medium (40% - 90%) was used for the dehydration of the cells for a period of 15 min each and absolute ethanol for 10 min. FE-SEM were employed to observe the cell attachment and morphology.

Cell Proliferation

MC3T3-E1 cells were seeded at a density of 5×10^4 cells/mL on 24-well plates and incubated for 1, 3, and 5 days, respectively. XTT (EZ-Cytox cell viability assay kit, Dogen, Korea) solution was poured to all well of the plate for each incubation period. The solution from the each well in the culture plate after incubation was shifted into a 96-well plate. A microplate reader with wavelengths of 450 nm and 630 nm (Multiskan Go with Skanlt software, Thermo Fischer Scientific, Waltham, MA, USA)

Alizarin Red Staining

Seeding of MC3T3-E1 onto the surface of the SLA and SLA-P group specimens were done at a density of 1 × 104 cells/mL and incubated for 21 days to evaluate the calcium matrix deposition on the substrate by alizarin red staining (ARS). After incubation, the attached cells on the substrates were washed twice with DPBS and fixed with 10% formalin for 15 at room temperature.

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Subsequently, 2% ARS solution was prepared by adjusting the pH (4.1-4.3) using ammonium hydroxide. Following cell fixation, the prepared ARS solutions were added to each well and incubated for 45 min at room temperature in the dark. Subsequently, the ARS was extracted with 10% cetylpyridinium chloride (CPC) solution and transferred into 96-well plates to measure the absorbance at 550 nm.

Results and Discussion

The surface characteristics of the SLA-treated and HA-coated specimens observed by FE-SEM are shown in Figure 1. The surface morphology remained unchanged after plasma treatment for both groups. The SLA-treated specimen exhibited an irregular and multifarious surface morphology (Figure 1a), whereas the HA-coated specimen showed a uniform and isotropic coating all over the surface; parallel slits were also observed on the disc, which were generated during polishing. Furthermore, the elemental composition on the treated disc confirmed the existence of Ca and P on the HA-coated sample. The surface roughness and surface topography revealed that there are no noteworthy changes in the samples with or without plasma treatment process; this was further confirmed from the FE-SEM image. In addition, the SLA-treated group showed considerably higher roughness than the HA-coated group (insets in Figure 1a & Figure 1b). The roughness values of each group were as follows: SLA, 0.81 0.01 Ra (μm); SLA-P, 0.86 0.01 Ra (μm); HA, 0.14 0.02 Ra (μm); and HA-P, 0.17 0.01 Ra (μm).

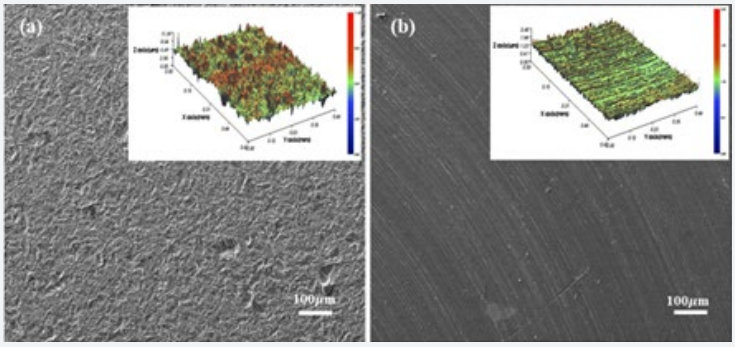

Figure 1: FE-SEM images and surface topography of (a) SLA-treated and (b) HA-coated specimens; the insets in 1a and 1b present the 3D surface profiles.

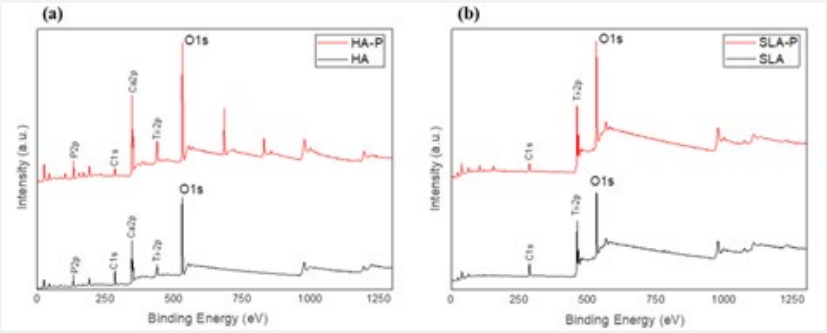

Figure 2: XPS profiles of surface-treated specimens before and after plasma treatment.

XPS was used to investigate the surface characteristics of the specimens. Figure 2 shows the XPS profiles of the SLA- and HA-coated specimens before and after plasma treatment. The XPS profiles were averaged by normalizing the peak value of each element through carbon (C1s) and oxygen (O1s) spectrum peaks. XPS results for the SLA-treated titanium surface revealed that the oxygen content on the surface increased by 47.26% and carbon content decreased by 28.35% after the plasma process (Figure 2a). Whereas, the oxygen content increased by 21.41% in the HA-coated titanium surface after plasma treatment (Figure 2b), while the carbon content decreased by 36.76%. The decrease in carbon content can be attributed to the cleaning effect of the organic substances remaining on the surface of the specimen due to plasma treatment [17]. Specifically, the pre-surface treated titanium surface of the specimens underwent low-temperature argon plasma bombardment, leading to modifications in the effects of the contaminated atoms. These contaminated atoms on the surfaces were scavenged in the course of the plasma process, which thereby decreased the carbon content. The surface hydrophilicity improved due to an increase in the oxygen concentration [18,19].

The surface wettability involves in a key role in implant surface modification and determines the biological response

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between bones and the implant interface [20,21]. Contact angle measurements were performed to investigate the surface hydrophilicity using a sessile drop method. The photographs of the water droplets on the surfaces of the pre- and post-treated samples are shown in Figure 3. The average contact angles of the SLA and HA groups before plasma treatment were 83.91 ± 5.52 and 38.8 ± 4.27, respectively. It was also observed that the measured contact angle was lower for the HA group (Figure 3c). In contrast, the contact angles measured for the SLA plasma and HA plasma groups after plasma treatment were 10° or less. The decrease in the contact angle is largely influenced by the argon plasma bombardment, which results in hydrophilicity of the surface. This result is also consistent with the XPS results. All four groups demonstrate a statically significant difference [18]. The general morphology and attachment pattern of the osteoblast cells after 1 and 3 days of incubation were witnessed by FE-SEM (Figure 4). After 1 day of incubation, MC3T3-E1 cells showed firm attachment onto the surface for all groups. Cells on the SLA and SLA-P surfaces were polygonal in shape with a cytoplasmic extension, whereas those on the HA and HA-P groups were more flattened and elongated in shape. After 3 days of incubation, the cells spread all over the surface of the specimens with long filopodia and firm attachment, resulting in increased density compared to that for 1 day of incubation [22,23].

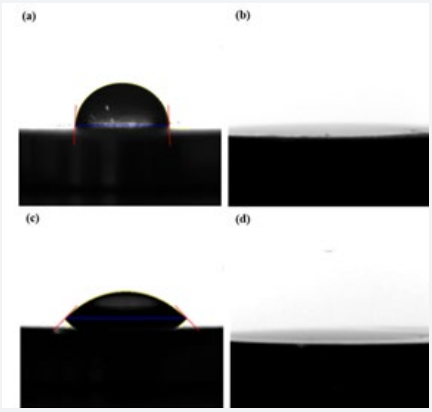

Figure 3: Photographs of the contact angle on the surface. (a) SLA, (b) SLA-P, (c) HA, (d) HA-P

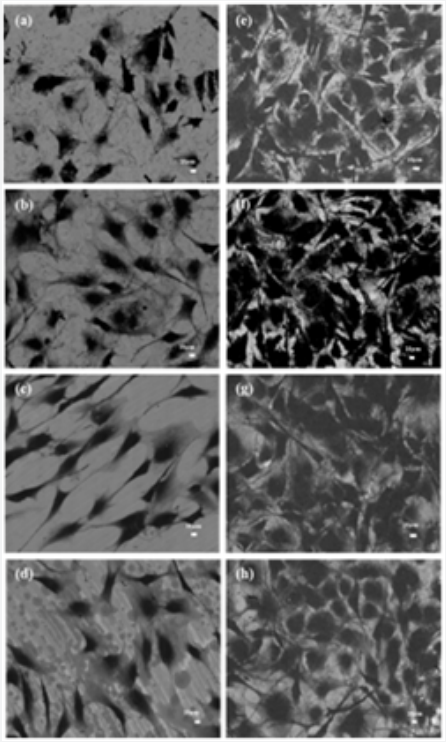

Figure 4: FE-SEM images of MC3T3-E1 cells on the surface of the specimens after 1 day (a-d) and 3 days (e-h) of incubation.

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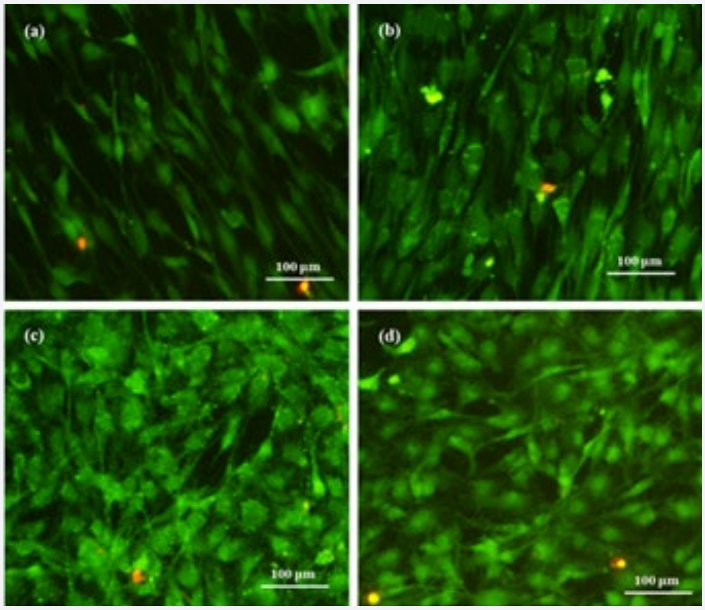

Figure 5: LIVE/DEAD staining showing the cell viability of MC3T3-E1 cells on the surface of the specimens after 1 day of incubation. (a) HA, (b) HA-P, (c) SLA, (d) SLA-P.

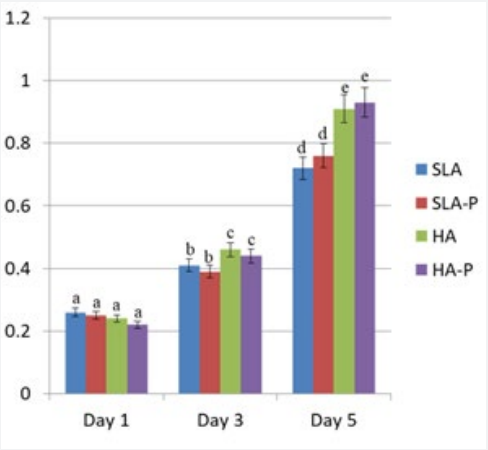

Figure 6: Cell proliferation of MC3T3-E1 cells after 1, 3, and 5 days of incubation. The different letters represent the significant difference ($P < 0.05$).

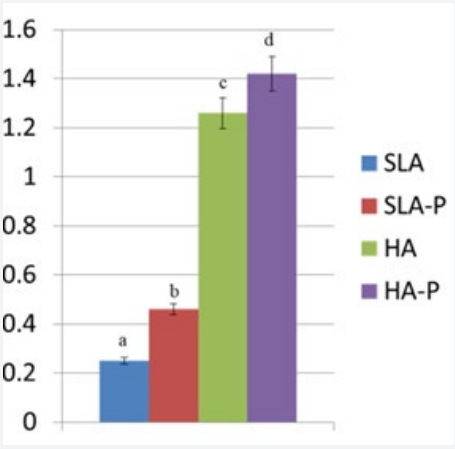

Figure 7: Quantification of mineral deposition by ARS extract. The different letters represent the significant difference ($P < 0.05$).

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The cytotoxicity's of the MC3T3-E1 cells before and after plasma treatment were evaluated using the LIVE/DEAD assay (Figure 5). After 1 day of incubation, the cells on the HA-coated group exhibited growth according to the coated pattern. The cells of the plasma-treated specimens for both groups were flatter and more viable, and well-adhered to the surface. Plasma treatment induces a strong impact on cell adhesion due to surface modification and the hydrophilic effect [24]. The proliferations of MC3T3-E1 cells incubated for 1, 3, and 5 days on the surface-treated and plasma-treated specimens are plotted in Figure 6. With increasing incubation time, the cell proliferation linearly increased. After 3 days of incubation, there were no significant differences among all groups However, a significant difference was observed for the SLA and HA-coated groups after 5 days of incubation. In addition, there was no significant difference amongst before and after the plasma treatments [18,25-27]. Deposition of the inorganic mineral matrix was observed through the osteogenic differentiation of the MC3T3-E1 cells. Figure 7 shows the ARS of 21-day MC3T3-E1 cells before and after the plasma treatment of the SLA and HA-treated specimens. The calcium deposition of the osteoblast cells on the SLA and HA surfaces after plasma treatment was greater than for the non-plasma-treated groups, possibly due to the difference in the surface energy [28,29].

Conclusion

In summary, this study demonstrated the effect of argon bombardment plasma treatment on SLA- and HA-treated titanium surfaces. The surface morphology and surface topography results revealed no significant differences before and after the plasma treatment. However, there was a considerable difference in the oxygen concentration, as determined by the surface chemical composition measurement (XPS study); this significantly controlled the surface hydrophilicity, as confirmed by contact angle measurements. The response of the osteoblast (MC3T3-E1) cell was higher in the HA-treated group than in the SLA group after 5 days of incubation. It was concluded that the effect of surface modification would have a significant effect on cell adhesion and cell differentiation. Thus, the utilization of plasma treatment for surface-treated titanium implants could provide further improvements, and therefore, be promising for application in clinical implants in the near future.

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