Article

Acceleration of Bone Formation and Adhesion Ability on Dental Implant Surface via Plasma Electrolytic Oxidation in a Solution Containing Bone Ions

Mosab Kaseem¹ and Han-Cheol Choe^{2,*}¹ Department of Nanotechnology and Advanced Materials Engineering, Sejong University, Seoul 05006, Korea; mosabkaseem@sejong.ac.kr² Advanced Functional Surface & Biomaterials Research Lab, Department of Dental Materials and Research Center of Nano-Interface Activation for Biomaterials, College of Dentistry, Chosun University, Gwangju 61452, Korea

* Correspondence: hcchoe@chosun.ac.kr

Abstract: The present study examined the in vitro and in vivo bone formation and adhesion ability on the surface of a titanium dental implant made by plasma electrolytic oxidation (PEO) in electrolytes containing bioactive ions. To achieve this goal, screw-shaped fabricated Ti-6Al-4V alloy implants were processed via PEO using an electrolyte solution containing calcium (Ca), phosphorous (P), magnesium (Mg), zinc (Zn), strontium (Sr), silicon (Si), and manganese (Mn) species. The screw implants doped with bioactive elements via PEO were placed in rabbit tibia, and the results were compared to the sand-blasted Ti-6Al-4V alloy implants. At eight-week post-surgery, there was no significant difference in the values of removal torque between sand-blasted and PEO-treated implants. However, it was observed that the PEO treatment of dental implants led to the formation of more periphery bone as compared to the case of sand-blasted implants. Accordingly, the PEO-treated implants have the potential to be used as promising materials for dental applications.

Keywords: dental implant; porous TiO₂; bone adhesion; rabbit tibia; removal torque

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1. Introduction

Titanium-based materials have been considered as promising metallic implants owing to their high strength/density ratios and low corrosion rate, favorably meeting the majority of the fundamental aspects in the development of metallic biomaterials [1–3]. However, an implant with a higher elastic modulus as compared to the bone might lead to stress or the shielding effect, allowing osteoporosis or poor osseointegration. In contrast, the implant with a low elastic modulus share would load with the bone to promote bone growth [4,5].

In the oral and maxillofacial area, therefore, efforts have been made for aesthetic, functional, and mental recovery by restoring lost teeth from restoration of single teeth to reconstruction of the maxillary defects after maxillofacial trauma or tumor surgery. Bone adhesion is necessary for the safety and long-term success of the implants, and the degree of new bone formation around the implant is considered to be very important in the prognosis. Therefore, several surface modification approaches, such as acid etching, sand blasting, plasma spray coating and anodizing have been largely studied [6–10]. In particular, plasma electrolytic oxidation (PEO) has been suggested by many research groups to overcome the aforementioned drawbacks [11]. Porous titanium oxide (TiO₂) coating made on Ti and its alloys via PEO in aqueous electrolytes has been reported to have acceptable mechanical properties and osteogenic ability [12,13]. Besides, the osteointegration of Ti-based materials can be significantly enhanced through the decoration of their surfaces with nanoscale coatings [14].

Considering the composition and crystal structure that is similar to the human bone, nanoscale hydroxyapatite (HA) was considered a promising material in biomedical appli-

cations due to its good ability to bond with bone tissue in vivo as well as stable chemical properties [15,16]. It was also claimed that HA can promote angiogenesis, which assists the osseointegration [17]. Although Ca and P elements, the major constituents of HA, can be easily incorporated and distributed homogeneously throughout the thickness of PEO coating, their impacts on the surface morphology of the oxide layer were insignificant [18]. However, the presence of a sufficient amount of those elements on the outmost surface would be necessary to induce the formation of nanoscale HA [19].

To promote the osteointegration, therefore, considerable interest has been given to form the endogenous nanoscale HA on the surface of the TiO₂ made via PEO along with the inclusion of bioactive elements into such oxide layers. Although the individual or dual incorporation of various combination of zinc (Zn), magnesium (Mg), silicon (Si), strontium (Sr), and manganese (Mn) elements into the TiO₂ coating via wet and dry plasma treatment helped to improve the bone formability in vitro [20–24], the impacts of Zn, Mg, Si, Sr, and Mn species on the in vivo osteointegration of dental implants have been little investigated. In our recent study [24], the effects of the addition of Zn, Mg, Sr, Si, and Mn ions into the solution were compared with the case when the electrolyte only contains Ca and P ions. The in vitro examination assessment revealed that the PEO treatment in an electrolyte containing Ca, P, Zn, Mg, Sr, Si, and Mn was an effective procedure to induce the proliferation of osteoblasts on the surface of Ti alloy samples when compared to the case of PEO treatment in solution only containing Ca and P ions [24]. Therefore, the developing Zn-, Mg-, Sr-, Si-, and Mn-doped TiO₂ coatings would be a promising strategy to improve the implant's osseointegration due to the combined effects originating from the porous structure of PEO coatings and the roles of those elements in the enhancement of the osteointegration. Well-known bioactive elements are the elements that facilitate biomineralization, which helps to reduce a typical adverse host response and facilitate the formation of direct bonding with the bone [11]. As demonstrated in [24], Ca, P, Mg, Zn, Sr, Si, and Mn are found to be bioactive elements. Therefore, the aim of this work was to improve the osseointegration activity of Ti-6Al-4V alloy through the incorporation of Ca, P, Mg, Zn, Sr, Si, and Mn into the porous TiO₂ coating made on the surface of Ti-6Al-4V alloy via PEO treatment. Accordingly, Ca-, P-, Zn-, Mg-, Sr-, Si-, and Mn-incorporated TiO₂ was fabricated by PEO treatment utilizing an electrolyte with ions of those elements. Since the sand-blasted implants are usually used in dental clinical application, we compared the osseointegration activity of sand-blasted implants with the implants treated via PEO. The removal torque and surface analysis of sand-blasted Ti-6Al-4V alloy and bioactive ion-doped Ti-6Al-4V surface implants in rabbit tibia were compared. The results provide a simple procedure to improve the bone adhesion at the surface of dental implants, increasing the contact of the bone and implant and improving clinical success.

2. Experimental

2.1. Fabrication and Modification of Ti Implants

Screw-shaped Ti-6Al-4V implants (2.9 mm in diameter and 7 mm in length) were fabricated and used as substrates in the present study. The surface of Ti-6Al-4V implants was either sand blasted or treated via PEO. For sand-blasting treatment, the implants were sand blasted with 250 µm alumina particles and passivated with HF/HNO₃ acid. To incorporate bone ions into implant material via PEO, implants made of Ti-6Al-4V alloy were treated via PEO in an electrolyte solution consisting of Ca, P, Zn, Mg, Sr, and Mn species. To prepare 7 ions solution, calcium acetate (0.12 M), calcium glycerophosphate (0.019 M), zinc acetate (0.0075 M), magnesium chloride (0.0075 M), manganese acetate (0.0075 M), and sodium metasilicate (0.001 M) were mixed together in 1000 mL of distilled water. The applied voltage was fixed to be 280 V for 3 min during the PEO process, which was conducted under direct current mode. Following PEO treatment, all coated samples were rinsed with distilled water and dried in hot air. Here, Ti64/SA describes the Ti-6Al-4V implants subjected to sand blasting, while Ti64/7 ions labels the Ti-6Al-4V implants treated via PEO in a solution containing ions of 7 elements.

2.2. Surface Characterization

The surface and cross-sectional morphologies of the samples were analyzed by scanning electron microscopy (SEM-HITACHI, S4800, Hitachi, Tokyo, Japan), with equipped energy-dispersive X-ray spectroscopy (EDS) for identifying chemical compositions of coatings. The measurements of the pore size and porosity were performed by SEM observations taken from at least ten different areas for each condition with the aid of image analyzer software (version 1.47 for Windows, 64 bit, free software, National Institutes of Health, Bethesda, MD, USA). Atomic force microscopy (AFM, Tosca™ Analysis, Anton Paar, Graz, Austria) was used to determine the surface roughness of the samples. The contact angles of the cleaned samples (sand-blasted and PEO-coated) were determined by a water contact angle goniometer (Kruss DSA100, Hamburg, Germany) with one drop (5 µL) of distilled water. It is worth mentioning that the sand-blasted and PEO-coated samples were not stored and aged before the contact angle measurements. The surface energy (solid-liquid) (γ_s) of the samples was calculated using Equation (1) [23]:

$$\cos\theta = 2\left(\frac{\gamma_s}{\gamma_L}\right)^{0.5} \exp[-\beta(\gamma_L - \gamma_s)^2] - 1 \quad (1)$$

where $\beta = 0.0001247 \text{ (m}^2/\text{mJ)}^2$, γ_L is the surface energy of water (72.8 mJ/m²), and θ is the average of the five measurements of contact angle.

2.3. Cell Culture

The MC3T3-E1 mouse osteoblasts (ATCC, USA) were used for the in vitro experiments to characterize the cells attachment on the surface of the tested implants. The cells were incubated in an atmosphere with a temperature of 37 °C and 95% O₂ and 5% CO₂, while minimum essential medium (MEM, WELGENE, Gyeongsan, Korea) was used as a culture medium. The sub-culture medium was altered every three days. The cultured cells were placed in phosphate-buffered saline (PBS) and then cultured in trypsin–ethylenediamine tetraacetic acid (EDTA) solution (0.05% trypsin, 0.53 mM EDTA, phenol red in Hank's balanced salt solution) at 37 °C for 10 min to separate the cells. Cells were seeded on a Ti alloy at a concentration of 9.2×10^6 cells/well on a 24-well plate and grown on the coated surface for 24 h. The samples were washed with PBS and fixed with 10% formaldehyde at 4 °C for 12 h. After fixing, the specimens were dehydrated with ethanol. The morphology of the attached cells was observed by using FE-SEM.

2.4. In Vivo Experiments

2.4.1. Surgical Procedures

Twelve adult male rabbits weighing 2 kg were utilized in the present work. To identify the effectiveness of PEO-treated Ti alloys on the bone adhesion at the surface of dental implants, implants were separated into two groups: the first group was a control group (C.G) which includes the sand-blasted dental implants (Ti64/SA) (diameter: 2.9 mm, length: 7 mm, 36 implants), while the second group was the experimental group (E.G) which consists of PEO-treated dental implants (Ti64/7 ions) (diameter: 2.9 mm, length: 7 mm, 36 implants). All surgical operations were conducted under sterile conditions where autoclave sterilization was performed for all surgical procedures related to animal experiments one day before the experiment. (121 °C, 15 pounds, 15 min). Then, the rabbits were weighed, and 3 mL of Zoletil (Zoletil50, Virbac Co, Carros, France) per kg was injected intramuscularly into the thigh to induce sedation and anesthesia. The rabbit was placed in the prone position and shaved on both tibias. To disinfect the surgical site, a povidone iodine, chlorhexidine gluconate solution, and isopropanol were used. For local anesthesia at the surgical sites, 100,000 invasive anesthesia was performed using lidocaine containing epinephrine. The incision was carried out using 15 blades and exfoliation of subcutaneous tissue and periosteum, exposing both tibias, as shown in Figure 1a.

Figure 1. Surgical procedures showing (a) incision and peeling; (b) preparation of implantation sites using a guide drill and twist drill; (c) implant replacement where Ti64/SA implants were placed on the right side, while Ti64/7 ion implants were placed in the left side; and (d) the scarified sample taken after 8 weeks of the experiments.

When the tibia was exposed, bone formation for implant placement under douche using a guide drill and twist drill was performed (Figure 1b). When the bone formation was completed, the control group (Ti64/SA implants) was inserted on the right side, the PEO-treated experimental group (Ti64/7 ion implants) was inserted on the left side, and three implants were implanted using an implant kit (Figure 1c). Periosteal sutures were performed using 2-0 vicryl, muscle sutures were used with 3-0 vicryl, and subcutaneous and skin closures were performed with 5-0 vicryl and 6-0 nylon. Before surgery, the site was sterilized using povidone-iodine, chlorhexidine gluconate solution, and isopropanol, and injected with first-generation cephalosporin antibiotics and Keromin. The rabbit's general condition, wound healing, and infection at the surgical site were checked daily and disinfected. After 8 weeks, the rabbits were sacrificed using a KCl solution, and bone fragments were collected using fracture diagrams with a margin of 7 mm or more around the implant, as shown in Figure 1d.

2.4.2. Histological and Histomorphological Analysis

After the tissue was fixed with plastic, the tissue was made using a grinder and subjected to Haemotoxylin and Eosin staining. Afterward, eighteen specimens were prepared for each of the control (Ti64/SA) and experimental group (Ti64/7 ions) implants. As a result of histological observation, the amount of new bone formation around the implant surface of the experimental group was increased compared to the control group. The BIC (bone-to-implant contact ratio), which is the ratio of the contact surface between the implant fixture and the bone, was measured. Four different measurements utilizing the Image J (1.52a, National Institutes of Health, Bethesda, MD, USA) program for each implant type were carried to calculate the bone-to-implant contact area ratio (BIC%) as well as new bone formation area ratio.

2.4.3. Removal Torque Value

A specially designed fixture mount for connecting the experimental implant and the torque meter was fastened to 35 Ncm and connected to a torque meter (MGT12, ELECTROMATIC Equipment Co. Inc., Oakland, CA, USA). The maximum torsional removal value at which the separation of the implant and bone occurred by rotating in the reverse direction of the implant placement was measured and recorded. Three values were obtained for each rabbit to calculate the mean value.

2.4.4. Statistical Analysis

Statistical analysis of the data (mean $\pm$ SD) was performed using an unpaired Student's *t*-test with Graph Pad Prism4 software Version 5.04 (GraphPad Software, San Diego, CA, USA). Values of *P* less than 0.05 were considered statistically significant.

3. Results

3.1. Surface Characterization In Vitro

Figure 2 shows the SEM images of the Ti64/SA and Ti64/7 ion implants. The Ti64/SA implants were characterized by a rough surface characterized by irregular cavities uniformly alternated with peaks and valleys (Figure 2a-2). The surface of Ti64/7 ion implants exhibited a porous structure with different characteristics in terms of the shape and size of pores (Figure 2b-2). The mean pore size and porosity in the surface of Ti64/7 ion implants were measured to be $1.13 \pm 0.4 \mu\text{m}$ and $9.98 \pm 1.1\%$, respectively. The pores are ascribed to the presence of different ions in the electrolyte during PEO which can affect the gas evolution during PEO coating [25]. The presence of small particles on the Ti64/7 ion implants suggested the inclusion of electrolyte species, namely, Ca, P, Zn, Mg, Mn, Sr, and Si into the oxide layer during PEO. As displayed in Figure 3, the Ti64/7 ion implants exhibited lower roughness value with an average value of mean surface roughness ($R_a = 1.43 \pm 0.40 \mu\text{m}$) as compared to the Ti64/SA implant, which showed a rougher surface with a value of ($R_a = 1.61 \pm 0.60 \mu\text{m}$). The thickness of the oxide layer formed on the Ti64/7 ion implant was found to be $\sim 3 \mu\text{m}$, as revealed by the cross-section image shown in Figure 4a.

Figure 2. The appearance and SEM images of the Ti-6Al-4V alloy implants treated via (a,a-1,a-2) sand blasting and (b,b-1,b-2) plasma electrolytic oxidation (PEO) in a solution containing ions of seven elements.

Figure 3. Atomic force microscopy (AFM) results of the implants: (a) Ti64/SA implants and (b) Ti64/7 ion implants.

Figure 4. (a) Cross-sectional image of the Ti64/7 ion implant; (b) EDS area spectrum for the Ti64/7 ion implant. Ca, P, O, Ti, V, Al, Si, Mn, Mg, Sr, and Zn were identified.

According to the EDS area results shown in Figure 4b and Table 1, Ca, P, O, Ti, V, Al, Si, Mn, Mg, Sr, and Zn elements were identified. The Ti, Al, and V originated from substrate alloy, while the presence of the other elements was attributed to the electrolyte solution. In our previous work, we found that anatase, rutile, and HA were the main phases in seven ion implants [24]. The wettability measurements revealed that the surface of Ti64/7 ion implants had a lower contact angle (85.9 ± 8.5) than Ti64/SA implants (99.8 ± 6.8), suggesting that a significant improvement in the hydrophilicity of the Ti-6Al-4V alloy implants was achieved via PEO. The surface energies for Ti64/SA and Ti64/7 ions surfaces were 3.53 ± 0.9 and 4.57 ± 0.5 mJ/m², respectively. In this study, the values of contact angle were somewhat in accordance with those obtained in other works. For example, contact angles in the range between $\sim 66.8^\circ$ and $\sim 77.4^\circ$ and the range between $\sim 76.9^\circ$ and $\sim 82.1^\circ$ were reported by Pegueroles et al. [26] for the pure titanium implants sanded by either SiC or Al₂O₃ particles, respectively. Raphel and coworkers [27] measured the values of contact angles of Ti-6Al-4V substrates irradiated with UV light in the presence of phosphate-buffered saline. This UV exposure resulted in a significant increase in surface hydrophilicity, with the contact angle decreasing from 50.83° to 26.33° , consistent with an increase in surface hydroxyls. On the other hand, lower values of contact angle below 15° were reported by Li et al. [28] for pure titanium treated via PEO in tetraborate electrolytes. As for surface energy, Kaseem and Choe [23] reported recently higher surface energies (~ 7.44 and ~ 8.13 mJ/m²) than those obtained in the present work for Ti-6Al-4V alloy treated via PEO in solutions containing Zn and Mg ions. Higher surface energy values in the range between ~ 65 to ~ 70 mJ/m² were reported by Marques and co-workers [29] for pure titanium coating via PEO in electrolytes containing Ca, P, and Si ions. Here, it is believed that the differences in the morphologies and the composition of treated implants would explain the variation in the values of contact angle and surface energy obtained in the present work in comparison to those described in the literature [23,26–29].

Figure 5 shows the SEM images of MC3T3-E1 cells cultured on the surface of Ti64/SA and Ti64/7 ion implants. Cells show the spinning form of filopodia (Ti64/SA implants) in lamellipodia (Ti64/7 ion implants) and grow in association with the cells. As can be seen from Figure 5b-1, the circular-type lamellipodia are noted near the pores as displayed in Figure 5b. Moreover, cell growth is actively found through interconnection with cells when compared with the Ti64/SA implants, as shown in Figure 5a.

Table 1. EDS results of the Ti64/7 ion implant showing the incorporation of Ca, P, Mg, Zn, Si, Sr, and Mn into the coating.

Element	Weight. %	Atomic. %
O K	43.12	67.68
Mg K	0.29	0.30
Al K	1.92	1.79
Si K	0.41	0.35
P K	5.53	4.49
Ca K	6.61	4.14
Ti K	33.84	17.74
V K	1.56	0.77
Mn K	3.53	1.61
Zn L	2.03	0.78
Sr L	1.16	0.35
Total	100.00	100.00

Figure 5. SEM images of the osteoblast cells cultured on the surface of Ti-6Al-4V alloy implants: (a,a-1) Ti64/SA implants and (b,b-1) Ti64/7 ion implants.

3.2. Clinical, Histological, and Histomorphologic Findings

The visual observations were as follows. When the implants were detached and the implants were observed, the fluctuations of all implant fixtures were hardly observed. The formation of inflammatory and granulation tissues around the implant fixture was not observed, and an almost smooth bone surface was confirmed. Figure 6 shows the tissue photograph eight weeks after implantation of the Ti64/SA and Ti64/7 ion implants, respectively. Eight weeks after implantation, there were no signs of inflammation at the bone–implant interface where all implants in both groups were histologically in direct contact with the surrounding cortical bone (red color) and bone marrow (gray color part) along existed implant threads, as shown in Figure 7a,b, after removing the implant from bone. Interestingly, the amount of newly formed bone (blue color) around the surface of the experimental group (Ti64/7 ions) was higher than those in those around the experimental groups (Ti64/SA), as shown in Figure 7c,d. However, in both groups, the bone fixation of the implant fixture was similarly high. From the results of histomorphometric analysis listed in Table 2, the ratio of new bone created nearby the implant fixture was more than doubled in the experimental group (Ti64/7 ions) in comparison to the control

group (Ti64/SA implants) ($p = 0.029$). As reported earlier [30], the bone formation would have originated from the implant surface properties, namely, chemical and physical design, which is referred to as “contact osteogenesis”, as well as from the bony margin of the surgical defect, i.e., “distance osteogenesis”. Therefore, we thought that the porous structure containing bioactive elements would be responsible for the higher amounts of bone formed in the case of Ti64/7 ion implants. However, there was no considerable difference between implant types in the values of BIC% (~ 69.3 for Ti64/SA implants vs. ~ 69.0 for Ti64/7 ion implants). This result was consistent with those obtained by He et al. [31] and Zhang et al. [32], who found that the incorporation of bioactive elements, such as Zn or Mg or Sr, into titanium implants would accelerate the surrounding bone formation and improve the amount of new bone formation in the rabbit model of osteoporosis. As such, Fernández-Yagüe et al. [33] found that the bioactive implants produced by plasma or thermochemical processes would exhibit more new bone formation when compared to the non-bioactive implants treated by shot blasting and acid etching. Furthermore, Aparicio et al. [34] demonstrated that the created microroughness by Al_2O_3 grit blasting accelerated in vitro apatite nucleation on alkali- and heat-treated titanium plates.

Figure 6. (a) Ti64/SA implants after 8 weeks of implantation, and (b) Ti64/7 ion implants after 8 weeks of implantation. As shown in (a,b), the implant fixture is well connected with the surrounding bone.

3.3. Removal Torque Analysis

The removal torque values measured in each animal group on Ti64/SA and Ti64/7 ion surface-treated implants are summarized in Table 3. As reported earlier [3], the interfacial shear stress can be reflected by the removal torque value strength. The degree of biomechanical anchorage determined by removal torque testing indicates the strength of implant integration in bone tissue [35]. As shown in Table 3, the average removal torque following eight weeks of implant placement was 49.7 Ncm for the control implants and 51.5 Ncm for the experimental implants. The values of removal torque reported by the present work were higher than those obtained in other studies. For instance, Cho and Park [36] measured the removal torque of dual acid-etched titanium screw implants inserted in rabbit tibia and reported that the average removal torque value was 38.7 Ncm. Cordioli et al. [37] reported that the average removal torque value was 25.28 Ncm for the machined implants, 26.85 Ncm for the grit-blasted implants, 29.57 Ncm for the plasma-sprayed implants, and 40.85 Ncm for the acid-etched implants under similar conditions. Thus, the greater torque rotation forces required to remove the implants in the present study would suggest higher strengths of osseointegration.

Figure 7. Histological images of tissue 8 weeks after implantation showing no signs of inflammation at the bone–implant interface where (a) Ti64/SA implants and (b) Ti64/7 ion implants are used. These images were observed after removing the implant from rabbits. By observing the tissue images of Ti64/SA and Ti64/7 ion implants, it can be seen that the dense bone in (b) is well-formed. The black part is judged as marrow, and the red represents bone. (c,d) represent the amount of newly formed bone around the surfaces of Ti64/SA and Ti64/7 ion implants, respectively. From high-magnification images shown as insets, the Ti64/7 ion implants were found to exhibit greater amounts of newly formed bone from original cortical bone compared with the Ti64/SA implants.

Table 2. Average bone-to-implant contact area (BIC) ratio and new bone formation rate measured in Ti64/SA and Ti64/7 ion implants.

Implants	Bone-to-Implant Contact Ratio (%)	New Bone Formation Ratio (%)
Ti64/SA	69.3 ± 6.9	28.4 ± 3.2
Ti64/7 ions	69.0 ± 7.1	58.4 ± 4.7

Table 3. Removal torque value measured in each animal group on Ti64/SA and Ti64/7 ion implants.

Rabbits	1	2	3	4	5	6
Ti64/SA	49.7 ± 14.5	50.4 ± 17.5	51.5 ± 16.7	46.3 ± 18.5	50.6 ± 14.2	49.7 ± 15.7
Ti64/7 ions	50.0 ± 18.5	52.5 ± 18.3	51.3 ± 15.4	49.5 ± 19.6	52.3 ± 19.5	53.4 ± 13.9

4. Discussion

The implant material is usually characterized as bio-tolerant (gold, cobalt-chromium, stainless steel, poly(methyl methacrylate), bio-inert (Ti), and bio-active (Ti-active surface) material. For bio-tolerant material, the bone formation is only on the bone surface and we observe only distant osteogenesis, while in other cases, it is found close to implant surface for bio-inert materials. The phenomenon of both distant osteogenesis and contact osteogenesis will be observed for bio-inert and bio-active material, but the formation rate is considered higher in the case of bioactive material. Bioactive surfaces are active because

of the presence of bio-bonding materials, such as calcium crystals, phosphate crystals, and hydroxyl appetite crystals. These crystals will act as nucleation sites to the newly formed bone cell and it will be bonded with the calcium ions of the implant interface. For this reason, we see a higher response of bone cell formation on the surface of the experimental group (Ti64/7 ions), as can be seen in Figure 7. The results of the present work revealed that Ti64/7 ion implants exhibited enhanced osteoblast differentiation as compared to Ti64/SA implants. In Figure 8, we simply explained the assumed mechanism for bone development in both cases of C.G (Ti64/SA), and E.G (Ti64/7 ions). During the initial few minutes after the implant is successfully checked for its primary stability, the blood irrigates the area around the implant. The white blood cells within the blood provide necessary resources for healing. Ions, proteins, and platelets start to adhere to the implant surface. Shortly, the bleeding stops due to the action of the platelets which fills the wounds by clotting and creating blood vessels and a fibrin-based provisional matrix that adheres to the implant. Days after surgery, fibroblasts migrate into the wound, and components such as collagen are synthesized which stabilize and protect the extracellular matrix of perivascular cells, which have characteristics of stem cells. These cells also migrate to wounded areas near implant surfaces forming new blood vessels that will restore oxygen supply and thereby promote tissue healing. After the first week, osteoclasts and osteoblasts adhere to the residual bone, resorbing it and creating space for the formation of new bone. Perivascular cells of progenitive nature also migrate to the implant surface where they will form new osteoblasts; these osteoblasts will form a mineralized matrix by incorporating calcium and phosphate. This collagen-free calcified matrix with proteoglycans makes the woven bone. However, this woven bone has very limited mechanical stability, but it permeates the growth of lamellar bone and remodeling and gives secondary stability due to good osseointegration [38].

Surface roughness, hydrophilicity, and surface activity of implants increase the bone healing response. Its higher reverse torque value and histomorphometric analysis suggest a quicker healing rate. On the basis of this, we propose that the bone formation shows contact osteogenesis and distant osteogenesis at a faster pace, as shown in Figure 8a, compared to its counterpart shown in Figure 8b. The faster bone response was attributed to the active ionic crystals which provided nucleation sites to the newly formed bone cells, and these bone cells find it easier to bond with the calcium and phosphorous ions of the implant interface. One of the main advantages of activating the interfacial surface with activating ions over the simple sand-blasting sample is the obtention of a better bone response in bodies that show a difficult healing response. This is because the bone formation in different patients of diabetes, cancers, and patients of radiotherapy is very slow; for a simple Ti-implant to complete osseointegration, 8–16 months are required; and in some cases, it is considerably challenging. Thus, the surface modification and incorporating these active seven ions will provide the active surface where the distant osteogenesis and contact osteogenesis rate will speed up, and, thus, we experience higher bone formation.

As mentioned above, the surface roughness, hydrophilicity, and surface activity of implants are the three important factors for their binding ability with fibrin-based matrix leading to good osseointegration and bone formation. Increased surface roughness would enhance the osseointegration of titanium implants [39–41], where Buser et al. [42] reported a direct relationship between surface roughness and bone formation.

In addition to surface roughness, Wennerberg et al. [43], reported that the osseointegration activity would be affected by other factors related to surface roughness, such as the pattern, size, and distribution of peaks and valleys. As the roughness values of both the studied implants are almost identical with the value of $R_a \sim 1.43 \mu\text{m}$ of the Ti64/7 ion implant and the value of $R_a \sim 1.61 \mu\text{m}$ of the Ti64/SA implant, we conclude that the faster bone formation is not mainly dependent on surface roughness values in our study. The other factor of improved osteoblast attachment on the surface of Ti64/7 ion implants would be related to its better hydrophilic property. Previous reports also suggest that hydrophilic surfaces promote stronger and faster osteoblasts attachment on their surfaces as compared

to the hydrophobic surfaces [44–46]. The calculation of surface energy also indicated that the highest surface hydrophilicity observed in the case of Ti64/7 ion implants would yield accelerated healing and an early osseointegration process [47,48].

Figure 8. The proposed mechanism for the new bone formation in (a) Ti64/7 ion implants and (b) Ti64/SA implants.

Hence, we can argue that the increased surface hydrophilicity and the surface chemistry of the Ti64/7 ion implant due to the incorporation of Ca, P, Sr, Mg, Zn, Si, and Mn elements would be the main factors affecting the performance of these implants during both in vitro and vivo tests. The effect of each ion separately is also important to briefly discuss, as we discussed previously the role of Ca and P ions in mineralization of the extracellular matrix. For example, Si ions are crucial for the promotion of osteoblasts maturation, while osteoclast proliferation can be prevented in the presence of Sr ions. Similarly, Mg and Mn ions would also improve the cell adhesion at the interface, thus enhancing the interaction with host bone tissues as both Mg and Mn ions can substitute Ca sites where it is required during the healing process. The small amounts of Zn and Si elements can lead to improvements in the corrosion performance of the oxide layers.

5. Conclusions

In the present study, the influence of sand blasting and PEO treatments on the surface properties of Ti-6Al-4V alloy was examined in vitro and in vivo. Ti64/SA and Ti64/7 ion implants were successfully inserted into the tibia of rabbits. The results showed that both Ti64/SA and Ti64/7 ions had high bone adhesion around implants. However, it was confirmed that the Ti64/7 ion implants formed more periphery bone than those treated via sand blasting, which was explained by the differences in surface chemistry owing to the incorporation of Ca, P, Mg, Zn, Si, Sr, and Mn elements. Therefore, the PEO treatment of Ti-6Al-4V alloy via PEO in a solution containing ions of seven elements would be an effective approach for improving the performance of dental implants since it combines the benefits of both a porous structure and bioactive elements incorporated into the oxide layer grown on the implant surface.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available as the data also forms part of an ongoing study.

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