Sol-Gel Fabricated TiO_2 Coating on Titanium Surface Promoted *In Vitro* Osteoblasts Differentiation

Keywords

Sol-Gel
Primary Human Osteoblasts
Titanium Dioxide
Osteogenic Differentiation

Authors

Assist. Prof. Dr. Thidarat Angwarawong*
(D.D.S., M.Sc., Ph.D)

Dr. Kavita Kanjanamekanant§
(D.D.S., Ph.D.)

Prof. Dr. Mansuang Arksornnukit§
(D.D.S., M.S., Ph.D.)

Prof. Prasit Pavasant^ †
(D.D.S., Ph.D.)

Address for Correspondence

Assist. Prof. Thidarat Angwarawong

Email: thidarat_ang@hotmail.com

* *Department of Prosthodontics, Faculty of Dentistry, Khon Kaen University, Khon Kaen, 40002*

§ *Department of Prosthodontics, Faculty of Dentistry, Chulalongkorn University, Pathumwan, Bangkok, 10330, Thailand*

^ *Department of Anatomy, Faculty of Dentistry, Chulalongkorn University, Pathumwan, Bangkok, 10330, Thailand*

† *Research unit of Mineralized Tissue, Faculty of Dentistry, Chulalongkorn University, Pathumwan, Bangkok, 10330, Thailand*

Received: 25.02.2019
Accepted: 31.07.2019

doi: 10.1922/EJPRD_01919Angwarawong09

ABSTRACT

Titanium has been used for biomedical devices due to its excellent biocompatibility, which is based partly on its 2-8 nm thick titanium oxide layer. However, the relatively poor surface hardness, wear resistance and metal release of these layers may cause some problems in clinical application. In this study, titanium surfaces were modified using a TiO_2 sol-gel coating, in order to improve surface properties and osteoblast function. No significant difference in surface roughness was observed between titanium and TiO_2 sol-gel discs. The surface of TiO_2 sol-gel discs possessed more wettability than titanium discs. The X-ray diffraction results showed amorphous TiO_2 phase on titanium discs, whereas TiO_2 sol-gel surfaces presented TiO_2 rutile and anatase phase. After 4 hours, the number of osteoblasts seeded on TiO_2 surface was significantly higher than those on titanium discs. The mRNA expression of bone sialoprotein and osteocalcin were also higher on day 5 and 7, respectively. Enzyme-linked immunosorbent assay(ELISA) analysis confirmed the increase of osteocalcin protein synthesis in osteoblasts grown on the TiO_2 sol-gel surface. Alizarin red-S staining showed higher amount of calcium deposition from osteoblasts cultured on TiO_2 surface than those on titanium discs at day 20. In conclusion, TiO_2 sol-gel coated-titanium could enhance osteoblasts differentiation and promote mineralization, indicating its potential in improving osseointegration for clinical application.

INTRODUCTION

Titanium (Ti) and its alloys are commonly used as biomedical devices in dental and orthopedics fields due to their remarkable characteristics such as excellent biocompatibility and corrosion resistance.[1] The major applications are bone-anchoring devices including hip-joint replacement devices and dental implants.[1,2] When Ti surface is exposed to air and/or physiological fluids such as body fluid and oxidizing media, the oxide layer is spontaneously formed within milliseconds through the so-called passivation process.[3,4] It has been reported that the excellent biocompatibility of Ti and its alloy is based on the native oxide layer, which is the first part that interact with biological tissue and therefore establishing the initial host responses.[2,5] The oxide layer also provides corrosion resistance as it acts as a barrier preventing the release of ions into surrounding tissue.[6] However, the corrosive process still occurs due to the breakdown of oxide layer. The released metal ions and debris consequently stimulate biological adverse reaction and also implant failure.[7] The passivating oxide layer of Ti is very thin, approximately 2-8 nm in thickness, usually amorphous and mainly consists of titanium dioxide (TiO_2).[2-4] With poor surface hardness and

ejprd.org - Published by Dennis Barber Journals. Copyright ©2019 by Dennis Barber Ltd. All rights reserved.

wear resistance, this naturally occurred oxide layer can be easily damaged.[2] It is proposed that the increase in the oxide layer thickness (about 100-200 nm) could increase corrosion resistance of Ti.[8,9] Therefore, the modification of Ti or its alloys to form a thicker TiO_2 layer might benefit long-term success of implant treatment.[2]

Several modification techniques have been developed to increase the thickness and stabilize TiO_2 layer.[10-13] Sol-gel process is one of the widely used techniques to obtain well-defined biomaterial surfaces while maintaining the overall favorable mechanical properties of material substrate. It is an inexpensive simple method that can be used on complex shaped substrate such as dental implant. Moreover, rapid deposition with high degree of purity and homogeneity of oxide layer can be achieved.[8,12,14]

Several studies indicated that sol-gel fabricated TiO_2 coating on material surfaces could improve osteoblast responses.[2,5,8,15-17] Sol-gel derived TiO_2 could improve cell attachment, proliferation, differentiation and *in vitro* mineralization of mouse osteoblast cell line (MC3T3-E1),[2,5,16] human osteosarcoma (HOS) cell lines,[8] and primary human osteoblast cells,[15] compared to bare Ti surface. In our previous work, we found that a single layer of 90 nm TiO_2 sol-gel film coated on glass slip could enhance osteoblast attachment, differentiation and *in vitro* calcification, when compared to uncoated surface.[16] The aim of this study was to fabricate and characterize sol-gel derived TiO_2 film coated on Ti discs. The function and behavior of primary human osteoblast cells grown on TiO_2 surface were also evaluated.

MATERIAL AND METHODS

TITANIUM DISC PREPARATION

Commercial pure Ti (cpTi grade 2, Morita Company, Japan) was prepared into Ti disc with a diameter of 12 mm and a thickness of 2 mm. Ti discs were polished with 1000-grit silicon carbide grinding paper in a polishing machine (DPS 3200, IMPTECH, South Africa). Before dipping process, specimens were ultrasonically washed for 5 minutes in acetone, 5 minutes in ethanol, three times rinsing with de-ionized water, and air dried at room temperature.

FABRICATION OF TIO_2 FILMS

The TiO_2 film was produced by simple sol-gel dip-coating procedure.[16] Briefly, the sols were prepared by dissolving tetrabutyl orthotitanate ($C_{16}H_{36}O_4Ti$) in absolute ethanol and mixed with fuming 37% hydrochloric acid (HCl) and acetylacetone ($C_5H_8O_2$) with a 1:30:0.5:1 molar ratio. After 1 hour of stirring at room temperature, Ti discs were mounted on a dipping machine and dipped into the sols at a speed of 10 cm/minute under ambient atmosphere. Only one cycle of sol-gel process was performed. After air drying for 24 hours, specimens were heat treated at a temperature of 550°C for 30 minutes in air at heating rate of 2°C/minute. All reagents were purchased from Fluka (Steinheim, Germany).

SURFACE ROUGHNESS MEASUREMENT

Surface roughness was measured using a profilometer (Talyscan 150, Taylor Hobson, UK). Surface value roughness (Sa) measurements were randomly taken at 5 different locations on each sample with a measurement speed at 2.0 mm/second.

CRYSTAL STRUCTURE ANALYSIS

The crystal structure of Ti disc and TiO_2 coated films were identified by X-ray diffraction (XRD; D8 Discover, Bruker AXS, Karlsruhe, Germany) at a scanning rate of 0.4 second/step, with an angle of incidence q = θ.2 in the range of 20º to 80º, CuKα radiation and wavelength (λ) of 1.54 ºA (40kV,40mA).

HYDROPHILICITY

Static contact angle measurement was performed using contact angle meter (DSA10, Krüss, Hamburg, Germany). Samples were kept in ambient environment for 15 minutes, a 10 µl sessile droplet of de-ionized water was drop vertically on specimen surface using micro-syringe without physical contact by hand. The contact angle was measured ten times for each surface.

PRIMARY HUMAN OSTEOBLAST CELL CULTURE

Primary human osteoblast cells were obtained from alveoloplasty or surgical removal of torus palatinus or torus mandibularis for prosthodontic treatment. All patients gave informed consent and the protocol was approved by the Ethical Committee, Faculty of Dentistry, Chulalongkorn University. Bone chips were washed in phosphate buffer saline (PBS, Gibco, Grand Island, NY, USA), cut into approximately 2×2 mm^2 pieces, and digested with 0.25% trypsin-ethylenediaminetetraacetic acid (Trypsin-EDTA; Gibco) solution. Bone pieces were then harvested and cultured in Dulbecco's modified Eagle's medium (Gibco), supplemented with 15% fetal bovine serum (FBS; ICP biologicals, Henderson, Auckland, New Zealand), 1% antibiotic-antimycotic (Gibco) under standard incubation conditions (5% CO_2, 100% humidified, 37°C). After cells were confluence, subculture was performed at a ratio of 1:3, and cells from passages 3 to 6 were used in this study. For all experiments, cells were seeded on each material, which was placed in each well of 24-well tissue culture plates at a density of $4×10^4$ cells per well. Prior to cell culture, Ti discs and glass cover slips were sterilized by washing with 70% ethanol for 10 minutes, followed by three times rinsing with de-ionized water and air dried, whereas TiO_2 coatings were sterilized using autoclave. The medium was changed every other day. Glass surface (12 mm round glass cover slip) was used as control.

Copyright ©2019 by Dennis Barber Ltd. All rights reserved.

MTT ASSAY

Cell viability was determined by MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-dipheyl tetrazolium bromide); USB Corporation, Cleveland, OH, USA) assay. After 0.5, 1 and 4 hours as well as 1 and 3 days of culture, the specimens were washed with PBS, followed by 30 minutes incubation in 5 mg/ml MTT solution. At the end of assay, the optical density of dissolved blue formazan crystal from each well was measured using Thermo Spectronic Genesys 10 UV-vis spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) at a wavelength of 570 nm.

CELL MORPHOLOGY EVALUATION BY SCANNING ELECTRON MICROSCOPY (SEM)

Cells were cultured on each surface for 0.5 and 1 hour. After removal of the culture medium, cells were fixed with 3% glutaraldehyde in PBS for 30 minutes and rinsed with PBS. After dehydration in a graded series of dimethyl sulfoxide (30%, 50%, 70%, 90% and 100%) for 2 minutes at each concentration and dried in air, samples were sputter-coated with gold and were examined by SEM (JSM-5410LV, JEOL, Tokyo, Japan).

REVERSE-TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

Expressions of osteocalcin (OC) and bone sialoprotein (BSP) messenger RNA (mRNA) were assessed using RT-PCR. After 5 and 7 days of cell culture, primary bone cells were extracted with TriPure Isolation Reagent (Roche Diagnostics, Indianapolis, IN, USA). The RNA samples were then reverse transcribed to obtain first strand cDNA using reverse transcriptase enzyme (ImProm-II Reserve Transcription System, Promega, Madison, WI, USA). The target genes were amplified by Tag Polymerase enzyme kit (Invitrogen, Sao Paulo, Brazil). PCR products were analyzed by separation on 1.8% agarose gel (USB Corporation) using electrophoresis (Power Pac Junior, Bio-Rad, Hercules, CA, USA) and visualized by ethidium bromide (Bio-Rad) staining. The stained bands were photographed under UV light, and the intensity was quantified with Scion Image Software (Scion Corporation, Walkersville, MD, USA). In this study, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ribosomal RNA was used as a reference gene.

OSTEOCALCIN ANALYSIS USING ELISA

To examine the OC protein synthesis, primary human osteoblasts were seeded on each sample and incubated for 5 and 7 days. The culture medium was changed 1 day prior to sample collection. The supernatants of cell seeded on each sample were collected and the osteocalcin protein expression were examined using intact human osteocalcin enzyme-linked immunosorbent assay (ELISA) kit (Biomedical Technologies, MA, USA).

ALIZARIN RED-S STAINING AND CALCIUM QUANTIFICATION

Calcium deposition was quantified by alizarin red-S staining. Primary bone cells were cultured on samples until confluence. The media were changed into osteogenic medium containing 10 mM β-glycerophosphate (Sigma), 50 µg/ml ascorbic acid (Sigma), and 100 nM dexamethasone (Sigma), and cells were cultured for another 20 days. At the end of culture period, cells were rinsed with PBS and fixed with cold methanol for 10 minutes, washed with deionized water and immersed in 1% alizarin red-S solution dissolved in 1:100 (v/v) ammonium hydroxide/water mixture (pH = 4.2).

The amount of calcium deposition was quantified by eluting with 10% cetylpyridinium chloride monohydrate (Sigma) in 10 mM sodium phosphate at room temperature for 15 minutes. The absorbance of the released alizarin red-S was measured at 570 nm using the UV-vis spectrophotometer.

STATISTICAL ANALYSIS

Data were analyzed by one-way analyses of variance (ANOVA), followed by a multiple comparison using the LSD or Tamhane pot hoc tests ($p < 0.05$), using statistical software SPSS® 15.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS

SURFACE CHARACTERIZATION

The surface characteristics of TiO_2 sol-gel coated surfaces were analyzed, compared to Ti discs and glass cover slips. The surface roughness was examined using profilometer. Results showed that the roughness of Ti disc and TiO_2 coated discs were comparable, with significant difference from glass surface *(Table 1)*. The hydrophilicity of each surface was examined through the measurement of water contact angle. TiO_2 coated Ti disc appeared to be the most hydrophilic surface, as the least water contact angle was observed *(Figure 1)*. To analyze the crystal structure of Ti discs and sol-gel derived TiO_2 film, XRD analysis was performed *(Figure 2)*. The XRD profiling of Ti discs showed the sole visible peak (s) of substrate cp-Ti, indicating this TiO_2 was amorphous *(Figure 2A)*. On the other hand, TiO_2 coated Ti surfaces demonstrated rutile (r) / anatase (a) two-phase structure *(Figure 2B)*.

Table 1. Surface roughness of glass, Ti discs and TiO_2 coated discs

Materials	Sa (nm)
Glass Surface	24.55 ± 0.89
Ti discs	221.12 ± 22.74*
TiO_2 coated discs	199.62 ± 10.27*

Data were presented as mean ± S.D. *Significant difference, $p<0.05$.

ejprd.org - Published by Dennis Barber Journals.
Copyright ©2019 by Dennis Barber Ltd. All rights reserved.

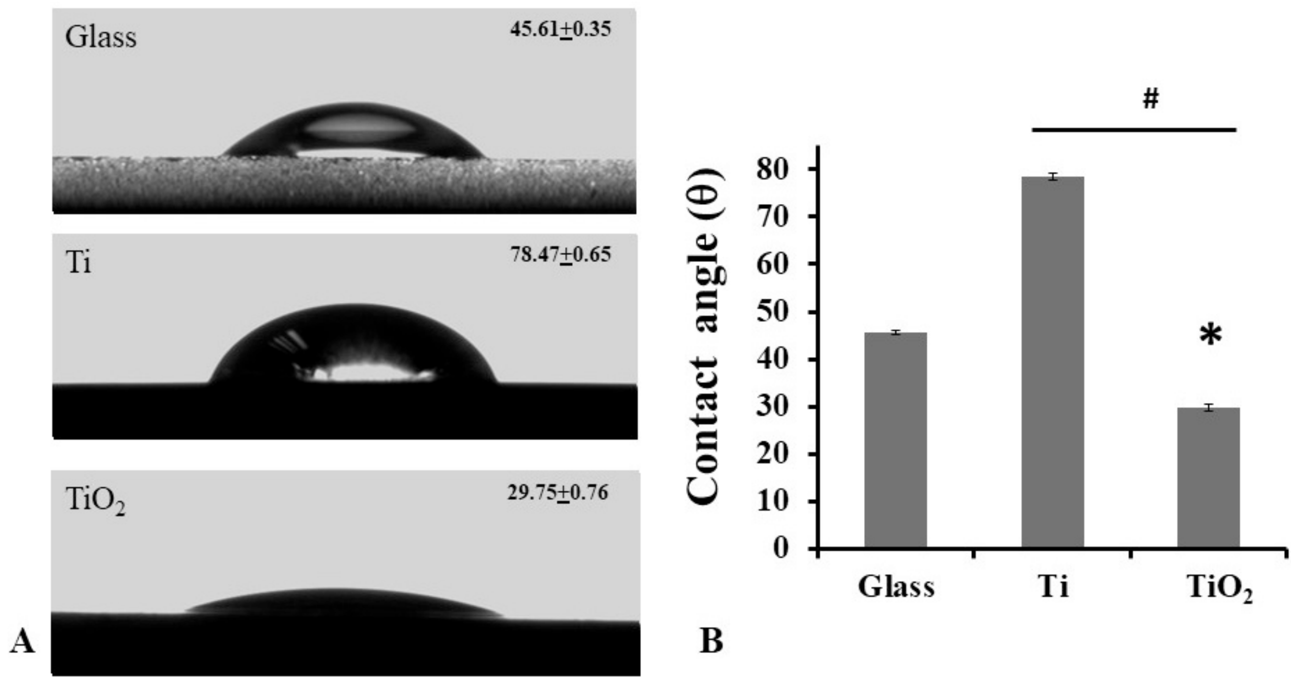

Figure 1: Water contact angle of glass, TiO_2 film, and Ti disc
(A) Phothograph of water droplet. (B) Water contact angle. Data are shown as mean ± S.D. * indicates statistical difference between glass cover slip and Ti disc or TiO_2 coated-disc; # indicates statistical difference between Ti disc and TiO_2 coated-disc, $p<0.05$.

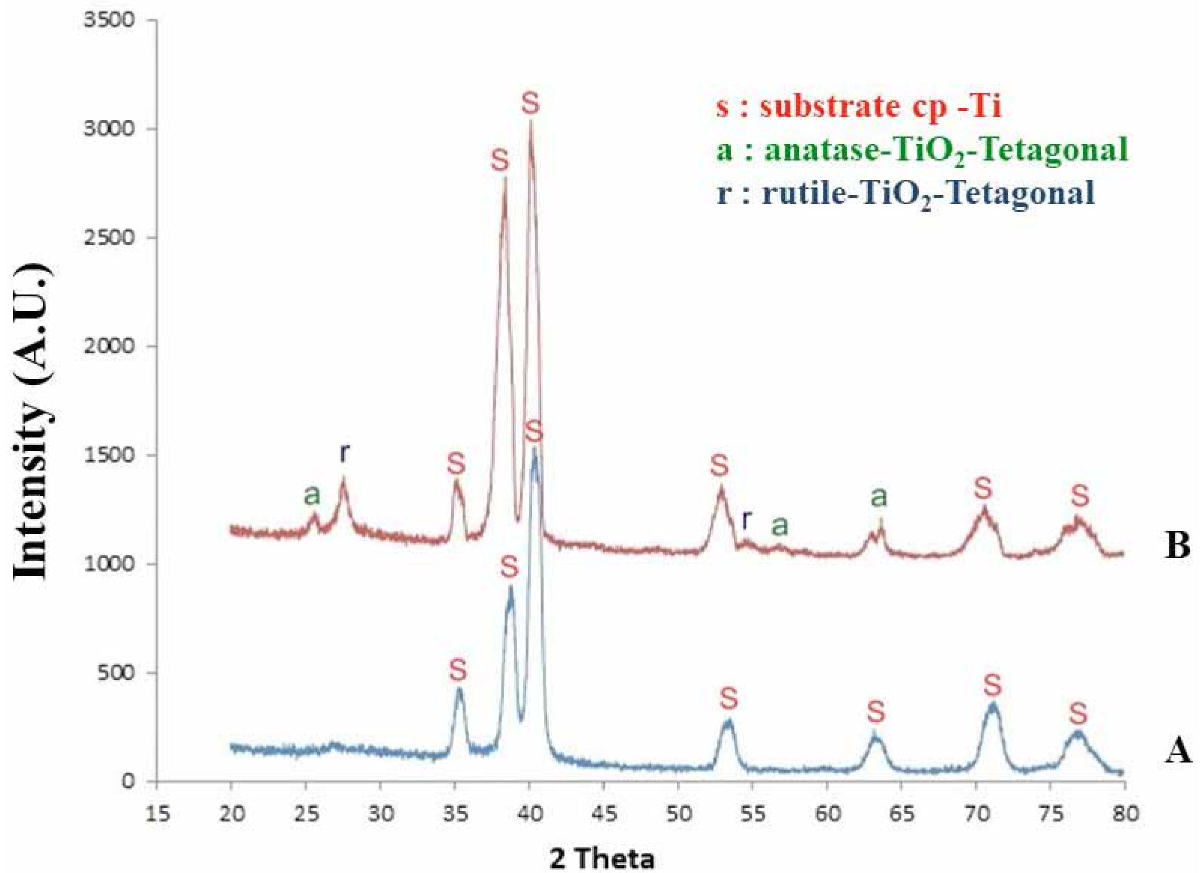

Figure 2: The crystal structure analysis

ejprd.org - Published by Dennis Barber Journals.
Copyright ©2019 by Dennis Barber Ltd. All rights reserved.

OSTEOGENIC DIFFERENTIATION AND MINERALIZATION

Primary human osteoblast-like cells were cultured on each surface. Cell morphology was examined by SEM. The result revealed normal morphology of cells on all surfaces after cell were seeded on materials for 0.5 and 1 hour *(Figure 3)*. The number of viable cells was further quantitated by MTT analysis *(Figure 4)*. A comparable number of viable cells adhered on all surfaces was observed at both 0.5 and 1 hour culture. However, when the culture was extended to 4 hours, 1 and 3 days, the highest number of viable cells was observed on glass surface, followed by TiO_2 film and Ti discs.

The expression of osteogenic markers OC and BSP was examined. RT-PCR analysis showed significant increase in OC and BSP mRNA expression of cells cultured on TiO_2 coated discs, compared to Ti discs and glass surface at day 5 and day 7 *(Figure 5)*. ELISA results confirmed a significant increase in OC protein synthesis of cells seeded on TiO_2 coated discs *(Figure 6)*.

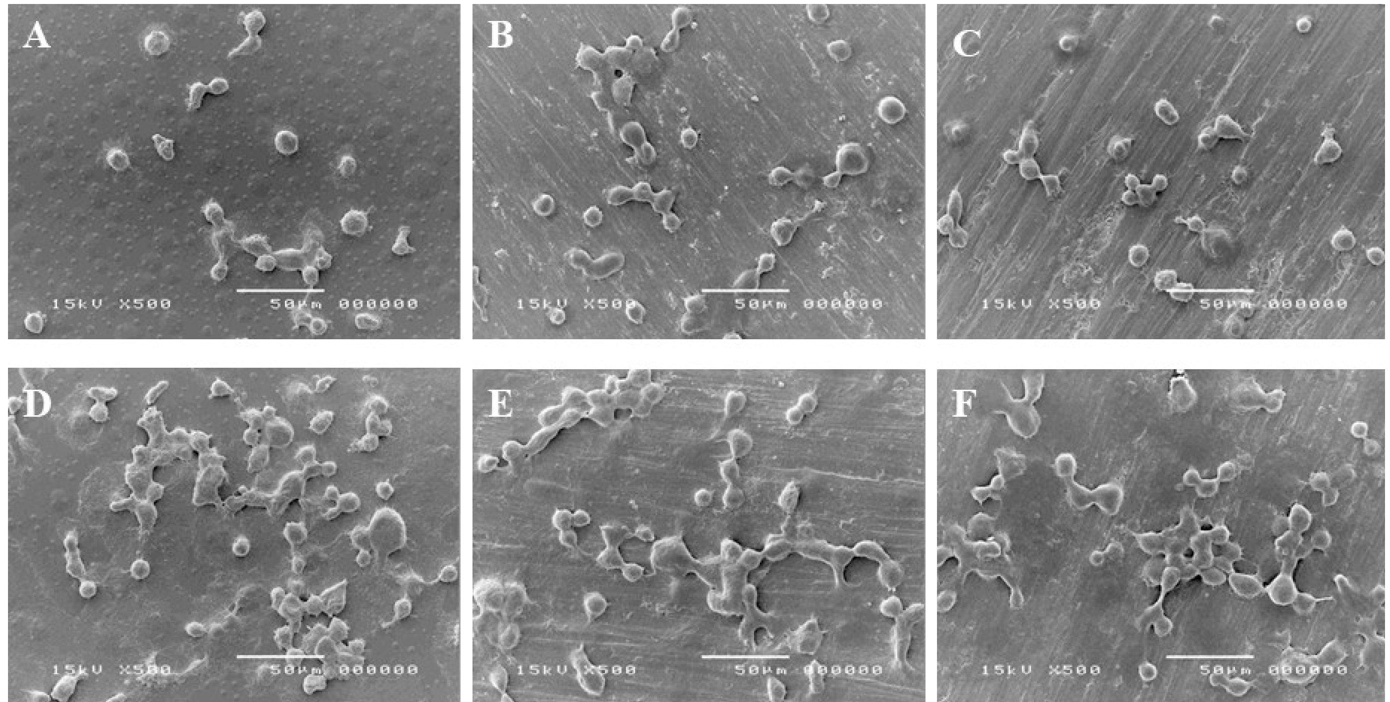

Figure 3: Cell morphology
Scanning electron micrograph of cells seeded on glass (A, D), Ti discs (B, E) and TiO_2 coated films (C, F) after 30 minutes (A-C) and 1 hour (D-F) incubation at magnification of 500x.

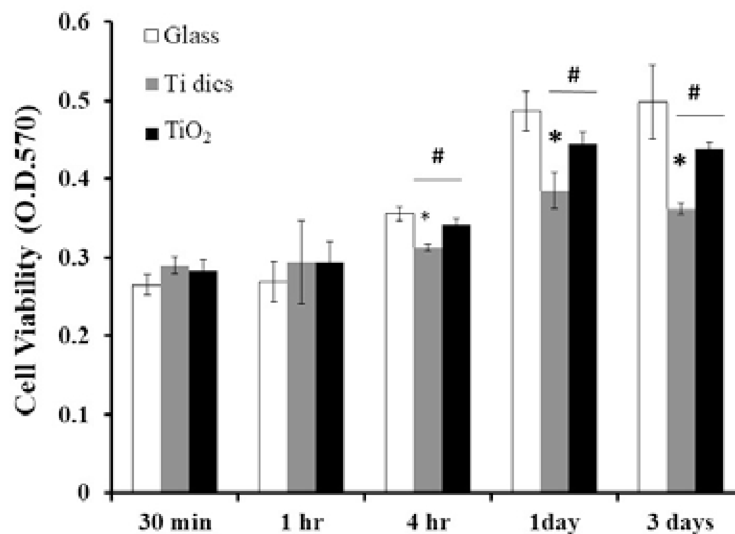

Figure 4: The number of viable cells on materials surface.
MTT assay showed the percentage of viable cells cultured on glass, Ti discs and TiO_2 coated discs after 0.5, 1 and 4 hours as well as 1 and 3 days incubation. Data are shown as mean ± S.D. * indicates statistical difference between glass cover slip and Ti disc or TiO_2 coated-disc; # indicates statistical difference between Ti disc and TiO_2 coated-disc, $p<0.05$.

ejprd.org - Published by Dennis Barber Journals. Copyright ©2019 by Dennis Barber Ltd. All rights reserved.

Figure 5: Osteogenic genes expression

The mRNA expression of bone sialoprotein (BSP) and osteocalcin (OC) of cell cultured on glass, Ti disc and TiO_2 coated disc after 5 days (A) and 7 days (B) incubation. Graphs showed relative band intensity normalized to GADPH. Data are shown as mean ± S.D. * indicates statistical difference between glass cover slip and Ti disc or TiO_2 coated-disc; # indicates statistical difference between Ti disc and TiO_2 coated-disc, p<0.05.

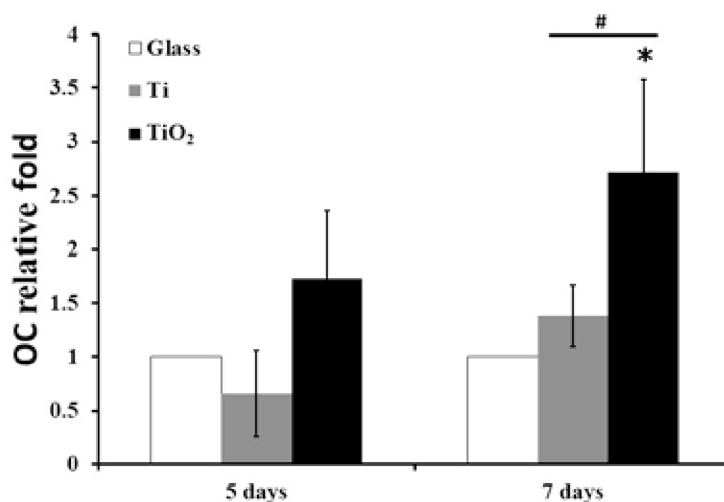

Figure 6: Osteocalcin protein expression.

Osteocalcin (OC) protein synthesis of cells cultured on glass, Ti disc and TiO_2 coated films at day 5 and 7 were determines using ELISA. Data are shown as mean ± S.D. *indicates statistical difference between glass cover slip and Ti disc or TiO_2coated-disc; # indicates statistical difference between Ti disc and TiO_2 coated-disc, p<0.05.

ejprd.org - Published by Dennis Barber Journals. Copyright ©2019 by Dennis Barber Ltd. All rights reserved.

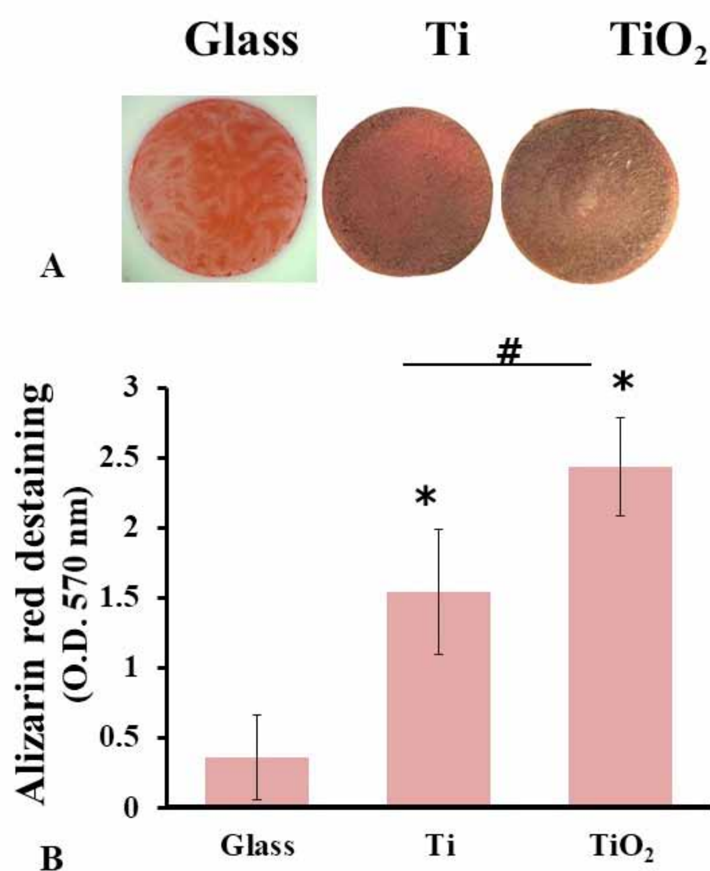

Figure 7: *In vitro* calcification analysis.
Alizarin red-S staining of cells cultured on glass, Ti and TiO_2 coated disc at day 20 (A). Graph showed quantification of calcium deposition by elution with cetylpyridinium chloride (B). Data are shown as mean ± S.D. * indicates statistical difference between glass cover slip and Ti disc or TiO_2 coated-disc; # indicates statistical difference between Ti disc and TiO_2 coated-disc, $p<0.05$.

An *in vitro* calcification was examined using alizarin red-S staining, which demonstrated more reddish deposition in cells cultured on TiO_2 coating films than those on glass and uncoated Ti discs at day 20 *(Figure 7A)*. The amount of calcium deposition on each surface was quantified colorimetrically by destaining in 10% cetylpyridinium chloride monohydrate. The highest calcium deposition level was also observed on TiO_2 coated surface *(Figure 7B)*.

DISCUSSION

In the present study, surface modification of Ti surface was created by a simple sol-gel technique to produce thicker TiO_2 films. Results indicated that TiO_2 derived sol-gel coated Ti surfaces could enhance osteoblast differentiation, as an increase in osteoblastic gene expression, as well as *in vitro* calcification were demonstrated.

Sol-gel process has been developed to fabricate TiO_2 film. Differences in sol reagents, aging time, coating technique and cycle of dipping are presented.[2,8,15] For example, the aging time used for preparing TiO_2 sol is ranging from 24 hours to 5 days. The coating techniques to immobilize TiO_2 on Ti surface include spinning and dipping. The simple spinning technique is only applicable on flat surface. On the other hand, dipping technique could be used on more complex surface, but multiple dipping cycles are usually required to achieve favorable result. In our study, we used one cycle dipping technique to fabricate a uniform and homogeneous single layer of TiO_2 film on both Ti and glass surface. Further, our sol-gel derived TiO_2 layer could support osteoblast differentiation and *in vitro* calcium deposition, similar to the results from other complicated sol-gel techniques.

Following biomaterial implantation, host responses are spontaneously occurred. Proteins from blood and surrounding tissue are immediately adsorbed onto the implant surface.[18,19] A conformational change occurs within the adsorbed protein, allowing subsequent biological interactions with biomaterial. Surface properties of implant biomaterials such as surface roughness, wettability and surface chemistry, not only affect the amount and type of protein bound, but also influence cell-biomaterial interactions including cell attachment, adhesion, proliferation, and differentiation around material surface.[19,20]

ejprd.org - Published by Dennis Barber Journals. Copyright ©2019 by Dennis Barber Ltd. All rights reserved.

The wettability of implant surface could influence the binding of proteins onto biomaterials. An increase in hydrophilicity of Ti and TiO_2 surfaces could increase the adsorption of fibronectin, a major protein that play crucial roles in osteoblast differentiation, *in vitro* nodule formation and osseointegration process.[11,21] In addition, hydrophilic surfaces could support adhesion of various cell types whereas hydrophobic surfaces often inhibit cell–surface interaction.[18,22] Webb *et al.* (1998) showed that moderate hydrophilic surface, with approximately 20-40 degree of water contact angle, could promote the highest levels of cell attachment.[22] Corresponded to these results, we found that a single layer of sol-gel derived TiO_2 coated Ti surface could enhance osteoblastic gene and protein expression, as well as *in vitro* calcification when compared to uncoated Ti discs. The surface roughness of material also affect protein adsorption and cellular response at the bone-implant interface.[23,24] However, no significant difference in surface roughness between the TiO_2 film coated and uncoated Ti surface was observed in our study.

Many studies reported that TiO_2 coating films could improve osteoblast function and differentiation.[2,13,16,17,25-28] Nevertheless, the crystal phase of TiO_2 that could better support cellular responses is still a big controversy. For example, Jokinen *et al.* (1998) work illustrated high bioactivity of amorphous TiO_2 film derived from dipping sol-gel technique.[25] Tsukimura *et al.* (2008) also reported that osteoblast functions such as proliferation, differentiation and calcification were increased in cell seeded on amorphous TiO_2 film than on Ti substrates.[26] Nonetheless, Kern *et al.* (2005) concluded that differences in oxide crystallinity did not affect protein adsorption and initial osteoblast attachment.[27] On the contrary, He *et al.* (2008) found that anatase TiO_2 showed the best performance on osteoblast adhesion, spreading, proliferation and alkaline phosphatase (ALP) activity when compared to amorphous and rutile phase.[28] Lu *et al.* (2018) noted that anatase TiO_2 surfaces could promote proliferation and ALP activity of MG-63 human osteoblast-like cells than amorphous TiO_2 surfaces.[11] Ochsenbein *et al.* (2008) study reported higher proliferation rate of MC3T3-E1 cultured on TiO_2 coating with a rutile/anatase two-phase structure, compared to uncoated cpTi surface.[2] Our results also demonstrated that a rutile/anatase two-phase structure of TiO_2 fabricated by sol-gel method could support proliferation and differentiation of primary human osteoblasts. The upregulation of BSP and OC mRNA and protein was also illustrated. BSP and OC are the late-stage markers of osteogenic differentiation and are correlate with the outcome of calcification.[29] The result was in accordance with study of Herle *et al.* (2006), which reported that TiO_2 coated-film on Ti surface could promote BSP mRNA expression in primary human osteoblast cells, when compared to cpTi disc.[15] Moreover, higher calcium deposition on TiO_2 coated surface was observed, when compared to uncoated Ti discs and glass surfaces. This finding was in agreement with Advincular *et al.* (2006)[5] study, which demonstrated that TiO_2 sol-gel coated films could induce calcium phosphate formation (bone-like apatite formation) in simulated body fluid when sintering sol-gel at 450-550°C.[30] The mechanisms of calcium phosphate layer formation are not fully understood. It is proposed that in an aqueous solution, hydroxyl (Ti-OH) groups can be formed in TiO_2 layer, which serve as nucleation sites for apatite crystals and favor deposition. The negatively charged OH- on the TiO_2 surface might interact with the positively charged Ca^{2+}, and later with phosphate groups (PO_4^{3-}), resulting in the formation of amorphous calcium phosphate.[10] In all, evidences suggested that the fabrication of TiO_2 film on Ti surface had a positive effects on osteoblasts proliferation and function.

CONCLUSIONS

In this study, simple sol-gel technique was used to fabricate TiO_2 coated film on Ti surface. The results showed that TiO_2 coated surface could promote primary human osteoblast cell adhesion, proliferation and differentiation, as indicated by the increase in BSP and OC expression, as well as *in vitro* calcification. This simple and cost effective sol-gel technique yielded favorable outcomes, and might be used to improve implant surfaces for clinical application in the future.

CONFLICTS OF INTEREST

None declared.

ACKNOWLEDGEMENTS

This work was supported by Chulalongkorn University Centenary Academic Development Project and Chulalongkorn University Dutsadi Phiphat Scholarship.

REFERENCES

1. Kulkarni M, Mazare A, Gongadze E, *et al.* Titanium nanostructures for biomedical applications. *Nanotechnology.*, 2015;**26**:062002.
2. Ochsenbein A, Chai F, Winter S, *et al.* Osteoblast responses to different oxide coatings produced by the sol-gel process on titanium substrates. *Acta Biomater.*, 2008;**4**:1506-17.
3. Eisenbarth E, Velten D, Schenk-Meuser K, *et al.* Interactions between cells and titanium surfaces. *Biomol Eng.*, 2002;**19**:243-9.
4. Zhu X, Chen J, Scheideler L, Reichl R, Geis-Gerstorfer J. Effects of topography and composition of titanium surface oxides on osteoblast responses. *Biomaterials.*, 2004;**25**:4087-103.
5. Advincula MC, Rahemtulla FG, Advincula RC, *et al.* Osteoblast adhesion and matrix mineralization on sol-gel-derived titanium oxide. *Biomaterials.*, 2006;**27**:2201-12.
6. Meachim G, Williams DF. Changes in nonosseous tissue adjacent to titanium implants. *J Biomed Mater Res.*, 1973;**7**:555-72.
7. Rodrigues DC, Valderrama P, Wilson TG, Jr. , *et al.* Titanium Corrosion Mechanisms in the Oral Environment: A Retrieval Study. *Materials.*, 2013;**6**:5258-74.
8. Kim HW, Koh YH, Li LH, Lee S, Kim HE. Hydroxyapatite coating on titanium substrate with titania buffer layer processed by sol-gel method. *Biomaterials.*, 2004;**25**:2533-8.

ejprd.org - Published by Dennis Barber Journals. Copyright ©2019 by Dennis Barber Ltd. All rights reserved.

9. Velten D, Biehl V, Aubertin F, *et al*. Preparation of TiO_2 layers on cp-Ti and Ti6Al4V by thermal and anodic oxidation and by sol-gel coating techniques and their characterization. *J Biomed Mater Res*., 2002;**59**: 18-28.
10. Das K, Bose S, Bandyopadhyay A. Surface modifications and cell-materials interactions with anodized Ti. *Acta Biomater*., 2007;**3**:573-85.
11. Lu R, Wang C, Wang X, *et al*. Effects of hydrogenated TiO_2 nanotube arrays on protein adsorption and compatibility with osteoblast-like cells. *Int J Nanomedicine*., 2018;**13**:2037-49.
12. Saini KK, Sharma SD, Chanderkant, *et al*. Structural and optical properties of TiO_2 thin films derived by sol–gel dip coating process. *Journal of Non-Crystalline Solids*., 2007;**353**:2469-73.
13. Vercellino M, Ceccarelli G, Cristofaro F, *et al*. Nanostructured TiO_2 Surfaces Promote Human Bone Marrow Mesenchymal Stem Cells Differentiation to Osteoblasts. *Nanomaterials (Basel)*., 2016;**6**:124.
14. Ramires PA, Wennerberg A, Johansson CB, *et al*. Biological behavior of sol-gel coated dental implants. *J Mater Sci Mater Med*., 2003;**14**:539-45.
15. Harle J, Kim HW, Mordan N, Knowles JC, Salih V. Initial responses of human osteoblasts to sol-gel modified titanium with hydroxyapatite and titania composition. *Acta Biomater*., 2006;**2**:547-56.
16. Petchsuphamit W, Angwarawong T, Namano S. Effect of sol-gel derived amorphous TiO_2 films on the adhesion, differentiation and *in vitro* calcification of MC3T3-E1. *IJOR*., 2010;**1**:1-10.
17. Petecchia L, Usai C, Vassalli M, Gavazzo P. Biophysical characterization of nanostructured TiO_2 as a good substrate for hBM-MSC adhesion, growth and differentiation. *Exp Cell Res*., 2017;**358**:111-19.
18. Ma Z, Mao Z, Gao C. Surface modification and property analysis of biomedical polymers used for tissue engineering. *Colloids Surf B Biointerfaces*., 2007;**60**:137-57.
19. Roach P, Eglin D, Rohde K, Perry CC. Modern biomaterials: a review - bulk properties and implications of surface modifications. *J Mater Sci Mater Med*., 2007;**18**:1263-77.
20. Mangano C, De Rosa A, Desiderio V, *et al*. The osteoblastic differentiation of dental pulp stem cells and bone formation on different titanium surface textures. *Biomaterials*., 2010;**31**:3543-51.
21. Sousa SR, Moradas-Ferreira P, Barbosa MA. TiO_2 type influences fibronectin adsorption. *J Mater Sci Mater Med*., 2005;**16**:1173-8.
22. Webb K, Hlady V, Tresco PA. Relative importance of surface wettability and charged functional groups on NIH 3T3 fibroblast attachment, spreading, and cytoskeletal organization. *J Biomed Mater Res*., 1998;**41**:422-30.
23. Mendonca G, Mendonca DB, Aragao FJ, Cooper LF. Advancing dental implant surface technology-from micron- to nanotopography. *Biomaterials*., 2008;**29**:3822-35.
24. Kulkarni M, Flasker A, Lokar M, *et al*. Binding of plasma proteins to titanium dioxide nanotubes with different diameters. *Int J Nanomedicine*., 2015;**10**:1359-73.
25. Jokinen M, Patsi M, Rahiala H, *et al*. Influence of sol and surface properties on *in vitro* bioactivity of sol-gel-derived TiO_2 and TiO_2-SiO_2 films deposited by dip-coating method. *J Biomed Mater Res*., 1998;**42**:295-302.
26. Tsukimura N, Kojima N, Kubo K, *et al*. The effect of superficial chemistry of titanium on osteoblastic function. *J Biomed Mater Res A*., 2008;**84**:108-16.
27. Kern T, Yang Y, Glover R, Ong JL. Effect of heat-treated titanium surfaces on protein adsorption and osteoblast precursor cell initial attachment. *Implant Dent*., 2005;**14**:70-6.
28. He J, Zhou W, Zhou X, *et al*. The anatase phase of nanotopography titania plays an important role on osteoblast cell morphology and proliferation. *J Mater Sci Mater Med*., 2008;**19**:3465-72.
29. Alford AI, Kozloff KM, Hankenson KD. Extracellular matrix networks in bone remodeling. *Int J Biochem Cell Biol*., 2015;**65**:20-31.
30. Peltola T, Patsi M, Rahiala H, Kangasniemi I, Yli-Urpo A. Calcium phosphate induction by sol-gel-derived titania coatings on titanium substrates *in vitro*. *J Biomed Mater Res*., 1998;**41**:504-10.

ejprd.org - Published by Dennis Barber Journals.
Copyright ©2019 by Dennis Barber Ltd. All rights reserved.