พฤติกรรมในห้องปฏิบัติการของเซลล์สร้างกระดูกบนผิวไทเทเนียมที่พ่นด้วยผงอะลูมิเนียมออกไซด์ และทรายแก้ว

นางสาวจิรัชญา พันธะไชย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาทันตกรรมประดิษฐ์ ภาควิชาทันตกรรมประดิษฐ์
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2554

IN VITRO BEHAVIOR OF OSTEOBLAST-LIKE CELLS ON TITANIUM SURFACE BLASTED WITH Al_2O_3 AND GLASS BEADS

Miss Jiratchaya Panthachai

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Prosthodontics

Department of Prosthodontics

Faculty of Dentistry

Chulalongkorn University

Academic Year 2011

Thesis Title	IN VITRO BEHAVIOR OF OSTEOBLAST-LIKE CELLS ON TITANIUM SURFACE BLASTED WITH Al_2O_3 AND GLASS BEADS
By	Miss Jiratchaya Panthachai
Field of Study	Prosthodontics
Thesis Advisor	Associate Professor Mansuang Arksornnukit, D.D.S., Ph.D.
Thesis Co-advisor	Professor Prasit Pavasant, D.D.S., Ph.D.

Accepted by the Faculty of Dentistry, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

……..Dean of the Faculty of Dentistry
(Associate Professor Wacharaporn Tasachan, D.D.S)

THESIS COMMITTEE

……..Chairman
(Assistant Professor Orapin Kaewplung, D.D.S., Ph.D.)

……..Thesis Advisor
(Associate Professor Mansuang Arksornnukit, D.D.S., Ph.D.)

……..Thesis Co-advisor
(Professor Prasit Pavasant, D.D.S., Ph.D.)

……..Examiner
(Thanaphum Osathanon, D.D.S., Ph.D.)

……..External Examiner
(Associate Professor Teerasak Damrongrungruang, D.D.S., Ph.D.)

จิรัชญา พันธะไชย: พฤติกรรมในห้องปฏิบัติการของเซลล์สร้างกระดูกบนผิวไทเทเนียมที่พ่นด้วยผงอะลูมิเนียมออกไซด์ และทรายแก้ว. (IN VITRO BEHAVIOR OF OSTEOBLAST-LIKE CELLS ON TITANIUM SURFACE BLASTED WITH Al_2O_3 AND GLASS BEADS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ทพ. ดร. แมนสรวง อักษรนุกิจ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ทพ. ดร.ประสิทธิ์ ภวสันต์, 98 หน้า.

การพ่นทรายบนผิวไทเทเนียม เป็นวิธีการเตรียมพื้นผิวให้มีลักษณะพื้นผิวและความหยาบ ที่เอื้อต่อการตอบสนองของเซลล์ได้ดีขึ้น การศึกษานี้เปรียบเทียบพฤติกรรมของเซลล์สร้างกระดูกของหนู (เอ็มซีสามทีสาม-อีหนึ่ง) บนผิวไทเทเนียมที่แตกต่างกัน 6 ชนิด คือ ผิวไทเทเนียมขัดเรียบ ผิวที่พ่นด้วยผงทรายแก้วขนาด 50 ไมโครเมตร ผิวที่พ่นด้วยผงทรายแก้วขนาด 100 ไมโครเมตร ผิวที่พ่นด้วยผงอะลูมิเนียมออกไซด์ขนาด 50 ไมโครเมตร ผิวที่พ่นด้วยผงอะลูมิเนียมออกไซด์ขนาด 100 ไมโครเมตร และผิวที่พ่นด้วยผงอะลูมิเนียมออกไซด์ขนาด 250 ไมโครเมตร เครื่องวัดความหยาบผิว แสดงผลความหยาบผิวที่ใกล้เคียงกันของพื้นผิวที่ผ่านการพ่นทรายด้วยผงขัดที่มีขนาดเท่ากัน ผิวที่พ่นด้วยผงทรายแก้วขนาด 50 ไมโครเมตรและผิวพ่นด้วยผงอะลูมิเนียมออกไซด์ขนาด 50 ไมโครเมตร มีค่าความหยาบผิว 0.5340 และ 0.5288 ไมโครเมตร ผิวที่พ่นด้วยผงทรายแก้วขนาด 100 ไมโครเมตรและผิวพ่นด้วยผงอะลูมิเนียมออกไซด์ขนาด 100 ไมโครเมตร มีค่าความหยาบผิว 0.6323 และ 0.6343 ไมโครเมตร ขณะที่ผิวที่พ่นด้วยผงอะลูมิเนียมออกไซด์ขนาด 250 ไมโครเมตร มีค่าความหยาบผิวสูงสุดคือ 1.5168 ไมโครเมตรผิวที่ผ่านการพ่นทรายทั้งสองชนิดจัดเป็นผิวที่มีคุณสมบัติชอบน้ำ โดยที่ผิวที่ผ่านการพ่นด้วยผงอะลูมิเนียมออกไซด์ สนับสนุนการสร้างไฟบรินบนผิววัสดุภายหลัง 5 นาที ได้มากกว่าผิวชนิดอื่นๆ นอกจากนี้ เซลล์บนผิวที่พ่นด้วยผงอะลูมิเนียมออกไซด์ขนาด 250 ไมโครเมตร แสดงการยึดเกาะบนผิวภายใน 30 นาทีได้เร็วกว่า มีอัตราการเจริญเติบโตของเซลล์ในวันที่ 2 สูงกว่า สามารถส่งเสริมเพิ่มการแสดงออกของคอลลาเจนชนิดที่หนึ่ง และออสตีโอแคลซินเอ็มอาร์เอ็นเอ ในวันที่ 7 ได้มากกว่าผิวชนิดอื่น การทดลองยังพบว่ากลุ่มผิวที่พ่นด้วยผงขัดอะลูมิเนียมออกไซด์ สามารถส่งเสริมการแสดงออกของออสตีโอแคลซินเอ็มอาร์เอ็นเอ ในวันที่ 14 และการสะสมแร่ธาตุเมื่อย้อมด้วยสีอะลิซาลินในวันที่ 14 ได้ดีกว่ากลุ่มผิวที่ผ่านการพ่นด้วยผงทรายแก้ว อย่างไรก็ตามไม่พบความแตกต่างอย่างมีนัยสำคัญของการตอบสนองเซลล์ ในกลุ่มผิวที่พ่นด้วยผงขัดอะลูมิเนียมออกไซด์ที่ใช้ขนาดผงแตกต่างกัน การศึกษานี้แสดงให้เห็นว่าผิวที่ผ่านการพ่นด้วยผงอะลูมิเนียมออกไซด์สนับสนุนการยึดเกาะ การแปรสภาพของเซลล์กระดูก และการพอกแร่ธาตุ ได้ดีกว่าผิวที่ใช้ผงทรายแก้วพ่น และผิวที่พ่นด้วยผงอะลูมิเนียมออกไซด์ขนาด 250 ไมโครเมตร สนับสนุนการยึดเกาะ การเจริญเติบโตของเซลล์ การแสดงออกของเอ็มอาร์เอ็นเอของเซลล์เอ็มซีสามทีสาม-อีหนึ่ง ในระยะต้นได้ดีที่สุด

ภาควิชา.....ทันตกรรมประดิษฐ์.....ลายมือชื่อนิสิต.....
สาขาวิชา.....ทันตกรรมประดิษฐ์.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก……
ปีการศึกษา……2554……ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

#5276107732 : MAJOR PROSTHODONTICS

KEYWORDS : BEHAVIOR OF OSTEOBLAST CELLS/ BLASTING/ SURFACE TOPOGRAPHY/ SURFACE ROUGHNESS/ Al_2O_3/ GLASS BEADS

JIRATCHAYA PANTHACHAI: IN VITRO BEHAVIOR OF OSTEOBLAST-LIKE CELLS ON TITANIUM SURFACE BLASTED WITH Al_2O_3 AND GLASS BEADS. ADVISOR : ASSOC. PROF. MANSUANG ARKSORNNUKIT, D.D.S., M.S., Ph.D., CO-ADVISOR : PROF PRASIT PAVASANT, D.D.S., Ph.D, 98 pp.

Blasting titanium with abrasive particle is the method used to generate surface topography and roughness to improve cellular responses. The behavior of MC3T3-E1 cells was compared on six different titanium surfaces: polished titanium (Ti-polish), titanium blasted with glass beads (SiO_2) particles of 50 or 100 μm in size ($50SiO_2$-Ti, $100SiO_2$-Ti) and titanium blasted with Al_2O_3 particles of 50,100 or 250 μm in size ($50Al_2O_3$-Ti, $100Al_2O_3$-Ti, $250Al_2O_3$-Ti). Profilometry showed the comparable roughness values for the surface blasted with the same size particle, (*Sa*= 0.5340, 0.5288 μm for $50SiO_2$-Ti and $50Al_2O_3$-Ti) (*Sa*=0.6323, 0.6343 μm for $100SiO_2$-Ti and $100Al_2O_3$-Ti). While the $250Al_2O_3$-Ti had the highest roughness values (*Sa*= 1.5168 μm). Both the SiO_2 and Al_2O_3 blasted surfaces were hydrophilic materials but only Al_2O_3 blasted surface could support higher amount of fibrin formation after 5 minutes. In addition, cells seeded on $250Al_2O_3$ –Ti showed faster rate of adhesion at 30 min, higher rate of proliferation at day 2, higher expression of collagen type I and osteocalcin at day 7 than the other surfaces. Moreover, increased expression of osteocalcin at day 14 and more alizarin red-S staining at day 14 were observed on Al_2O_3 blasted surfaces compared to the SiO_2 blasted surfaces. However, no significant differences in cell response among the groups, which prepared by different size of Al_2O_3 were detected. The results of this study indicated that Al_2O_3 blasted surface could support the osteoblast adhesion, differentiation and mineralization better than SiO_2. These results suggested that the $250Al_2O_3$ –Ti supported the greatest initial adhesion, proliferation and initial gene expression of MC3T3-E1 cells.

Department : Prosthodontics Student's Signature .....

Field of Study : Prosthodontics Advisor's Signature .....

Academic Year : 2011 Co-advisor's Signature .....

ACKNOWLEDGEMENTS

My thesis could not have been completed without the helpfulness of these people.

First of all, I would like to express my deepest gratitude and thanks to my thesis advisor and co-advisor, Associate Professor Mansuang Arksornnukit and Professor Prasit Pavasant who are my role model of the good teacher and inspiration for research. They have given me valuable advices, guidance for conducting this research worth their kind helpful. I have learnt a great deal from them and enjoyed working with both over my time at Faculty of Dentistry, Chulalongkron University.

I also would like to thank the thesis committee, Assistant Professor Dr. Orapin Kaewplung, Dr. Thanaphum Osathanon and Associate Professor Dr. Teerasak Damrongrungruang for the important comments, suggestions in my thesis.

I am very much appreciated everyone at the Research unit of Mineralized Tissue, Faculty of Dentistry, Chulalongkorn University, for their friendly and willingness help. This thesis would also have not been possible without funding by Chulalongkorn University Centenary Academic Development Project.

Finally, with all my heart, I would like to thank my parents, Terdchai and Nittaya Panthachai, for their love, spirit, supports and encouragement in everything. They have always been there for me and believed in me. My lovely sister, Chirawan Panthachai, and my close friends, for all your endless help and encouragement.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

2D	Two-dimentional
3D	Three-dimentional
ANOVA	Analysis of Variance
Al_2O_3	Aluminium Oxide
BIC	Bone-Implant-Contact
CaO	Calcium Oxide
cDNA	Complementary DNA
Col I	Type I collagen
Cp Ti	Commercially pure titanium
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethylsulfoxide
EDS	Energy Dispersive X-ray Spectroscopy analysis
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HA	Hydroxyapatite
HMDS	Hexametryldisilazane
mRNA	Messenger RNA
Na_2O	Sodium oxide
OC	Osteocalcin
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
Ra	The arithmetic mean of the height variation on the roughness profile

Rq	The root mean of the height variation on the roughness profile
Rsm	The mean spacing of surface peaks
Rt	The maximum height the profile
Rz	The 10-points average roughness
Sa	The arithmetic mean deviation of a surface
Sds	The peak number per area (mm^2)
SiO_2	Silicon dioxide (this study used it for glassbeads)
SLA	Sand-blasted, Large grit, Acid-etched
St	The maximum height of the surface
TiO2	Titanium dioxide
V	Vanadium

CHAPTER I

INTRODUCTION

The titanium-based implant materials have been generally used in orthopedic and dental for endosseous implants due to the excellent biocompatibility and corrosion resistance. The success of implant depends on the intimate contact between the bone structure and biomaterial surface without fibrous tissue growing at the interface so called osseointegration, the condition that indicate the long term stability of implant and optimal bone regeneration[1].

One of the factors, influencing the success of osseointegration, is the surface characteristics, such as surface topography, surface chemistry, wettability and surface roughness[2]. When an implant is surgically placed, the initial interaction between host and implant surfaces is conditioned by tissue fluids. These interactions affect the amount and quality of cell adhered on the implant's surface[3, 4].

For these reasons, numerous surface modifications have been suggested to enhance the cellular response in achieving a stable mechanical bone-implant contact. The current methods include turning, blasting, acid-etching, porous sintering, anodic oxidation, hydroxyapatite-coating surfaces, ion implantation and biomolecule-based engineering[5-7].

In biomedical materials, the sand-blasting technique is commonly used to clean surface and to produce micro-retentive topography that can be sensed by individual cell. Generally, micro-roughness varied by size, shape and type of abrasive materials. In addition, roughness contains specific topographical features across a range from the nanometer to the millimeter scale[8]. Previous reports demonstrated that bone anchorage on titanium implants is markedly improved by surface roughness with *Ra* ranging from 0.5-1.5 μm[9-12].

Several abrasive materials such as Al_2O_3, SiC, glass beads, iron, corumdum, rutitle and hydroxyapatite (HA) have been used to improve the surface topography and chemical composition of biomaterials[13]. Among these materials, Al_2O_3

is the most widely applied abrasive material, which was shown to produce an appropriate topography and roughness of the implant surface by its ultra-hard and sharp angular characteristic[4]. The major component of abrasive is 99% Al_2O_3. There are many dental implant manufacturers which treat titanium surfaces with Al_2O_3 such as EVL (SERF, Decine, France), STI (The Allfit, Switzerland) and Ankylos (Densply-Friadent, Germany)[14]. Apart from Al_2O_3, glass bead is another choice of abrasive material, which has approximate properties, price and supply. It is spherical shape abrasive and used for blasting to create a rough surface of titanium implants in hip arthroplasty[15]. The major component of glass bead is ~70% SiO_2 along with Na_2O and CaO as the remaining component[16]. However, the effects of different types of abrasive materials on osteoblast behavior are still largely unknown.

The objectives of this study were to compare the effect of using variables, such as abrasive materials (Al_2O_3 and glass beads) and abrasive particle size (50,100,250 μm) on titanium surface characterization and MC3T3-E1 cell response. This *in vitro* study determined osteoblastic cell attachment, morphology, proliferation, mineralization and gene expression.

RESEARCH QUESTIONS

1. Whether the blasting titanium surface with different types or size of abrasive materials affect the surface characteristics of titanium.
2. Whether the surface prepared by different types or size of abrasive materials affect the behavior of osteoblast-like cells *in vitro*.

RESEARCH OBJECTIVES

1. To examine the surface roughness, topography, chemistry, hydrophilicity and fibrin clot formation on titanium surface blasted with Al_2O_3 and glass beads.
2. To examine the surface roughness, morphology, topography ,hydrophilicity and fibrin clot formation on titanium surface blasted with different abrasive particle size (50,100,250 μm).
3. To compare behavior of osteoblast-like cells on titanium surface blasted with Al_2O_3 and glass beads.
4. To compare behavior of osteoblast-like cells on titanium surface blasted with different abrasive particle size (50,100,250 μm).

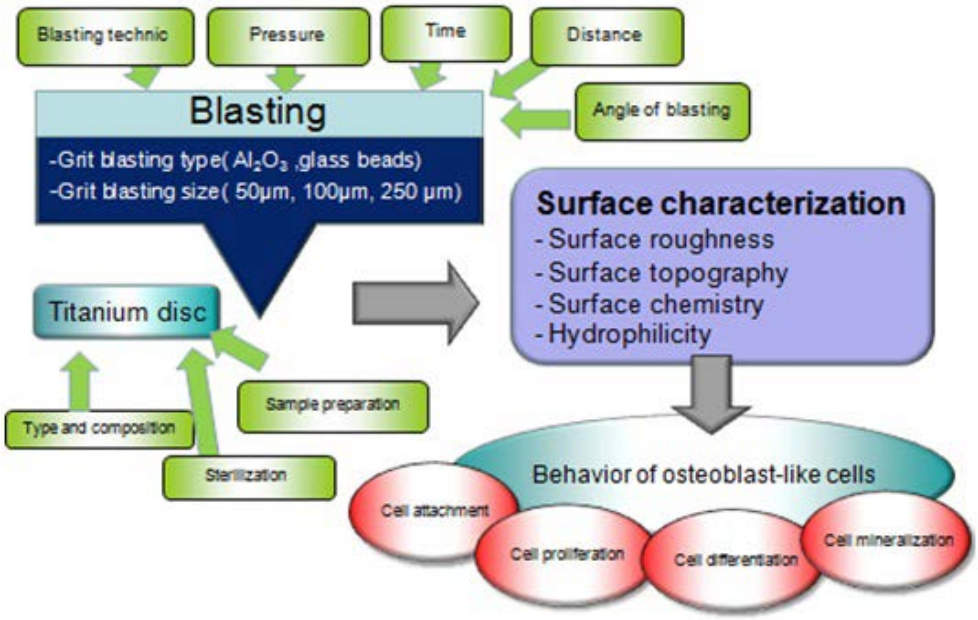
RESEARCH HYPOTHESIS

1. Blasting with different types or size of abrasive materials can affect the surface characteristics of titanium.
2. Surface prepared by different types or size of abrasive materials can affect the behavior of osteoblast-like cells *in vitro*

RESEARCH DESIGN

Laboratory experimental research

CONCEPTUAL FRAMEWORK



KEYWORDS

Behavior of osteoblast-like cells

Blasting

Surface topography

Surface roughness

Al_2O_3

Glass beads

RESEARCH EXPECTATION

1. To understand the effect of using different abrasive materials and particle size on titanium surfaces characterization.
2. To understand the effect of using different abrasive materials and particle size on the *in vitro* osteoblast-like cells response.
3. To establish basic knowledge for formulate a guideline to select the proper abrasive materials for blasting and manufacturing the dental implant.

CHAPTER II

REVIEW OF RELATED LITERATURE

Titanium and titanium alloys as an Implant materials

Titanium and titanium alloys are also used in biomedical implantation due to excellent biocompatibility, elegant mechanical properties, low level of electronic conductivity, high corrosion resistance, thermodynamic state at physiological pH values and low ion-formation in aqueous environments[17]. The biocompatibility and corrosion resistance of titanium and titanium alloys are associated with its thin (approximately 4 mm) surface oxide layer. The thin film occurs on implant surfaces and forms naturally in the presence of trace amounts of oxygen. The thin film is insoluble, resistant to body environments and strong adhered to titanium surface. This reaction leads to the prevention of fibrous tissue formation around implant[18, 19].

Commercially pure titanium (Cp) is used for endosseous dental implant applications. There are currently four Cp Ti grades (ASTM F 67) and one titanium alloy specially made for dental implant applications (Ti-6Al-4V, ASTM F 1472). Titanium grade 2 is used for industrial dental implant applications because its biocompatibility and lower modulus of elasticity (Young 's modulus) are more closely match of the bone which lead to a lower incidence of bone degradation[19]. Titanium alloy (Ti-6Al-4V) is the most widely used in medical implants because its high strength. Moreover, this alloy is used in dental implants for any patients who have parafunctional habits or history of implant fracture[20]. However, titanium alloy (Ti-6Al-4V) has a possible toxic effect resulting from released of vanadium and aluminum. For this reason, vanadium-and aluminum-free alloys have been introduced for implant applications. These new alloys include Ti-13Nb-13Zr (ASTM F1713), and Ti-12Mo-6Zr (ASTM F1813)[19].

Osseointegration

Osseointegration was first termed by Brånemark and later defined in a paper by Albrektsson*et al*. in 1981. This term was defined as the condition and the

process for having a loaded implant in direct contact with bone[21]. Mechanisms of the osseointegration process are similar to those occurring during bone fracture repair and involve a cascade of various cellular and extracellular events[3, 22].

When an implant is surgically placed, the initial interaction between host and implant surfaces is conditioned by tissue fluids elicited by the inflammatory response associated with wound healing. Studies reported that the implant surface was covered with the layer macromolecules of plasma (biofilm) and extracellular matrix component, such as immunoglobulins, vitronectin, fibrinogen, and fibronectin[23, 24]. This biofilm formed within a short time of contact. The fibrin network and the migrating effects of growth factors expressed as the important role in the establishment of osteoprogenitor reservoir at interface[24]. Then, during the first 3 days the mesenchymal cell recruitment occurs and ends with cell attachment to the implant surface. Osteoblast differentiation and proliferation occur after 3-6 days. The matrix calcification subsequently occurs after the first up to the third week. After 3 weeks, the formation and remodeling of new bone around implant occur in the regions[25]. All these processes illustrate in figure 2.1.

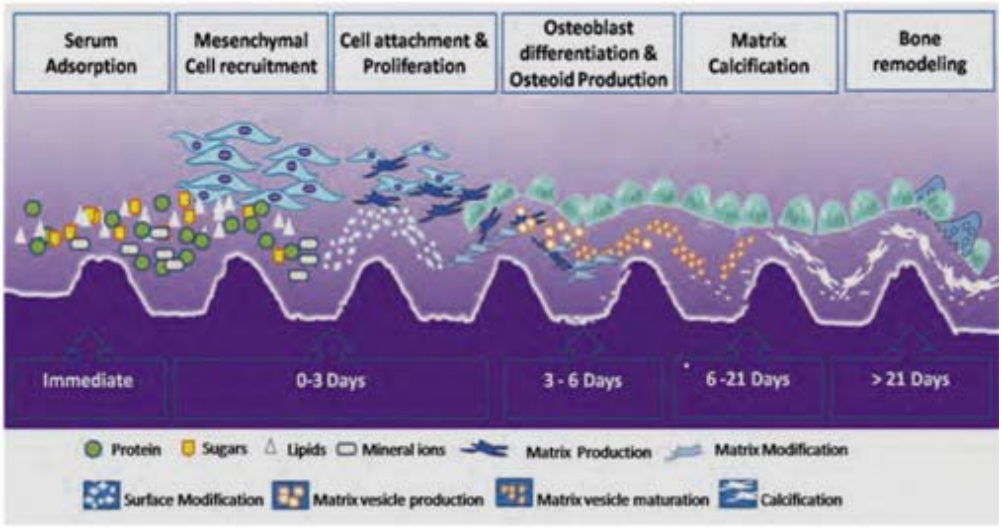


Figure 2.1 Illustration showing the cellular phenomena at the implant bone interface during healing of implant[26].

Influence of implant surface characteristics on osseointegration

Albrektsson et al., presented six factors for obtaining osseointegration, such as biocompatibility, design, surface properties, status of host tissue, surgical technique and loading condition[27]. The first three factors are related to all implant properties and many researchers attempt to develop the implant devices by focusing the surface properties.

The titanium surface, including topography, chemistry, wettability and surface roughness, has been described as the important factor to influence osseointegration. Amount of bone-to-implant contact (BIC) is an important determinant in long-term success of dental implants. Consequently, maximizing the BIC and osseointegration has become a goal of surface modification[26, 28].

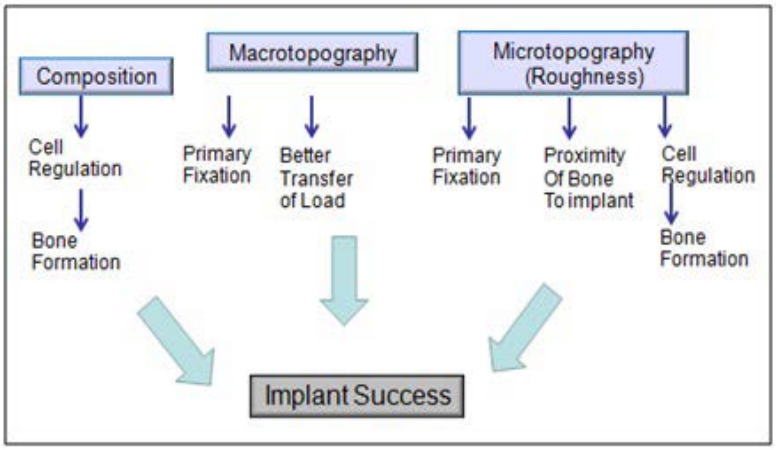


Figure 2.2 This schematic shows the implant success depends on the composition and structural features[8].

Dental implants have been designed to provide textures and shapes that may enhance cellular activity and direct bone apposition (Figure 2.2). For many years, the machined surface of the Brånemark implant was the gold standard for implant surfaces. The macrotopography such as screw like contours of implant can promote a mechanical interlock with surrounding bone. However, the actual surface of machined implant is smooth, which leads to fibrous formation at the interface of implant. These

outcomes had led to decrease bone formation. Moreover, implant manufacturers recently attempt to modify surfaces roughness that can be sensed by individual cell and contains topographical features across a range from the nanometer to the millimeter scale. It is their hope that the in-migration of new bone (osteoconduction) will be enhanced to specifically designed microtopography feature on the implant surface[8, 26, 29].

Several evaluations (see table 2.1) have demonstrated that implant with rough surfaces (modified titanium) show better bone apposition and BIC than implants with smooth surfaces (machined titanium)[30-32].

Table 2.1 Clinical evidence of commercially pure titanium topography effects on bone-to-implant contact[33].

Authors	Duration of Healing (Average)	Bone-to-Implant Contact	
		Machined c.p. titanium implant	Modified c.p. titanium implant
Ivanoff et al.	6.3 months	9%	37%(TiOBlast)[a]
Trisi et al.	12 months	7%	77%(Grit-blast)[b]
Lazzara et al.	6 months	34%	73%(Osseolite)[c]

[a] P=0.0001

[b] P<0.05

[c] P=0.0129

The main idea behind the establishment of a rough topography was to increase the surface area of the implant contact to bone and to improve the cell adhesion[2]. In addition, it has been reported that surface topography can alter a number of inter & intracellular reactions. For example, osteoblastic proliferation, platelet adhesion and collagen synthesis increased on rough surfaces, fibroblasts and epithelial

cells also adhered more strongly to smooth surfaces[8, 34, 35]. The mineralized nodule production is increased on titanium surfaces with deep grooves[36].

The chemical composition of implant surface often differs from the bulk composition and surface treatments. The surface layer may contain reactive bonds and various ions influences the binding of proteins to the surface and the subsequent cell reactions[37]. Buser et al., found the chemical enhanced SLA surface (Sand-blasted, Large grit, Acid-etched) was significantly enhanced BIC during the first 4 weeks of bone healing than the standard SLA surface. This study has supported the use of alterations in surface chemistry to modify osseointegration events[38].

Wettability and surface energy influence the adsorption of proteins, and increase adhesion of osteoblasts on the implant surface. The cell behavior on a hydrophilic surface is different from that on a hydrophobic surface[26]. There are usually reported that biomaterial surfaces with moderate hydrophilicity promoted the highest level of cell attachment and cell growth[39, 40].

However the responses of cells to surface characteristics are not specific. Many diverse responses and interactions are involved so that the response on any single test may not predict overall performance.

Implant surface topography

The topography of a surface is defined in terms of *form, waviness* and *roughness* (figure 2.3). Waviness and roughness are often presented together under the term *texture.* In the analysis, data describing form and waviness are first determined and then the roughness is assessed. The roughness describes the smallest irregularities in the surface, while form relates to the largest structure (profile)[41, 42].

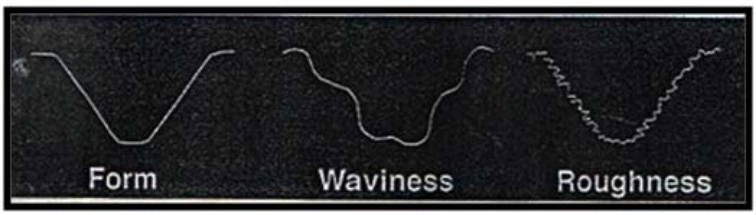


Figure 2.3 Illustration of surface topography [42]

For a proper topographical characterization of a surface, instruments and methods must be used to provide both numerical and visual data. There are three groups of instruments that may provide such information;

1. Mechanical contact stylus instruments.
2. Optical instruments.
3. Scanning probe microscope (SPM).

The surface characteristics of screw-type oral implants, only optical instruments may provide proper information. However, three-dimensional (3D) measurements are more reliable than two-dimensional (2D) determinations due to the increased amount of data obtained in the 3D assessment[43].

Surface roughness

Surface roughness can be divided into three levels depending on the scale of the features: macro-, micro- and nano-sized topography[44].

The macro level is defined for topographical features as being in the range of 10 μm-1 mm. This scale is directly related to implant geometry, with threaded screw and macroporous surface treatments. The primary implant fixation and long-term mechanical stability can be improved by an appropriate macro roughness[8, 26].

The micro-topographic profile of dental implants is defined for surface roughness as being in the range of 1–10 μm. This range of roughness increases the interlocking between mineralized bone and implant surface. Studies supported by some clinical evidence suggested that the micron-level surface topography resulted in greater accrual of bone at the implant surface[37, 45].

The nano- topographic profile is composed of nano-sized materials with a size range between 1-100 nm. Nanotopography modifications are commonly described in the literature both as nano-roughness and nano-features. Topographical features in the nanometer ranges play an important role in the adsorption of proteins, adhesion of osteoblastic cells and thus the rate of osseointegration[46]. However, reproducible surface roughness in the nanometer range is difficult to produce with

chemical treatments. In addition, the optimal surface nano-topography for selective adsorption of proteins leading to the adhesion of osteoblastic cells and rapid bone apposition is unknown. Thus, there is a need for more in vitro, in vivo and the long-termed study on the potential importance of nanostructure[27, 44].

Methods of surface modifications of implants

Surface modifications of implants can be divided into three main categories: physical, chemical, and biochemical[4].

Physical treatments: To modify surface characteristics by the application of external actions, shaping or removal of the material surface by another solid material. The physical methods of implant surface include cutting and turning, smoothing and blasting[4].

Chemical treatments: To produce modifications in the chemical composition of native materials, with specific regards to the surface layer. The chemical methods of implant surface modifications include chemical treatment with acidic or alkaline, hydrogen peroxide treatment, sol-gel, chemical vapor deposition, and anodization. Chemical surface modification of titanium has been widely applied to alter surface roughness and composition and enhance wettability/surface energy[38]. There are dental implant manufacturers which have produced surfaces in the chemical treatment such as Tiunite implants (Noble Biocare; anodized technique), Osseotite implants (Biomet; dual acid etching by hydrochloric and sulphuric acids)[47].

Biochemical treatment: To guide the enrichment of a biocompatible and bioresorbable carrier with the active molecule as coating material on implant surface. These biochemical methods include the covalent attachment, the peptide inclusion into carrier materials treatment and the adsorption treatment[48].

Blasting

In biomedical application, blasting techniques are mainly used for cleaning and improving the surface roughness. Blasting process requires abrasive particles to be forced against the surface by using compressed air, flowing through an

ejector and sucks particles up. Due to the dynamic of the contact between forced particles and surface, blasting treatment can produce higher roughness values with specific topography and the blasted particles are used to modify the surface chemical composition[4].

In dental implants manufacturing, such as SLA (Straumann, Switzerland) and Frialit-2 implants (Densply-Friadent, Germany), these surfaces are produced by a large grit 250-500 μm blasting process and followed by etching with hydrochloric/sulfuric acid[47]. Sandblasting results in surface roughness and acid etching leads to microtexture and cleaning[49]. The method is suggested for better osseointegration.

Bowers et al., reported that the irregular rough surface produced by sandblasting appears to be more conductive to osteoblast attachment than other surfaces roughened by polishing or acid etching[50]. Similarly, the study of Deligianni et al., focused on the short- and long-term response of human bone marrow cells in vitro and protein adsorption on titanium alloy Ti-6Al-4V with three values of surface roughness. The results showed the cell attachment and proliferation were increased as the roughness of Ti alloy increased[11]. On the other hand, several reports indicate that increased surface roughness cannot enhance cell function and bone formation[51-53].

Abrasive materials and biological response

Grit blasting (also called abrasive blasting) is based on bombardment of the surface by hard particles of high velocity. The particles lead to local plastic deformation and removal of the material surface[13]. Al_2O_3 is the most common abrasive suggested in preparation because of so easily acquired, affordable price and easily removed in acidic solution[4].

Al_2O_3 is used due to its hardness, strength and sharp angular characteristic. It is widely used as a coarse or fine abrasive. In addition, its low heat retention and low specific heat have made it widely used in grinding operations. The major component of abrasive is 99% Al_2O_3, along with fine particle of less than 1%

crystallized silica[54]. There are many dental implant manufactures which treat surfaces with Al_2O_3 grit blasting such as EVL (SERF, Decine, France), STI (The Allfit, Switzerland) and Ankylos (Densply-Friadent, Germany)[14].

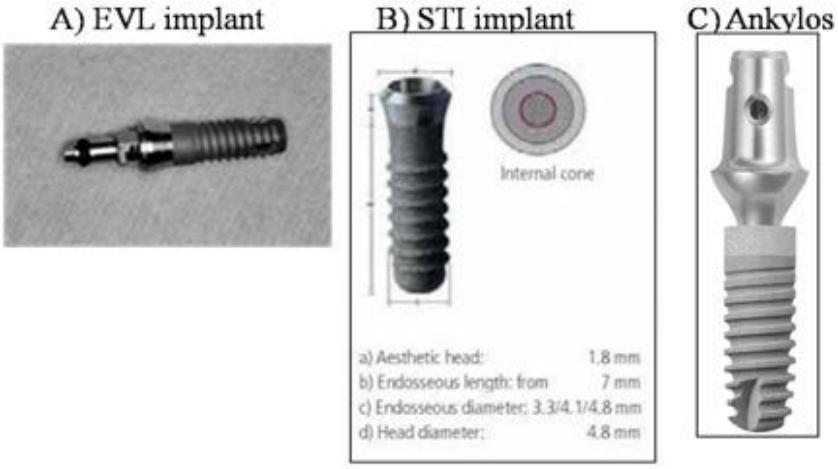


Figure 2.4 Images of implants blasted by Al_2O_3. The EVL implant, one-step, cylindro-conical, self-tapping endosseous screw-shaped implant made of grade 2 titanium(A), The STI implant produced from grade 4 titanium (B), The Ankylos implant (C)[55-57].

Mueller et al., compared implant blasted with Al_2O_3 and bioceramic particle. Average surface roughness (*Ra*) was estimated to be around 0.5 μm for both modifications. No significant difference was found in the bone response[58].Similarly the study of Wennerberge et al., who reported TiO_2 and Al_2O_3 blasting particles resulting in *Sa* values of about 1 μm. The biological results were not significantly different. It is interesting to note that in the study, they did not find any negative bone tissue effect of the Al ions which probably were present on the Al_2O_3-blasted titanium surfaces[59]. This is in contrast to the case of Ti-6Al-4V alloy, there is a potential for continuous release of Al (and V) ions into the tissue, while the Al_2O_3-blasted surface presents a transient and limited releasing of Al ions[60].

Contrarily, Esposito et al., found the releasing of remnants from blasting materials has been suggested to impair bone mineralization and repair through a competition between Al and Ca ions[52].

Glass bead is a unique air blasting abrasive for cleaning and conditioning surfaces. It is manufactured from high - grade glass, annealed in its spherical shape to equalize internal stresses and resist fracture. The inherent strength of glass bead is such that it can survive against multiple impacts, allowing for continuous recycling and reducing cost[16].

In biomedical applications, glass bead is used for providing a level of roughness as well as a suitable surface topography such as Wong et al., who modified the surface treatment by blasting glass bead and hydroxyapatite (HA) coated on Ti6Al7Nb[61]. Schuh et al., used glass bead blasting to create a rougher surface of Titanium implants in hip arthroplasty[15]. The major component of commercial abrasive is SiO_2 ~70% along with Na_2O and CaO[16]. These compositions are bioactive materials. Chang et al., found that the Ti disc blasted with commercial spherical-shaped glass (glass bead-Ti) can enhance cell growth with culturing up to 7 days. There was no significant difference with respect to the bioactive glass particles with a composition of $70SiO_2.25CaO.5P_2O_5$,which were prepared by a sol gel method[7]. The summarized studies which used the Al_2O_3 or glass bead particle for creating the roughed surfaces as showed in Table 2.2.

Table 2.2 Studies of blasting by variable abrasive materials on bone to implant contact or the cellular response.

Material/surface			Ref
	Model	Bone to implant contact	
Ti blasted with 25 μm TiO_2 VS 25 μm Al_2O_3	Rabbit	25 μm TiO_2 = 25 μm Al_2O_3	[59]
Ti blasted with 25 μm Al_2O_3 VS 75 μm Al_2O_3	Rabbit	75 μm Al_2O_3 >25 μm Al_2O_3	[62]
Ti blasted with 25 μm Al_2O_3 VS 250 μm Al_2O_3	Rabbit	25 μm Al_2O_3 =250 μm Al_2O_3	[63]
Ti blasted with 110 μm Al_2O_3 VS 50 μm bioceramic	Rabbit	110 μm Al_2O_3 = 50 μm bioceramic	[58]
Ti,Ti alloy blasted with 150-250 μm glass beads VS 300-400 μm corundum	Dog	150-250 μm glass beads =300-400 μm corundum	[61]
Ti blasted with 50 μm glass beads VS bioactive glass	*In vitro*	**Cell growth 7 days** 50 μm glass beads= bioactive glass	[7]

Properties and required information to describe blasted surfaces[64]

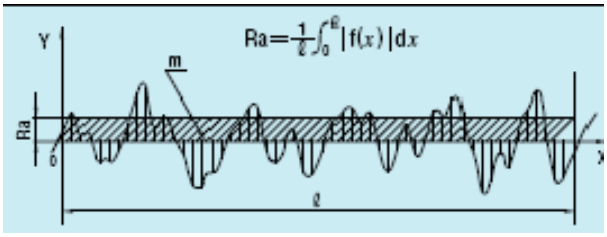
When using the blasting techniques to produce the specific topography and various chemical compositions of titanium surface, the optimal surface properties were required such as.

- Morphology, Texture, Roughness and Form property

The typical information needed: Type and distribution of morphological features, size and distribution of open or close porosity, 2D and 3D parameters were describing the surface roughness. Waviness and form are ranging from the atomic or nanometer (nm) to the micrometer (μm) to the mm or cm scale[64].

Several roughness parameters exist to describe surface topography. The 2D parameters *Ra*, *Rq*, *Rz* and *Rt* are the most commonly used parameters and 3D parameters *Sa*, *Sq*, *Sz* and *St*are used to provide better characterization for all modern implant surface, a description is as follow[43].

Ra (*Sa* for 3D) is the arithmetic average of the absolute height values of all points of the profile (*Ra*) or a surface (*Sa*). This is a stable height-descriptive parameter.



$$R_a = \frac{1}{L}\int_0^L |y(x)|dx$$

Figure2.5 Show the calculation of *Ra*. *Ra* measurement for sample length " L " is the mean height of the surface profile (Peaks and inverted valleys).

Rq (*Sq* for 3D) is the root mean of the values of all points of the profile (*Rq*) or surface (*Sq*). *Rq* gives almost the same information as *Ra* but is slightly more sensitive to high peaks and low valleys.

$$R_q = \sqrt{\frac{1}{L}\int_0^L y^2(x)dx}$$

Rz (*Sz* for 3D) is the 10-points average roughness i.e. the average of the five lowest valleys and the five highest peaks within the profile (*Rz*) or the surface (*Sz*).

Rt (*St* for 3D) is the maximum peak to valley of the profile (*Rt*) or the surface (*St*).

The roughness parameters were estimated from the topography data using the software of its own instrument. They depend on the length scale selected. The average length of an osteoblast is about 10 μm. Thus, the proper scan sizes should be larger than the cell size length[34, 65].

In an early study published in 1972, Predecki et al., found a certain degree of surface roughness (*Ra*=0.508 μm) to be necessary for fixation and growth of bone toward the implant surface[66]. The previous reports have demonstrated that primary bone anchorage of titanium implants was markedly improved by surface roughness with *Ra* ranging from 0.5-1.5 μm[9-12].

Albrektsson & Wennerberg reviewed the topographic and classified surface roughness. They suggested smooth surfaces to have an *Sa* value of <0.5 μm, minimally rough surfaces were identified with an *Sa* of 0.5–1 μm, moderately rough surfaces with *Sa* 1–2 μm and rough surfaces with an *Sa* of >2 μm[37].

The surface roughness can be varied by the process parameters and particle size. For example, alumina particles in the size 25-75 μm result in *Ra* range 0.5-1.5 μm[62, 67], while *Ra* in the range 2-6 μm are reported for surfaces blasted with particle size of 200-600 μm[68, 69].

- Chemical composition property

The typical information needed: type of inorganic compounds, oxidation states of elements, molecular structure of organic compound, distribution parallel of chemical composition to surface, distribution perpendicular of chemical composition to surface.

Particles are likely to become embedded in the titanium surface during blasting. This is frequently observed with alumina and silica particles[62]. The blasting particles can be used to modify the surface chemical composition for example, blasting with bioactive glass particles or hydroxyapatite which enriches the surface in Ca and P[7, 53, 70]. On the other hand, blasting particles are contamination and can lead to impair the cell response[52].

- Surface energetics property

The typical information needed: wettabilty by polar/nonpolar solvents, hydrophilic/hydrophobic properties

Surface energy is an indicator of potential cellular adhesion. The surface with high energy has a high affinity for adsorption and show stronger osseointegration than implant with a low surface energy. A practical way to measure surface energy is contact angle measurements. The method also used to define the surface is hydrophilic or hydrophobic[37]. The classification of hydrophilic/hydrophobic properties can be divided with the contact angle values (Table 2.3).

Table 2.3 Classification of hydrophilicity[71]

Classification	Contact angle
Hydrophilic surface	0° to 90°
Hydrophobic surface	>90°
Superhydrophobic surface	>150°

There were studies found that the hydrophilic property was influenced by surface roughness[65, 72]. The studies showed an increased surface area of titanium by blasting should lead to decrease the values of the contact angles which indicated more hydrophilicity. However, it is expected that the wettability of a surface relate to its free energy, polar character, surface charge, roughness and chemical composition. Therefore, the study of the wetting surface interactions needs further investigation[73].

CHAPTER III

RESEARCH METHODOLOGY

1. Titanium samples preparation and blasting method

Titanium (Ti) discs, 15 mm in diameter and 3 mm in thickness, were prepared from commercially available pure titanium grade-2 (KVM Heating Element Co.,Ltd. Thailand). All Ti discs were machine-polished (DPS 3200, IMPTECH, South Africa) with silicon carbide paper on a rotative polisher at 150 rounds per minute (rpm) for 30 seconds and randomly divided into six groups;

1) Control (no blasting, Ti polish)

2) Blasting with 50 μm particles of glass beads ($50SiO_2$-Ti, Shofuinc, Accord)

3) Blasting with 50 μm particles of Al_2O_3 ($50Al_2O_3$-Ti, Tec line, Dental vision)

4) Blasting with 100 μm particles of glass beads ($100SiO_2$-Ti, Tec line, Dental vision)

5) Blasting with 100 μm particles of Al_2O_3 ($100Al_2O_3$-Ti, Kepler)

6) Blasting with 250 μm particles of Al_2O_3 ($250Al_2O_3$-Ti, Tec line, Dental vision)

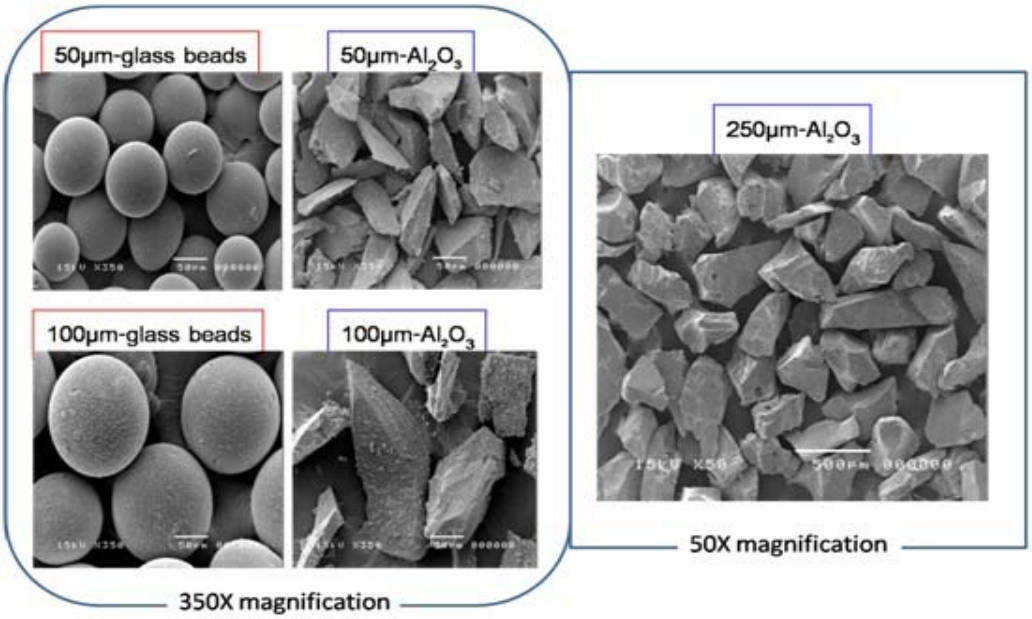


Figure 3.1 Picture from SEM showed the abrasive particles that used in this study

All Ti discs were blasted under pressure blasting (Druckminderer, typ5417, Germany) at a constant distance of 2.5 inch at air pressure of 3-4 bars for 30 seconds and blasting angle of 90º (Figure 3.2). Subsequently, discs were ultrasonically cleaned with deionized water for 10 minutes then consequently rinsed with 70 % ethanol and sterile by autoclave.

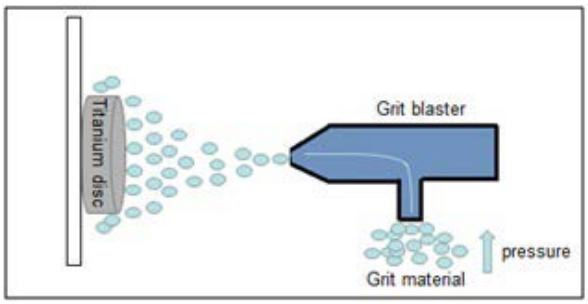


Figure3.2 Illustration of blasting method

2. Surface characterization analysis

2.1 Surface roughness and topography

The surface roughness parameters were measured using a surface profilometer (Talyscan 150, Taylor Hobson, UK, n=10). Five different locations (2x2 mm) on each sample (ASTM D7127-05) were scanned with filter/cut-off = 0.08 mm (0.08 mm ignored at the beginning and the end of the profile), 2000 μm/s speed and one way direction measurement. Results were express as *Ra* (arithmetic mean of the height variation on the roughness profile), *Rt* (maximum peak to valley of the profile) and *RSm* (mean spacing of surface peaks), The value of *Sa* (arithmetic mean deviation of a surface), *St* (maximum peak to valley of the surface), and *Sds* (peak number per area (mm^2), were also calculated.

Surface topography of the Ti discs was also described by roughness parameters. Moreover, the qualitative profiles of the textured titanium surfaces were made with the software of profilometer program.

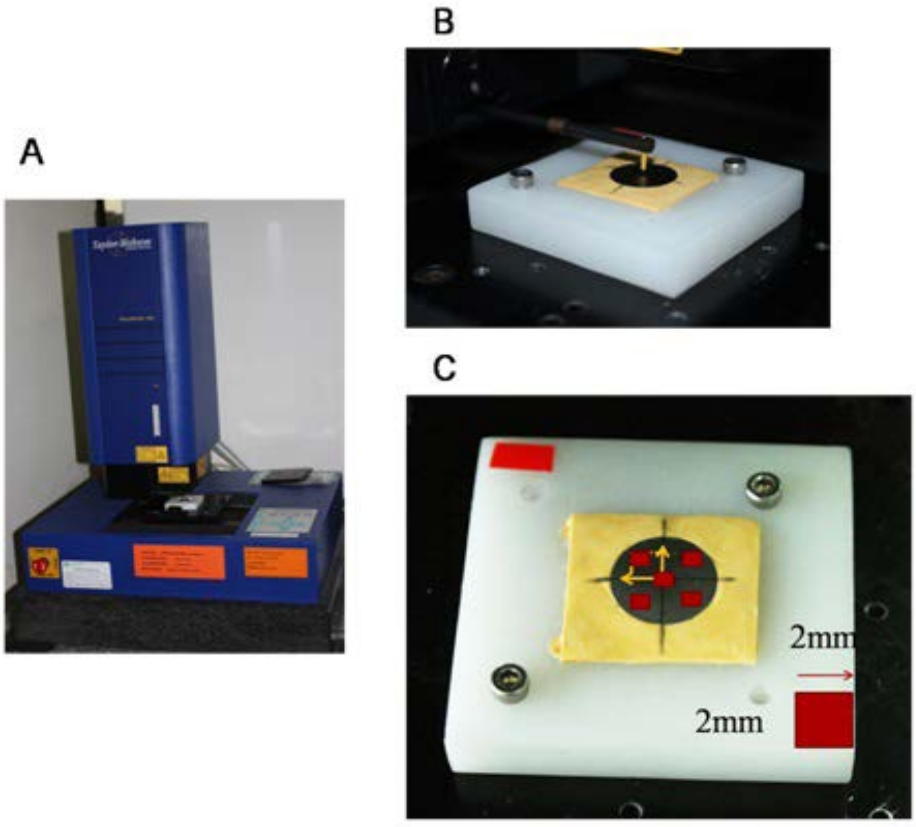


Figure 3.3 Picture showed the profilometer (Talyscan 150, Taylor Hobson) (A), The diamond inductive stylus gauge showed the location for scanning on each sample (B, C).

2.2 Surface morphology

Surface morphology of the Ti discs was also examined using a scanning electron microscope (JSM 5410LV, JEOL, Japan), in order to qualitatively evaluate the different blasted surface.

2.3 Surface chemical analysis

The composition of the Ti discs was confirmed using Energy Dispersive X-ray Spectroscopy (EDS) analysis (Link ISIS 300, Oxford, England). After ultrasonically cleaned, the chemical elements were randomly measured at three different locations on each sample.

2.4 Hydrophilicity

Static contact angle measurement was performed using a contact angle meter (DSA 10, Krüss, Hamburg, Germany) at ambient temperature. The 10 µl sessile droplet of deionized water was drop vertically on the specimen surface without physical contact using micro-syringe onto the surface. The contact angles were measured ten times and reported as mean $\pm$ standard deviation.

2.5 Fibrin clot formation

The 150 µl of fresh blood was dropped on the Ti surface and covered with glass cover slip immediately. After 5 minutes, the specimens were rinsed three times in 0.1M PBS. They were dehydrated in a graded series of alcohol and then critical point dried with 100% hexamethyldisilazane (HMDS, Fluka, Steinheim, Germany) for 5 min. The fibrin structure can be determined using scanning electron microscope (JSM 5410LV, JEOL, Japan).

This procedure used non anti-coagulated whole blood. The protocol was approved by the ethical committee, Faculty of Dentistry, Chulalongkorn University.

3. Analysis of function and behavior of osteoblast-like cells on titanium surfaces

3.1 Cell culture

MC3T3-E1 cells (ATCC CRL-2593) is a non-transformed cell line established from newborn mouse calvaria. Cells were grown in alpha minimum essential medium (HyQ® MEM/EBSS, Hycone, Logan, Ultah, USA) supplemented with 10% fetal bovine serum (FBS, ICP biologicals, Henderson, Auckland, New Zeland), 2 mM L-glutamine, 100 unit ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 0.25 µg ml^{-1} amphotericin B (Gibco,Grand Island, New York, USA). Cells were subcultured once a week.

3.2 Cell morphology in scanning electron microscopy (SEM)

Cells were seeded on the Ti-polish discs and Ti-blasted discs for 30 min, 4 and 16 hours. Cells were fixed with 3% glutaraldehyde solution (Fluka, Steinheim, Germany) for 30 min, rinsed with 0.1M PBS, dehydrated in a graded series of alcohol (30%, 50%, 70%, 90% & 100% ethanol), critical point dried using 100% hexamethyldisilazane (HMDS, Fluka, Steinheim, Germany), coated with a thin layer of gold and examined under scanning electron microscope (JSM 5410LV, JEOL, Japan).

3.3 Cell attachment and proliferation

Cells were cultured on Ti discs in 24 well culture plates at ~30,000 cells/well. The attachments were determined by scanning electron microscopy (SEM) and MTT assay after for 30 min, 4 and 16 hours in culture. The proliferation rate was studied using MTT assay cells after 16 hours, 2 and 3 days culture. The MTT assay is based on the reduction of tetrazolium salt to formazan crystals by dehydrogenase enzymes secreted from the mitochondria of active cells. The amount of purple formazan crystals relates to the number of viable cells. In brief, cells were incubated with 250 µL/well of MTT solution (0.5 mg/ml in DMEM without phenol red) at 37 °C. After 30 min, the formazan crystal was dissolved in dimethylsulfoxide (DMSO, Sigma-Aldrich, Seelze,Germany) (900 µL/well) and glycine buffer (pH = 10) (125 µL/well). The absorbance was read with Thermospectronic Genesis10 UV-vis spectrophotometer at a wavelength of 570 nm. The data represented the number of viable cells.

3.4 Gene Expression

Cells were seeded on materials for 7 days and 14 days. Expressions of type I collagen (Col I) and osteocalcin (OC) messenger RNA (mRNA) were assessed using qRT-PCR. Total RNA was extracted with TriPure Isolation Reagent according to manufacturer's instruction. One µg of each RNA sample was converted to cDNA by avian myeloblastosis virus (AMV) reverse transcriptase (Promega, Fitchburg, WI, USA) for 1.5 h at 42°C followed by performing polymerase chain reaction (PCR) using primers, prepared from the following reported sequences from GenBank (NM_007742.3,

NM_001032298.2 and XM_001476723.1 for Col I, OC and GAPDH, respectively). The oligonucleotide sequences of the primers were as follows:

Col I sense 5´GGTGCCCCCGGTCTTCAG3´
antisense 5´AGGGCCAGGGGGTCCAGCATTTC3´
OC sense 5´CTTGGGTTCTGACTGGGTGT3´
antisense 5´AGGGAGGATCAAGTCCCG3´
GAPDH sense 5´ACTTTGTCAAGCTCATTTCC3´
antisense 5´TGCAGCGAACTTTATTGATG3´

The PCR products were electrophoresed on 1.8 % agarose gel (Usb, Cleveland, OH, USA) and visualized by ethidium bromide fluorostaining (EtBr; Bio-Rad, Hercules, CA, USA). The density of band was determined using Scion Image Software (Scion Corporation, USA).

3.5 Mineralization

In vitro mineralization was quantified by Alizarin red-S staining (Alizarin Red S –certified, Sigma, St.Louis, MO, USA) after 14 days of cells culture. Cells were fixed with cold methanol for 20 min and stained with 1% Alizarin red in 1:100 (v/v) ammonium hydroxide/water (pH 4.2) for 3 min. The amount of calcium deposition was quantified by destained with 10% cetylpyridinium chloride monohydrate (Sigma, St. Louis, MO, USA) in 10mM sodium phosphate at room temperature for 15 min. The absorbance was measured at 570 nm using the UV-vis spectrophotometer (Thermospectronic Genesis10 UV-vis, Madison, WI, USA).

4. Statistical analysis

Data were expressed as mean$\pm$ standard deviation. Statistical analysis was carried out by the one-way analysis of variance (one-way ANOVA), follow by Scheffe test or Dunnett test (SPSS®17.0 for Windows, SPSS, Chicago, IL, USA). A probability of < 0.05 was considered significant.

CHAPTER IV

RESULTS

1. Surface characterization analysis

1.1 Surface roughness and topography

The roughness parameters of the polished Ti and blasted Ti surfaces were shown in Table 4.1. No statistically difference ($p>0.05$) was from in both *Ra* and *Sa* values of SiO_2 and Al_2O_3 blasted surfaces prepared from the same particle size. However, the statistically difference ($p<0.05$) were observed among the groups of surfaces prepared from different particle size. Data from Table 4.1 revealed that both *Ra and Sa* values increased according to the increased particles sized used. The other parameters such as *Rt*, *Rsm*, *St*, and *Sds*, were used to present the surface topography on each sample. These values appeared to be similar to the topography profiles by profilometry analysis (see figure 4.1).

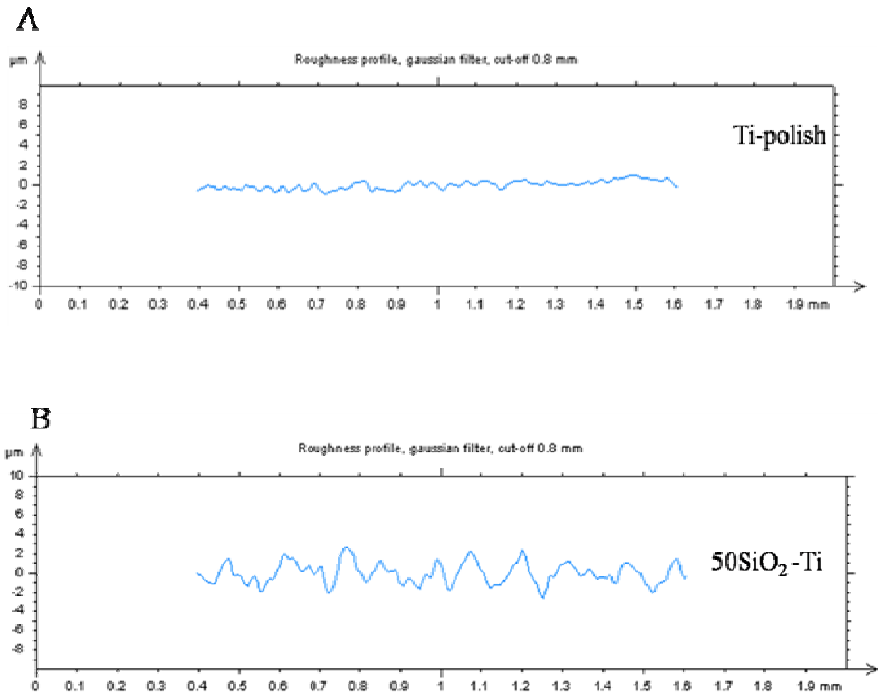
Table4.1 Surface roughness parameters of titanium samples

Materials	*Ra* (μm)	*Sa* (μm)	*Rt* (μm)	*Rsm* (μm)	*St* (μm)	*Sds* (peak/mm^2)
Ti-polish	0.0630±0.0100^a	0.1341±0.0152^a	0.461±0.076	0.0388±0.002	1.638±0.501	1563.40±132.63
Ti-50SiO_2	0.2265±0.0106^b	0.5340±0.0213^b	1.632±0.096	0.0433±0.002	6.888±0.488	939.18±28.99
Ti-50Al_2O_3	0.2339±0.0110^b	0.5288±0.0159^b	2.065±0.199	0.0390±0.002	11.016±1.673	1218.72±17.82
Ti-100SiO_2	0.2513±0.0112^c	0.6323±0.0242^c	1.970±0.209	0.047±0.001	10.614±0.935	760.50±22.56
Ti-100Al_2O_3	0.2593±0.0117^c	0.6343±0.0143^c	2.298±0.222	0.0409±0.001	14.428±1.909	1089±17.17
Ti-250Al_2O_3	0.5882±0.0284^d	1.5168±0.0535^d	4.874±0.213	0.048±0.001	34.948±4.952	787.89±8.58

Data are presented as mean ± SD (n=10). Statistical significance was observed on Ti-samples prepared by different particle size compare to Ti-polish (p<0.05, show as the different superscript letters). Same superscript letters indicate statistical insignificance at each sample.

Ra = arithmetic mean of the height variation on the roughness profile, *Sa* = arithmetic mean deviation of a surface, *Rt* = maximum height the profile, *RSm* = mean spacing of surface peaks, *St* = maximum height of the surface and *Sds* = peak number per area (mm^2)

Surface topographic analysis using profilometry was shown in Figure 4.1(A-F). The Al_2O_3 blasted surface presented many sharp peaks with deep valleys (Figure 4.1C, E, F) while blasting with SiO_2 produced more regular shallow peaks with rounded and wide pits (Figure 4.1B, D). Similar results were observed by SEM analysis, which presented as the surface morphology (Figure4.2). Note that the surface topography of Ti-polish appeared as the longitudinal grooves with some scratches, resulting from the grinding operation (Figure4.1A, 4.2A).



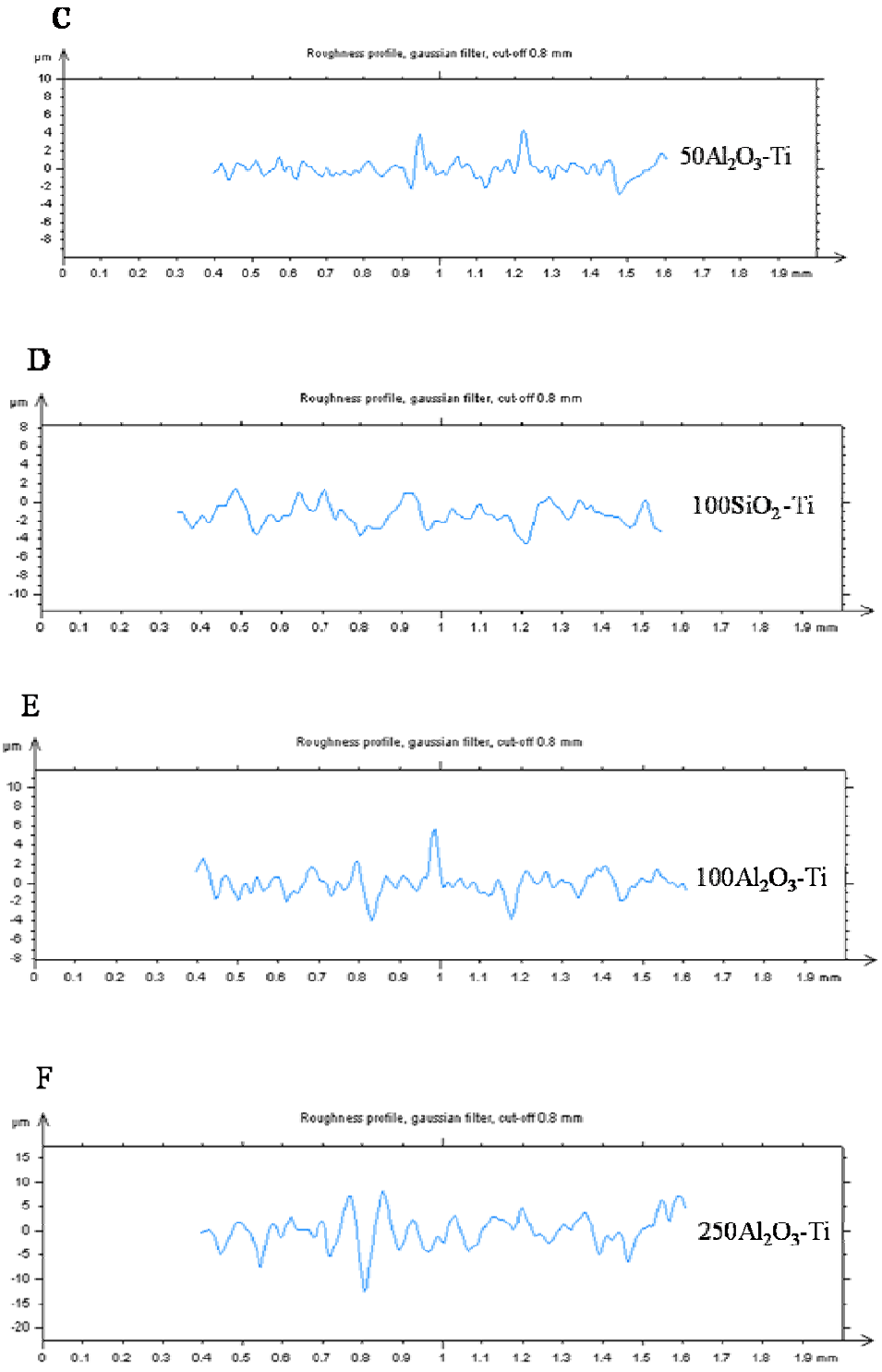


Figure 4.1 Profile topography generated from profilometer Ti-polish (A), 50SiO_2-Ti (B), 50Al_2O_3-Ti (C), 100SiO_2-Ti (D), 100Al_2O_3-Ti (E), Ti-250Al_2O_3 (F).

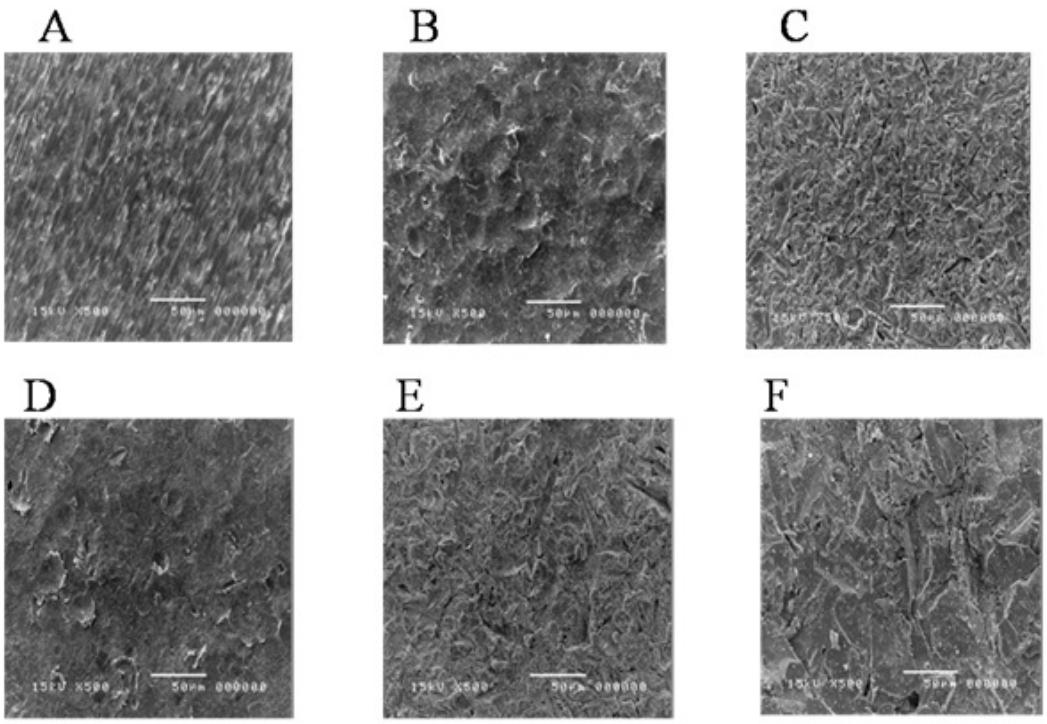


Figure 4.2 SEM surface morphology of Ti-polish (A), 50SiO_2-Ti (B), 50Al_2O_3-Ti (C), 100SiO_2 -Ti (D), 100 Al_2O_3-Ti (E), 250Al_2O_3-Ti (F): 500X magnification.

1.2 Surface chemical analysis

The chemical compositions of the material surface obtained from EDS analysis was shown in Table 4.2. Both the polished and blasted surfaces contained Ti, N and C, however, the SiO_2 blasted surface also contained O and trace amount of abrasive materials such as Si, Na and Ca. Interestingly, the Al_2O_3 blasted surface showed the higher ratio of O compared to the SiO_2 blasted surface. Moreover, the presence of Al was around 10% in the $50Al_2O_3$ and $100Al_2O_3$ blasted surfaces while the titanium surface blasted with $250Al_2O_3$ contained Al around 5%.

Table 4.2 Quantitative Energy Dispersive X-ray Spectroscopy (EDS) analysis of the different titanium surface

element(%)	Ti-polish	Ti-50SiO_2	Ti-50 Al_2O_3	Ti-100 SiO_2	Ti-100 Al_2O_3	Ti-250 Al_2O_3
Ti	85.8	61.8	33.8	53.7	37.1	49.5
N	9.7	6.4	4.8	5.6	3.5	6.3
C	4.5	3.2	3.9	4.2	3.5	3.5
O		26.3	45.7	34.7	45.4	36
Si		0.9		1.2		
Na		0.3		0.4		
Ca		0.1		0.2		
Al			10.5		10.2	4.8

1.3 Hydrophilicity

The surface hydrophilicity was determined by measuring the contact angle of a water-drop on the surfaces. The results were shown as shown as graph in Figure 4.3A and Figure 4.3(B-G). The polished titanium surface showed the highest angle degree (82.37°) compared to other blasted surfaces, indicating that blasted surfaces possessed a greater hydrophilicity than the polished surfaces. In addition, the contact angle of water decreased on SiO_2 blasted surfaces compared to the Al_2O_3 blasted surface (p<0.05) suggesting the better hydrophilicity of SiO_2 blasted surfaces. Among the blasted surfaces, the $250Al_2O_3$ blasted surfaces showed the highest contact angle (75.09°) indicated the least hydrophilicity compared to other surfaces.

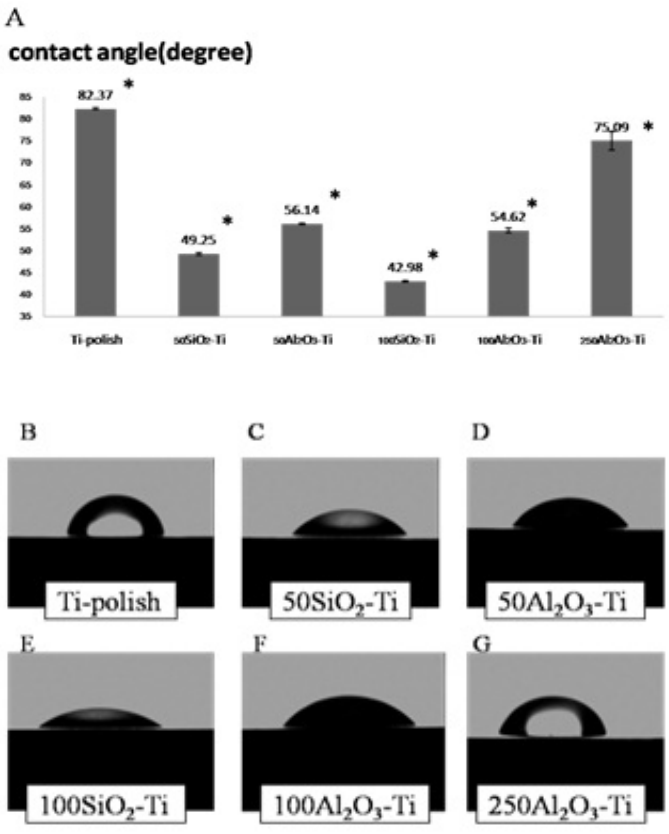


Figure 4.3 The water contact angle of titanium surface (A). Data were shown as the mean ± standard deviation (n=10). * designated statistically significant, p<0.05. The images of water dropped on different titanium surfaces (B-G).

1.4 Fibrin clot formation

The ability of Ti surface to support fibrin formation was determined. Fresh blood was dropped on the titanium surfaces for 5 minute and then washed thoroughly. The fibrin formation on the surface was examined by SEM as shown in Figure 4.4 (A- F). The amount of fibrin formation on Al_2O_3 blasted surfaces (Figure 4.4 (C, E, F)) was obviously higher than that on SiO_2blasted and polished surfaces (Figure 4.4 (A, B, D)). However, the amount of fibrin formation was comparable among the Al_2O_3blasted surfaces.

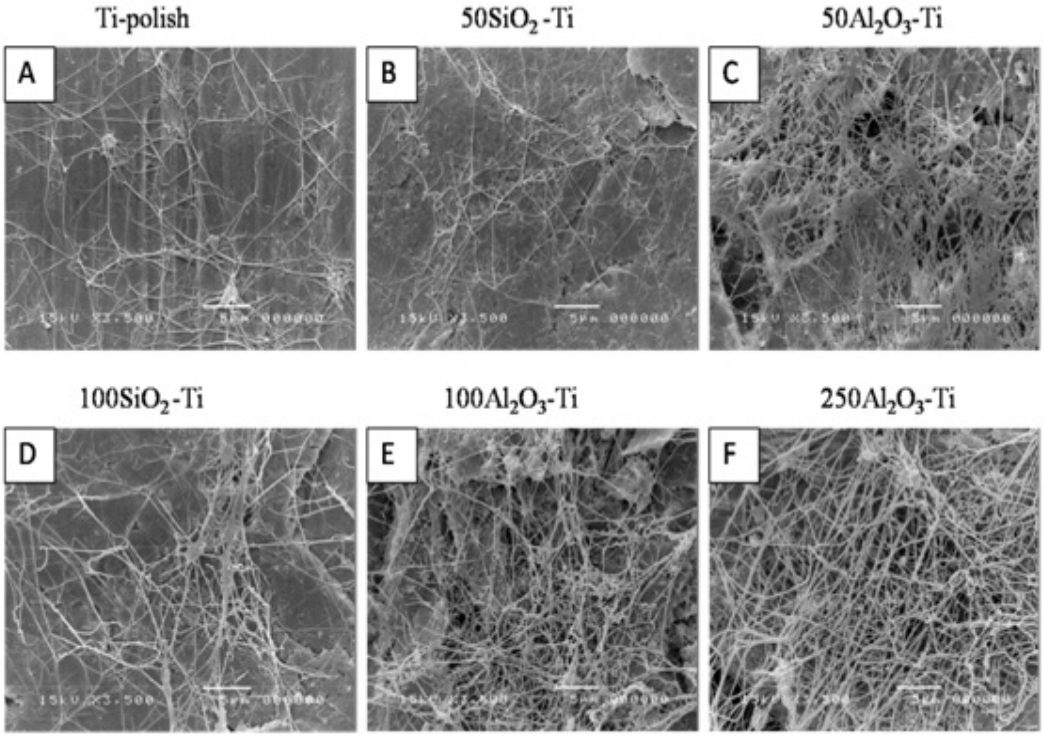


Figure 4.4 SEM, shows the fibrin clot formation at 5 min on different titanium surfaces: Ti-polish (A), $50SiO_2$-Ti (B), $50Al_2O_3$-Ti (C), $100SiO_2$ -Ti (D), $100Al_2O_3$-Ti (E), $250Al_2O_3$-Ti (F): 3500X magnification.

2. Cell morphology and cell attachment

Cell morphology and cell attachment were analyzed after seeding for 30min, 4 and 16 hours. The result was assessed by SEM and shown in Figure 4.5(A-R). After 30 min and 4 hours, cells on Ti-polish, $50SiO_2$ and $100SiO_2$ blasted surfaces appeared round and exhibit few cytoplasmic protrusions (Figure 4.5 A, B, D, G, H, J). Cells cultured on $50Al_2O_3$, $100Al_2O_3$ and $250Al_2O_3$ blasted surfaces were well spreaded and possessed long fine cytoplasmic extensions forming intercellular connections (Figure 4.5 C, E, F, I, K, L). At 16 hours, cells on all samples appeared flattened and started to form cell-cell contact (Figure 4.5 (M-R)).

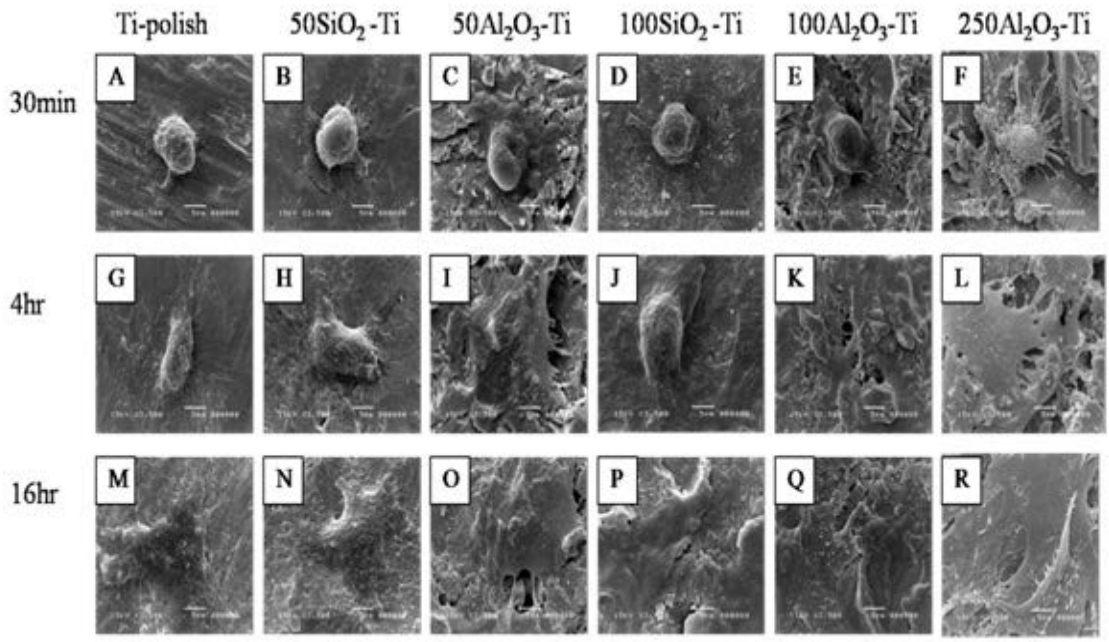


Figure 4.5 SEM, morphology and attachment of MC3T3-E1 cells on different titanium surfaces. At 30min (A-F) at 4 hours (G-L) and 16 hours (M-R): 3500X magnification.

3. Cell viability

The viability of cells was assessed using MTT assay, as presented in Figure 4.6. At 30 min, and 4 hours, the cell numbers and cell attachment appeared to be significant higher on Al_2O_3 blasted surfaces compared the polish surfaces and SiO_2 blasted surfaces. At 16 hours, the 250Al_2O_3 blasted surfaces shown higher cell number than the other surfaces and significant difference from the polish surface. However, no statistical difference in cell number was observed among the groups blasted by different Al_2O_3 particle size (50 μm, 100 μm and 250 μm).

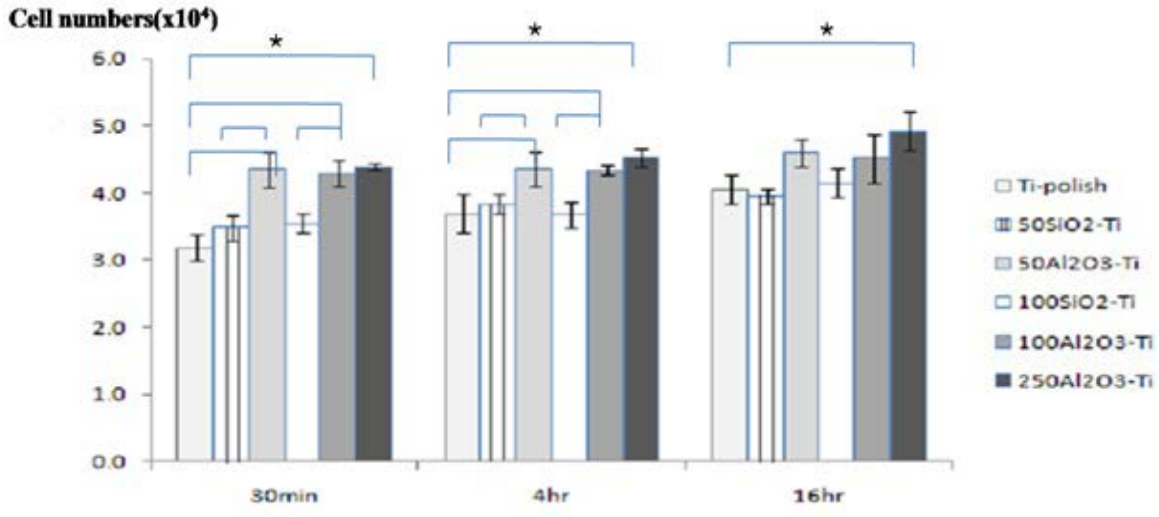


Figure 4.6 The cell numbers were estimated by the MTT assay after 30 min, 4 and 16 hours incubation. Data were shown as the mean ± SD. * Statistically significant (p<0.05) (n=3).

Cell proliferation, as determined by MTT assay, was shown in Figure 4.7. The proliferation rate of cells on the surfaces blasted with particle size 50 μm appeared to be similar to the rate found on the surfaces blasted with particle size 100 μm (Data of $100SiO_2$-Ti and $100Al_2O_3$-Ti were not shown in Figure 4.7). Cells grew on Al_2O_3 and SiO_2 blasted surfaces proliferated faster than cells on Ti-polish on the first two days after seeding. However, at day 3 no different in cell number was observed between cells seeded on Al_2O_3 and SiO_2 blasted surfaces.

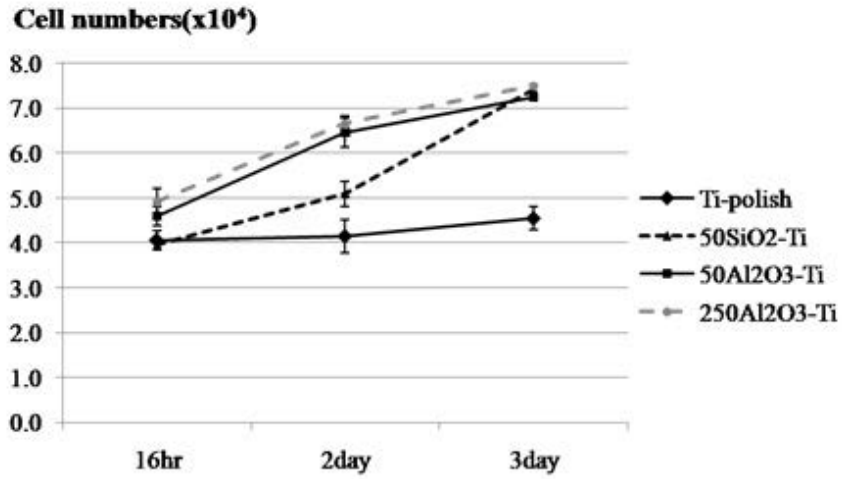


Figure 4.7 Shows cell proliferation on different titanium surfaces after 16 hr, 2 and 3 day incubation. Data were shown as the mean ± standard deviation.

4. Osteoblastic gene expression

The expression of Col I and OC was determined by RT-PCR at day-7 and day-14. After 7days of culture, the expression of both genes in the groups blasted with 50 μm or 100 μm particle size showed a similar pattern. The results indicated that cells cultured on Al_2O_3 blasted surfaces expressed higher level of Col I and OC compared to those expressed by cells on SiO_2 blasted surfaces (Figure 4.8). The density of bands was quantitated and normalized to GAPDH mRNA level (Figure 4.9).This result revealed that $250Al_2O_3$ blasted surfaces displayed higher gene expression than the other surfaces and significant different from the polish surface and the SiO_2 blasted surface.

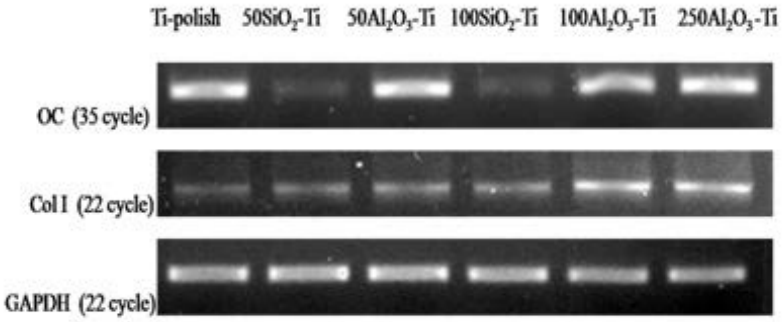


Figure 4.8 Show osteoblastic gene expression of the MC3T3-E1 cells on different surfaces quantitated by RT-PCR analysis at day 7.

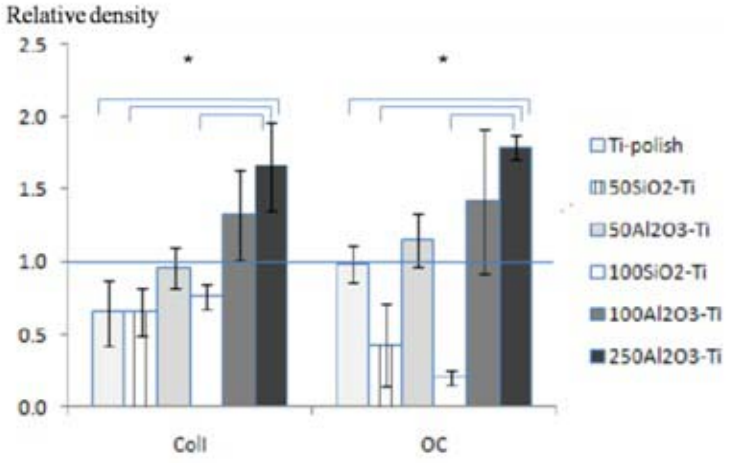


Figure 4.9 Graph shows the relative density of PCR products at day 7 which were quantitated and normalized to GAPDH expression. The expression level of tissue culture

plate was mark as 1 fold (line). Data were shown as the mean ± SD. * Statistically significant (p<0.05) (n=3).

The expression of OC in cell cultured on the Al_2O_3 blasted surfaces ($50Al_2O_3$-Ti, $100Al_2O_3$-Ti and $250Al_2O_3$-Ti) was examined after 14 days in culture (Figure 4.10). The results were shown in graph as the relative band intensity normalized to GAPDH. No significant difference was observed among the cells on Al_2O_3 blasted surfaces.

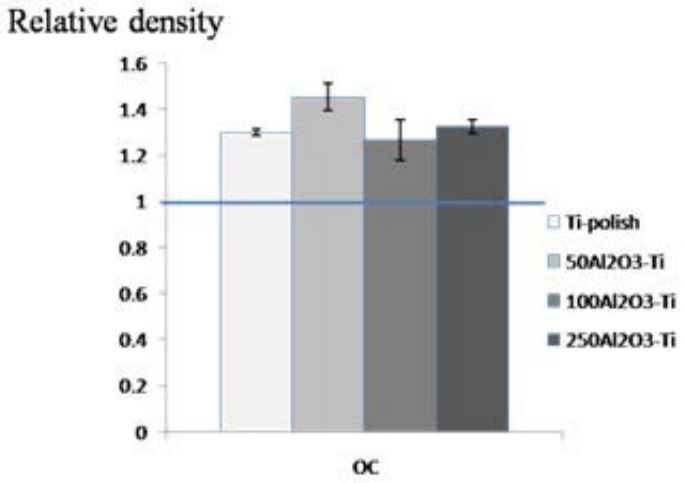


Figure 4.10 Graph shows the relative density of PCR products at day 14 which were quantitated and normalized to GAPDH expression. The expression level of tissue culture plate was mark as 1 fold (line). Data were shown as the mean ± SD.

5. *In vitro* mineralization

The mineralization of the cells on titanium surfaces was examined by Alizarin red-S staining after culturing for 14 days. The optical image of the stained surfaces was shown in Figure 4.11B. The amount of calcium deposition was quantified by eluting with 10% cetylpyridinium chloride monohydrate and the optical density was shown in Figure 4.11C.

The cells cultured on Al_2O_3 blasted surfaces showed a significant higher calcium deposition than the $100SiO_2$ blasted surfaces. Moreover, the amount of calcium deposition in cell culture on $100Al_2O_3$ blasted surfaces was slightly higher than other Al_2O_3 blasted surfaces, but no significant difference was observed among the groups. (Figure 4.11D).

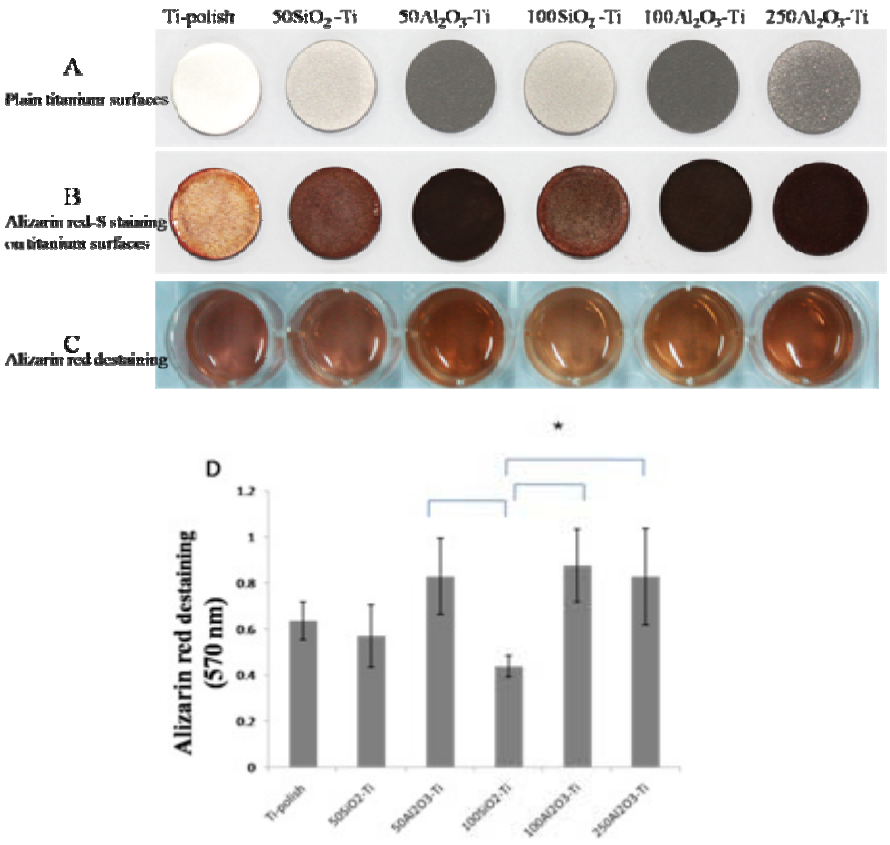


Figure 4.11 Images of plain titanium surfaces (A). The stained titanium surfaces (B). Alizarin red elution (C).The amount of *in vitro* calcium deposition at day14 was quantified by eluting 10% cetylpyridinium chloride monohydrate and measured the absorbance at 570 nm. (*Statistically significant, $p<0.05$) (D).

CHAPTER V

DISCUSSION AND CONCLUSION

DISCUSSION

Sandblasting is one of the preferable methods used to modulate the surface topography of dental implant in order to improve osseointegration. Generally sandblasting can generate the surface roughness in the level of micrometers. There were numerous in vitro and in vivo studies demonstrated the influence of the roughened surface on cell attachment, proliferation and differentiation[7, 11, 53, 59]. In this study, two types of abrasive materials (Al_2O_3 and glass beads) were used for sandblasting. For each type of materials, different particle sizes from 50,100 and 250 μm were used depending on the availability. Surface characteristics and the response of MC3T3-E1 in culture had been examined.

The results from this study demonstrated that Al_2O_3 blasted surface could support osteoblast adhesion, differentiation and mineralization better than glass bead (SiO_2) blasted surfaces. This study also provided the evidence that Al_2O_3 blasted surface has the ability to support fibrin formation, which implies the ability of Al_2O_3 blasted surface for protein adsorption.

For determining the effect of abrasive type on cell response, we used the same particles size (50,100 μm) of both Al_2O_3 and glass beads (SiO_2) to prepare Ti surface. The result showed that the comparable roughness value (*Ra* and *Sa)* on both blasted surface. This method was selected to minimize the influence of roughness values.

However, the pattern of roughness from both blasted surface was different. The difference was possible due to the shape and hardness of the particles used. Surface topography showed that blasting with Al_2O_3 generated many sharp peaks with deep valleys than that generated by SiO_2. This resulted from the shape of Al_2O_3particle, which was irregular in shape and sharp angle. It was appeared that the pattern generated by Al_2O_3 could support better adhesion of cells as evidence from SEM

results showing the faster attachment and better spreading. Moreover, the Al_2O_3 blasted surface showed higher *Sds* values than the SiO_2 blast surface. *Sds* is roughness parameter and is defined in the peak number per area (mm^2). The high Sds values of Al_2O_3 blasted surfaces are expressed as the frequency of peak and tend to present more micro-texture appearance. Our result supported the work of Wennerberg & Albrektsson, who suggested that the three-dimensional roughness parameters such as *Sdr* and *Sds* could provide a proper data of implant surface characterization on cell response[41].

In the present study, we demonstrated that fibrin formation could be formed on Al_2O_3 blasted surface better than SiO_2 blasted surfaces. Fibrin is generally formed after bleeding and the clot provide the suitable environment for cells adhesion leading to tissue healing[2, 24, 33]. Therefore, it is tempting to speculate that Al_2O_3 blast surface provide a better environment for the protein adsorption that is suitable for cell adhesion leading to a faster activity and healing process.

Our result is also in agreement with Mohammadi et al. In their study, they determined the effect of two grit materials (Al_2O_3 and SiO_2) on adhesion strength of plasma–sprayed hydroxyapatite coating. Their results indicated the Al_2O_3 blasted surfaces could support a better adhesion of HA than the SiO_2 blasted surfaces[13]. These data supported the surface topography affected the adhesion and adsorption property on the material.

The surface wettability is another parameter that affects the cell-surface interaction, which is directly related to the adhesion and adsorption processes[74, 75]. In this study, the contact angle of Al_2O_3 and SiO_2blasted groups were 54.62°-75.09° and 42.98°-49.25°, respectively. These results indicated the good hydrophilicity of both types of blasted surfaces. However, Al_2O_3 blasted surfaces gave a better cell attachment property. Although many studies indicated that hydrophilic surfaces could support cell attachment, cell spreading, and cytoskeletal organization[26, 39, 76], it is obvious that hydrophilicity alone is not the role factor to support the cell-implant interaction. In addition, effects of surface wettability on cellular response were still

controversy. There were studies, which found insignificant difference of cell attachment, area and shape between the hydrophilic and hydrophobic surfaces, when cultured the cells in the presence of 15 % serum[77, 78]. These finding supported the surface wettability may not play the important role on cellular response.

It is possible that parts of abrasive particles are remained on the blast surfaces, therefore, the response of cells observed might not due to the surface topography but due to the particles remained on the surface. In this study, we found the presence Al and Si on each of the grit-blasted surfaces. The influence of Al on the surfaces on osteoblast response is still controversy. It has been shown that Al ions may inhibit normal bone mineralization[52]. On the contrary, Piattelli et al. did not find significant differences in bone-implant contact for alumina blasted and decontaminated implants[79]. Our result showed the Al_2O_3 blasted surface could support osteoblast mineralization. However, it is difficult to correlate the cellular result with the chemical nature of the residual particles. Therefore, the effect of chemical ions on cellular response needs further investigation.

Our results showed the up-regulation of collagen type I (Col-I) and osteocalcin (OC) when cells were cultured on the blasted surfaces. Col-I is the early marker in bone formation and is synthesized by pre-osteoblast during the initial period of proliferation and matrix production[75]. In this study, cells on Al_2O_3 blasted surfaces showed the higher level of Col I than the SiO_2 blasted surfaces at day 7. This result indicated that Al_2O_3 blasted surfaces could promote the differentiation rate toward the matrix formation stage. Moreover, OC is considered as the late stage marker of osteoblast differentiation[80]. Therefore, the expression of OC at day 14 indicated that Al_2O_3 blasted surfaces possess the ability to support osteoblast differentiation. The osteoblast differentiation was supported by cells seeded on Al_2O_3 blasted surfaces showed faster rate of in vitro mineralization compared to the other surfaces.

Our result showed that using the different sizes of the grit-blasting particles could generate different surface roughness. However, in case of SiO_2, no significant was found between cell response and surface roughness. It is possible that

both *Sa* values of SiO_2 were identified as minimally rough surfaces (*Sa* 0.5-1μm), which was reported as insufficient roughness value for stronger bone response. Many commercial dental implants have minimal rough surfaces such as Brånemark (Noble Biocare, Sweden), and 3I Osseotite implants (Biomet, USA)[37]. However, the cellular response when cultured on surfaces prepared from different size of Al_2O_3 revealed that surface prepared from 250 μm Al_2O_3 blasted surfaces showed greater cell attachment, cell proliferation, higher Col-I and OC expression at day 7 compared to other surfaces. The *Sa* value of the 250 Al_2O_3 blasted surfaces was 1.5168 μm and was identified as moderately rough surfaces(*Sa* 1–2 μm)[37]. Moreover, we found the *Ra* value was 0.5882 μm and there were studies supported the range roughness for enhancing adhesion and differentiation properties of the implants[9-12, 66]. The example of implant manufacturers which have moderately roughened surfaces such as TiOblast™and OsseoSpeed™ surfaces (Astratech, Sweden), TiUnite (NobelBiocare, Sweden), SLA (Straumann, Switzerland) and Cellplus designs (Densply-Friadent, Germany)[37, 47]. Although the results from this study showed relation of the increase surface roughness on early cell response but we did not find the relation of the increase roughness on OC expression at day 14 and cell mineralization. Therefore, the increase surface roughness in this study may not directly correlate to osteoblast differentiation and study in animal model is needed to confirm.

In the present study, evidence suggests that the surface topography plays major role on cellular behaviors. However, it is possible that, during the blasting process, the surface modification process by itself could alter other surface properties such as surface topography, chemistry, roughness and wettability. Thus, it is difficult to identify the genuine effect of just one factor. It is interesting to further explore the methods of surface modification that will affect just one parameter. Among the techniques, for example, the Laser assisted direct imprint (LADI) is suggested to be able to determine the effect of just topography on cellular response. It is a technique for patterning nanostructure in solid substrates that does not require etching. A single or multiple laser pulses melt a thin surface layer of substrate material and a mold is

embossed into the resulting liquid layer[81, 82]. The use of such technique might be required to provide more precise results of surface properties on the cellular responses.

CONCLUSION

The Al_2O_3 blasted surfaces provided the suitable environment for cell spreading, attachment, proliferation and osteoblastic gene expression than the SiO_2 blasted surfaces. Our result demonstrated the surface roughness affected early cell response, but not cell expression in late stage and mineralization. These findings indicated the importance of materials to be used in sandblast process of dental implant and should be concerned as an information for implant selection in clinic.

REFERENCES

[1] Linez-Bataillon, P., Monchau, F., Bigerelle, M. and Hildebrand, H. In vitro MC3T3 osteoblast adhesion with respect to surface roughness of Ti6Al4V substrates. Biomol Eng 19 (2002) : 133-141.

[2] Ramazanoglu, M. and Oshida, Y. Osseointegration and Bioscience of Implant Surfaces - Current Concepts at Bone-Implant Interface. In Turkyilmaz, I. (ed.), Implant Dentistry - A Rapidly Evolving Practice, pp.57-58. InTech, 2011.

[3] Francois, P., Vaudaux, P., Taborelli, M., Lew, D. and Descouts, P. Influence of surface treatments of developed for oral implants on the physical and biological properties of titanium(II) Adsorption isotherms and biological activity of immobilized fibronectin. Clin Oral Impl Res 8 (1997) : 217-225.

[4] Bagno, A. and Bello, C. Surface treatments and roughness properties of Ti-based biomaterials. Journal of Material Science: materials in medicine 15 (2004) : 935-949.

[5] Liu, Y., de Groot, K. and Hunziker, E.B. Osteoinductive Implants: The Mise-en-scene for Drug-bearing Biomimetic Coatings. Annals of Biomedical Engineering 32 (2004) : 398-406.

[6] Juodzbalys, G., Sapragoniene, M., Wennerberg, A. and Baltrukonis, T. Titanium dental implant surface micromorphology optimization. Journal of Oral Implantology 33 (2007) : 177-185.

[7] Choi, C.R. et al. Bone cell responses of titanium blasted with bioactive glass particles. J Biomater Appl 25 (Aug 2010) : 99-117.

[8] Boyan, B.D., Dean, D.D., Lohmann, C.H., Cochran, D.L., Sylvia, V.L. and Schwartz, Z. The titanium bone cell interface in vitro: the role of the surface in promoting osteointegration. In Brunette, D.M., Tengvall, P., Textor, M. and Thomson, P. (ed.), Titanium in medicine, pp.561-586. New York : Springer, 2001.

[9] Thomas, K., Kay, J., Cook, S. and Jarcho, M. The effect of surface macrotexture and hydroxylaptite coating on the mechanical strengths and histologic profiles of titanium implant materials. J biomed Mater Res 21 (1987) : 1395-1414.

[10] Martin, J.Y. et al. Effect of titanium surface roughness on proliferation, differentiation, and protein synthesis of human osteoblast-like cells (MG63). J Biomed Mater Res 29 (Mar 1995) : 389-401.

[11] Deligianni, D.D., Katsala, N., Ladas, S., Sotiropoulou, D., Amedee, J. and Missirlis, Y.F. Effect of surface roughness of the titanium alloy Ti-6Al-4V on human bone marrow cell response and on protein adsorption. Biomaterials 22 (Jun 2001) : 1241-1251.

[12] Citeau, A. et al. In vitro biological effects of titanium rough surface obtained by calcium phosphate grid blasting. Biomaterials 26 (Jan 2005) : 157-165.

[13] Mohammadi, Z., Ziaei-Moayyed, A.A. and Sheikh-Mehdi Mesgar, A. Grit blasting of Ti-6Al-4V alloy: Optimization and its effect on adhesion strength of plasma-sprayed hydroxyapatite coatings. Journal of Materials Processing Technology 194 (2007) : 15-23.

[14] Szmukler-Moncler, S., Zeggel, P., Perrin, D., Bernard, J.P. and Neumann, H.G. From microroughness to resorbable bioactive coatings. In Ellingsen, J. and Lyngstadaas, P.S. (ed.), Bio-implant interface improving biomaterials and tissue reactions, pp.73-100. CRC Press LLC, 2003.

[15] Schuh, A., Holzwarth, U., Kachler, W., Goske, J. and Zeiler, G. [Surface characterisation of shot peened implants with glas beads in total hip arthroplasty]. Zentralbl Chir 129 (Jun 2004) : 225-229.

[16] Available from : http://www.shofu.com.sg/MSDS%20PDF/GlassBeads.pdf. [2010, May 18]

[17] Hossain, M.M. and Gao, W. How is the Surface Treatments Influence on the Roughness of Biocompatibility?Trends Biomaterial. Artif. Organ 22 (2008) : 144-157.

[18] Zhu, X., Chen, J., Scheideler, L., Reichl, R. and Geis-Gerstorfer, J. Effects of t opography and composition of titanium surface oxides on osteoblast responses. Biomaterials 25 (Aug 2004) : 4087-4103.

[19] Elias, C.N., Lima, J.H.C., Valiev, R. and Meyers, M.A. Biomedical Applications of Titanium and its Alloys. JOM Journal of the Minerals, Metals and Materials Society 60 (2008) : 46-49.

[20] McCracken, M. Dental Implant Materials: Commercially Pure Tita&um and Titanium Alloys. J Prosthodont 8 (1999) : 40-43.

[21] Branemark, P.I., Adell, R., Albrektsson, T., Lekholm, U., Lundkvist, S. and Rockler, B. Osseointegrated titanium fixtures in the treatment of edentulousness. Biomaterials 4 (Jan 1983) : 25-28.

[22] Marco, F., Milena, F., Gianluca, G. and Vittoria, O. Peri-implant osteogenesis in health and osteoporosis. Micron 36 (2005) : 630-44.

[23] Nygren, H. and Tengvall, P. The initial reactions of TiO2 with blood J Biomed Mater Res 34 (1997) : 487-492.

[24] Iorio, D., Traini, T., Degidi, M., Caputi, S., Neugebauer, J. and Piattelli, A. Quantitative evaluation of the fibrin clot extension on different implant surfaces: An *in vitro* study. Journal of Biomedical Materials Research Part B: Applied Biomaterials 74B (2005) : 636-642.

[25] Neugebauer, J., Khoury, F. and Zoller, J.E. Influence of the implant surface on the success rate for implants in grafted bone. In Khoury, F. and Antoun, H.M., P (ed.), Bone Augmentation in Oral Implantology, pp.67-75. Quintessence, 2006.

[26] Anil, S., Anand, P.S., Alghamdi, H. and Jansen, J.A. Dental Implant Surface Enhancement and Osseointegration. In Turkyilmaz, I. (ed.), Implant Dentistry - A Rapidly Evolving Practice, pp.83-108. InTech, 2011.

[27] Albrektsson, T., Branemark, P.I., Hansson, H.A. and Lindstrom, J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. Acta Orthop Scand 52 (1981) : 155-170.

[28] Soskolne, W.A., Cohen, S., Sennerby, L., Wennerberg, A. and Shapira, L. The effect of titanium surface roughness on the adhesion of monocytes and their secretion of TNF-alpha and PGE2. Clinical Oral Implants Research 13 (2002).

[29] Pilliar, R.M. Implant surface design for development and maintenance of osseointegration. In Ellingsen, J. and Lyngstadaas, P.S. (ed.), Bio-implant interface improving biomaterials and tissue reactions, pp.43-58. New York : CRC Press LLC, 2003.

[30] Lazzara, R.J., Testori, T., Trisi, P., Porter, S.S. and Weinstein, R.L. A human histologic analysis of osseotite and machined surfaces using implants with 2 opposing surfaces. Int J Periodontics Restorative Dent 19 (Apr 1999) : 117-129.

[31] Trisi, P., Rao, W. and Rebaudi, A. A histometric comparison of smooth and rough titanium implants in human low-density jawbone. Int J Oral Maxillofac Implants 14 (1999) : 689-698.

[32] Ivanoff, C.J., Hallgren, C., Widmark, G., Sennerby, L. and Wennerberg, A. Histologic evaluation of the bone integration of TiO(2) blasted and turned titanium microimplants in humans. Clin Oral Implants Res 12 (Apr 2001): 128-134.

[33] Cooper, L.F. Cellular interaction at commercially pure titanium. In Ellingsen, J. and Lyngstadaas, P.S. (ed.), Bio-implant interface improving biomaterials and tissue reactions, pp.165-181. New York : CRC Press LLC, 2003.

[34] Brunette, D.M. Spreading and orientation of epithelial cells on grooved substrata. Exp Cell Res 167 (Nov 1986) : 203-217.

[35] Park, J.Y. and Davies, J.E. Red blood cell and platelet interactions with titanium implant surfaces. Clin Oral Implants Res 11 (Dec 2000) : 530-539.

[36] Perizzolo, D., Lacefield, W.R. and Brunette, D.M. Interaction between topography and coating in the formation of bone nodules in culture for hydroxyapatite- and titanium-coated micromachined surfaces. J Biomed Mater Res 56 (Sep 15 2001) : 494-503.

[37] Albrektsson, T. and Wennerberg, A. Oral implant surfaces: Part 1--review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. Int J Prosthodont 17 (Sep-Oct 2004) : 536-543.

[38] Buser, D. et al. Enhanced bone apposition to a chemically modified SLA titanium surface. Journal of dental research 83 (2004) : 529-533.

[39] Webb, K., Hlady, V. and Tresco, P.A. Relative importance of surface wettability and charged functional groups on NIH 3T3 fibroblast attachment, spreading, and cytoskeletal organization. J Biomed Mater Res 41 (1998) : 422-430.

[40] Ponsonnet, L. et al. Relationship between surface properties (roughness, wettability) of titanium and titanium alloys and cell behaviour. Materials Science and Engineering 23 (2003) : 551-560.

[41] Wennerberg, A. and Albrektsson, T. Suggested guidelines for the topographic evaluation of implant surfaces. Int J Oral Maxillofac Implants 15 (May-Jun 2000) : 331-344.

[42] Wennerberg, A. and Albrektsson, T. Bone response to surface roughness: measurements and results from experimental and clinical studies. In Ellingsen, J. and Lyngstadaas, P.S. (ed.), Bio-implant interface improving biomaterials and tissue reactions, pp.59-72. New York : CRC Press LLC, 2003.

[43] Wennerberg, A. and Albrektsson, T. Effect of titanium surface topography on bone integration: a systematic review. Clin oral impl res 20 (2009) : 172-184.

[44] Le Guehennec, L., Soueidan, A., Layrolle, P. and Amouriq, Y. Surface treatments of titanium dental implants for rapid osseointegration. Dent Mater 23 (2007) : 844-854.

[45] Shalabi, M.M., Gortemaker, A., Van't Hof, M.A., Jansen, J.A. and Creugers, N.H. I mplant surface roughness and bone healing: a systematic review. J Dent Res 85 (Jun 2006) : 496-500.

[46] Brett, P.M. et al. Roughness response genes in osteoblasts. Bone 35 (Jul 2004) : 124-133.

[47] Albrektsson, T. and Wennerberg, A. Oral implant surfaces: Part 2--review focusing on clinical knowledge of different surfaces. Int J Prosthodont 17 (Sep-Oct 2004) : 544-564.

[48] Nanci, A., Wuest, J., Peru, L., Brunet, P., Sharma, V. and Zalzal, S. Chemical modification of titanium surfaces for covalent attachment of biological molecules. J Biomed Mater Res 40 (1998) : 324-335.

[49] Galli, C. et al. Comparison of human mandibular osteoblasts grown on two commercially available titanium implant surfaces. J Periodontol 76 (Mar 2005) : 364-372.

[50] Bowers, K.T., Keller, J.C., Randolph, B.A., Wick, D.G. and Michaels, C.M. Optimization of surface micromorphology for enhanced osteoblast responses in vitro. Int J Oral Maxillofac Implants 7 (Fall 1992) : 302-10.

[51] Wennerberg, A., Albrektsson, T. and Andersson, B. Bone tissue response to c ommercially pure titanium implants blasted with fine and coarse particles of aluminum oxide. Int J Oral Maxillofac Implants 11 (Jan-Feb 1996) : 38-45.

[52] Esposito, M., Hirsch, J., Lekholm, U. and Thomsen, P. Biological factos contributing to failures of osseointegrated oral implants(II). Etiopathogenesis. Eur J Oral Sci 106 (1998) : 721-764.

[53] Mustafa, K., Saliva Lopez, B., Hultenby, K., Wennerberg, A. and Arvidson, K. Determining optimal surface roughness of TiO_2 blasted titanium implant material foe attachment, proliferation and differentiation of cells derived from human mandibular alveolar bone. Clin Oral Impl Res 12 (2001).

[54] Available from : http://www.shofu.com/msds(new).html. [2010, May 18]

[55] Froum, S., Tarnow, D., Jalbout, Z., Brun, J. and Fromental, R. Histological Evaluation of the Serf EVL Evolution Implant: A Pilot Study in a Dog Model. Implant Dentistry 12 (2003): 69-74.

[56] Available from : http://www.dentistrytoday.com/top25implant-d/5557-dentsply-tulsa-dental-specialties. [2012, May 10]

[57] Available from :http://osseosource.com/dentalimplants/product_info.php?products_id=903. [2012, May 10]

[58] Mueller, W.D. et al. Evaluation of the interface between bone and titanium surfaces being blasted by aluminium oxide or bioceramic particles. Clin Oral Implants Res 14 (Jun 2003) : 349-356.

[59] Wennerberg, A., Albrektsson, T., Johansson, C. and Andersson, B. Experimental study of turned and grit-blasted screw-shaped implants with special emphasis on effects of blasting material and surface topography. Biomaterials 17 (1996): 15-22.

[60] Johansson, C., Albrektsson, T., Thomsen, P., Sennerby, L., Lodding, A. and Odelius, H. Tissue reactions to titanium-6aluminium-4vanadium alloy. Eur J Exp Musculoskel Res 1 (1992) : 161-169.

[61] Wong, M., Eulenberger, J., Schenk, R. and Hunziker, E. Effect of surface topology on the osseointegration of implant materials in trabecular bone. J Biomed Mater Res 29 (Dec 1995) : 1567-1575.

[62] Wennerberg, A., Albrektsson, T. and Lausmaa, J. Torque and histomorphometric evaluation of c.p. titanium screws blasted with 25- and 75-microns-sized particles of Al2O3. J Biomed Mater Res 30 (Feb 1996) : 251-260.

[63] Wennerberg, A., Hallgren, C., Johansson, C. and Danelli, S. A histomorphometric evaluation of screw-shaped implants each prepared with two surface roughnesses. Clin Oral Implants Res 9 (1998) : 11-19.

[64] Voros, J., Wieland, M., Ruiz-Taylor, L., Textor, M. and Brunette, D.M. Characterization of the titanium surfaces. In.Brunette, D.M., Tengvall, P., Textor, M. and Thomsen, P. (ed.), Titanium in medicine, pp.87-144. New York : Springer, 2001.

[65] Rosales-Leal, J.I., Mazzaglia, G., Ramon-Torregrosa, P.J. and al, e. Effect of roughness, wettability and morphology of engineered titanium surfaces on osteoblast-like cell adhesion. Colloids and Surfaces A: Physicochem. Eng. Aspects 365 (2010) : 222-229.

[66] Predecki, P., Stephan, J., Auslaender, B. and Mooney VL, K.K. Kinetics of bone growth into cylindrical channels in aluminum oxide and titanium. J Biomed Mater Res 6 (1972) : 375-400.

[67] Baleani, M., Viceconti, M. and Toni, A. The effect of sandblasting treatment on endurance properties of titanium alloy hip prostheses. Artif Organs 24 (Apr 2000) : 296-299.

[68] Buser, D. et al. Interface shear strength of titanium implants with a sandblasted and acid-etched surface: a biomechanical study in the maxilla of miniature pigs. J Biomed Mater Res 45 (May 1999) : 75-83.

[69] Hacking, S.A., Bobyn, J.D., Tanzer, M. and Krygier, J.J. The osseous response to corundum blasted implant surfaces in a canine hip model. Clin Orthop Relat Res (Jul 1999) : 240-253.

[70] Ishikawa, K., Miyamoto, Y., Nagayama, M. and Asaoka, K. Blast coating method: new method of coating titanium surface with hydroxyapatite at room temperature. J Biomed Mater Res 38 (1997) : 129-134.

[71] Förch, R., Schönherr, H. and A. Jenkins, A.T. Surface design: applications in bioscience and nanotechnology. Wiley-VCH, 2009.

[72] Bathomarco RV, Solorzano G, Elias CN and Prioli R. Atomic force microscopy a nalysis of different surface treatments of Ti dental implant surface. Appl Surf Sci 233 (2004) : 988-994.

[73] Pegueroles, M., Gil, F.J., Planell, J.A. and Aparicio, C. The influence of blasting and sterilization on static and time-related wettability and surface-energy properties of titanium surfaces. Surface & Coatings Technology 202 (2008) : 3470-3479.

[74] Lim, J., Shaughnessy, M., Zhou, Z., Noh, H., Vogler, E. and Donahue, H. Surface energy effects on osteoblast spatial growth and mineralization. Biomaterials 29 (2008) : 1776-1784.

[75] Klein, M., Bijelic, A., Toyoshima, T., Götz, H., Duschne, H. and et al. Long-term response of osteogenic cells on micron and submicron-scale-structured hydrophilic titanium surfaces: sequence of cell proliferation and cell d

ifferentiationClinical Implant Dentistry and Related Research 21 (2010) : 642-649.

[76] Ruardy, G., Schakenraad, J.M., Van der Mei, H.C. and Busscher, H.J. Adhesion and spreading of human skin fibroblasts on physocochemically characterized gradient surfaces. J Biomed Mater Res 29 (1995) : 1415-1423.

[77] Schakenraad, J.M., Busscher, H.J., Wildevuur, C.R. and Arends, J. The influence of substratum surface free energy on growth and spreading of human fibroblasts in the presence and absence of serum proteins. J Biomed Mater Res 20 (Jul-Aug 1986) : 773-784.

[78] Vanoni, C. Production and Physiochemical Characterization of Self–Assembled Monolayers on Titanium Surfacesand their Influence on Fibroblast Behavior, Faculty of Dentistry. Laboratory for Surface Science and Technology, ETH Zurich, 2000.

[79] Piattelli, A., Degidi, M., Paolantonio, M., Mangano, C. and Scarano, A. Residual aluminum oxide on the surface of titanium implants has no effect on osseointegration. Biomaterials 24 (Oct 2003) : 4081-4089.

[80] Enomoto, H. et al. Cbfa1 is a positive regulatory factor in chondrocyte maturation J Biol Chem 275 (2000) : 8695-8702.

[81] Chou, S.Y., Keimel, C. and Gu, J. Ultrafast and direct imprint of nanostructures in silicon. Nature 417 (2002) : 835-837.

[82] Cui, B. Ultrafast Fabrication of Metal Nanostructures Using Pulsed Laser Melting. In Cui, B. (ed.), Recent Advances in Nanofabrication Techniques and Applications, pp.113-138. Intech, 2011.

APPENDICES

APPENDIX A

EDS Spectrum of blasted surface

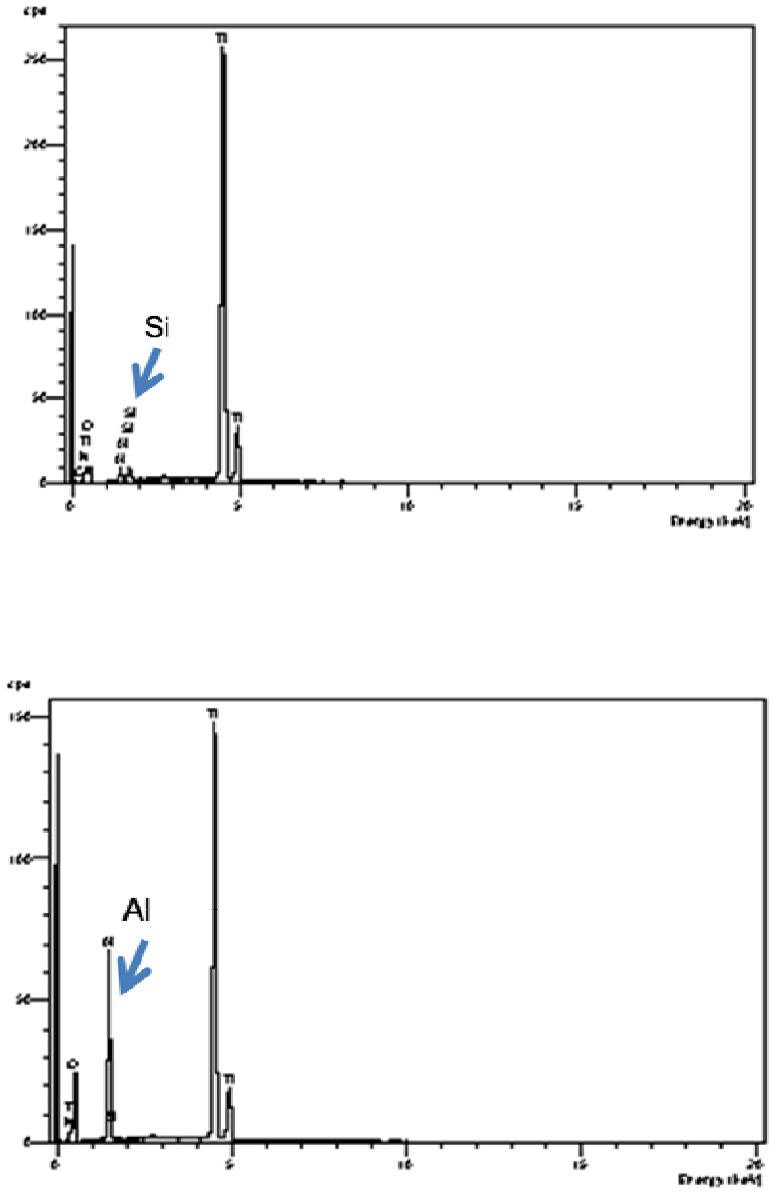


Figure A1 EDS spectrums of SiO_2 and Al_2O_3 blasted surface

SEM

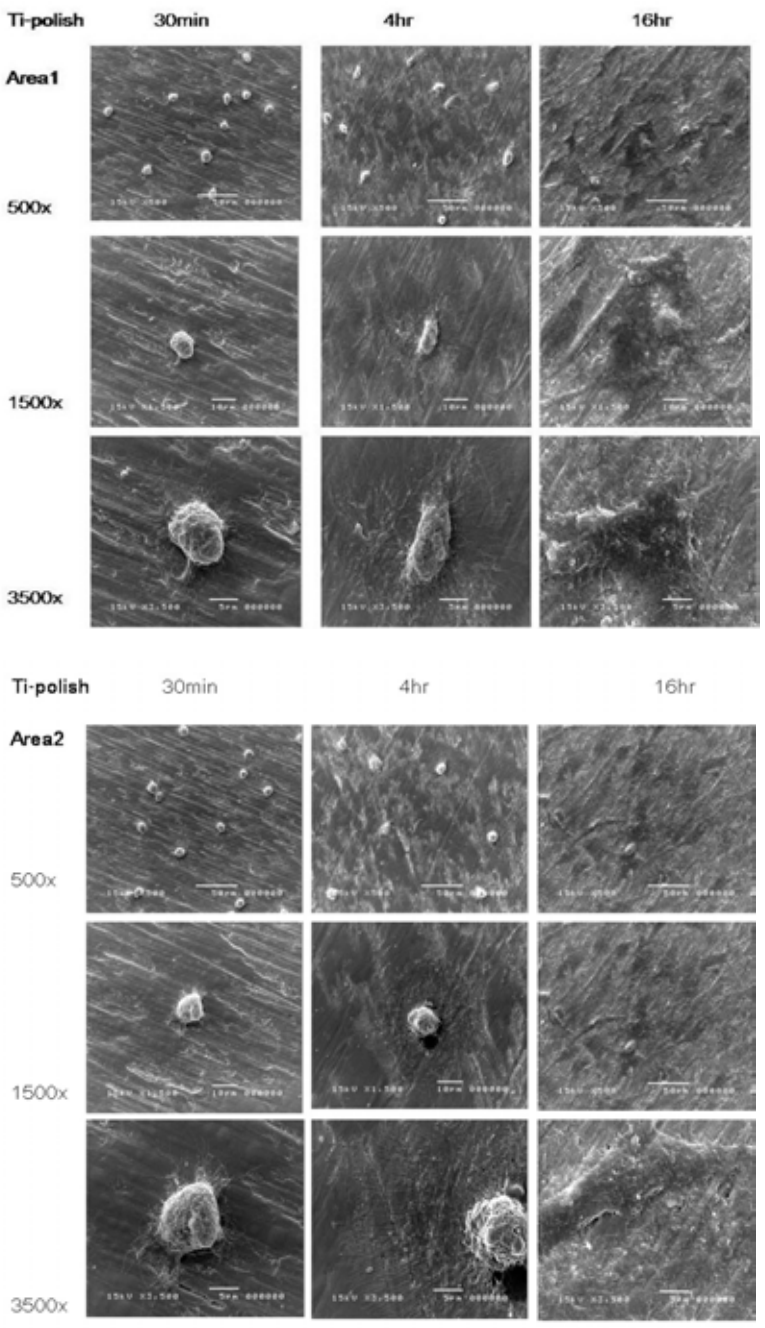


Figure A2 SEM, morphology and attachment of MC3T3-E1 cells on polished titanium

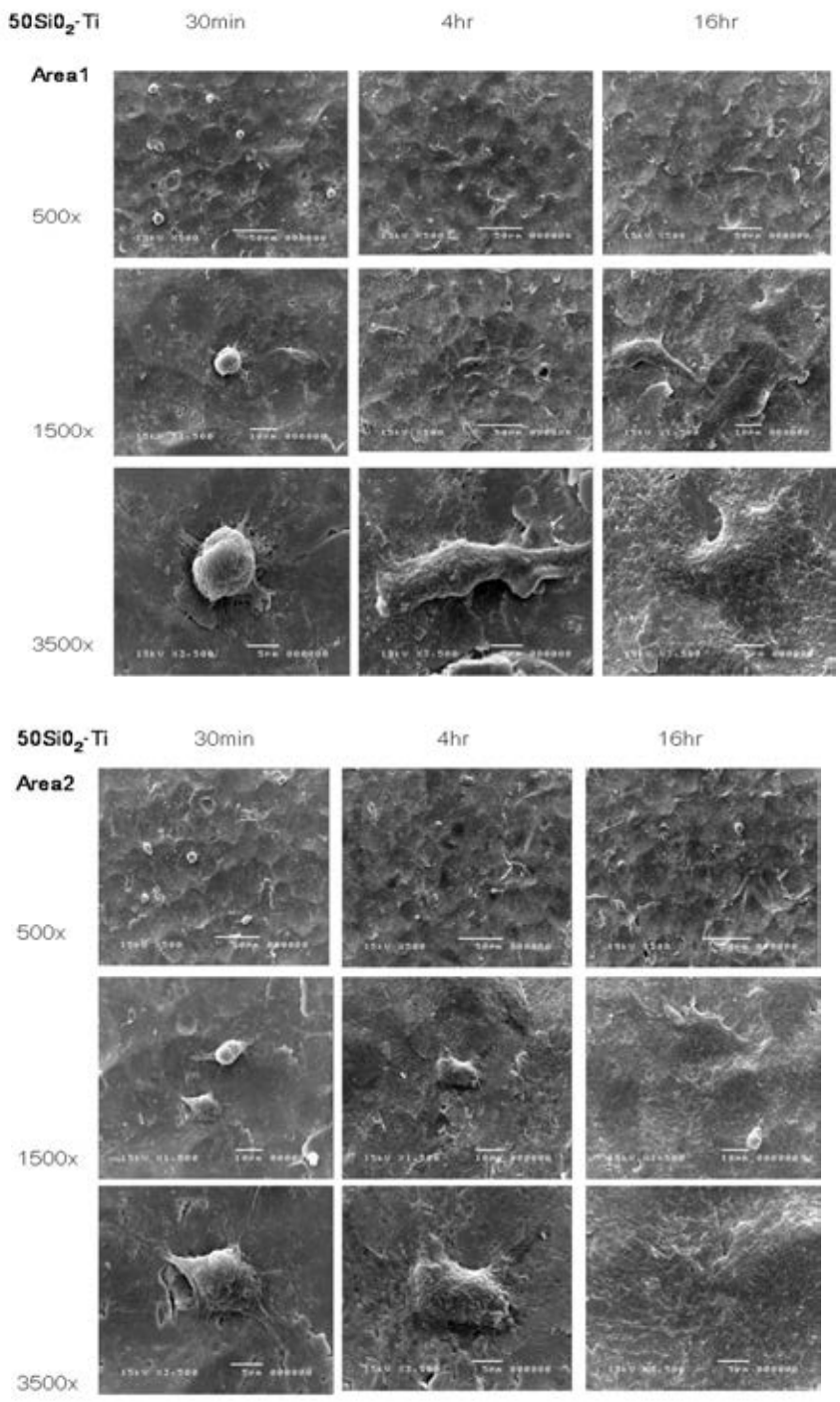


Figure A3 SEM, morphology and attachment of MC3T3-E1 cells on $50SiO_2$ blasted surface

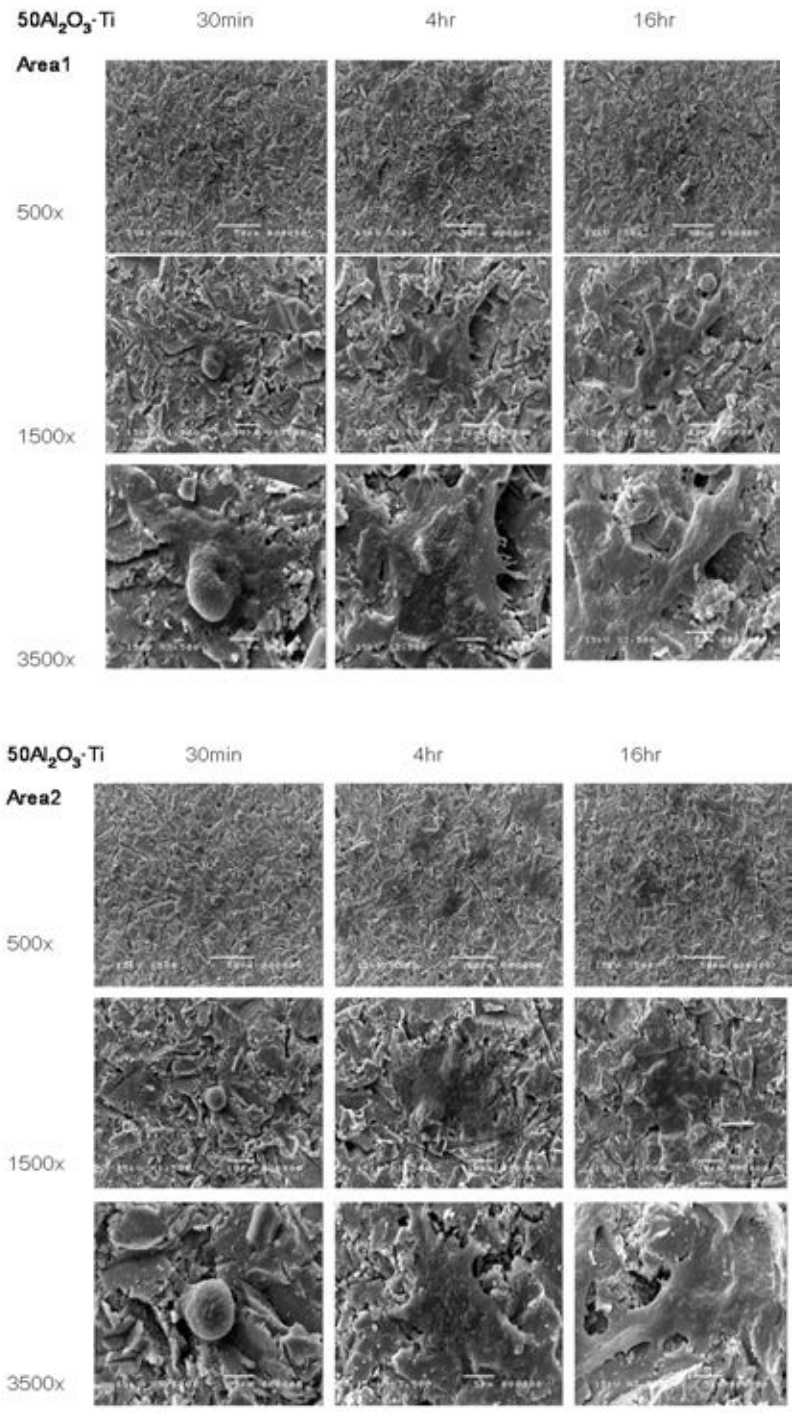


Figure A4 SEM, morphology and attachment of MC3T3-E1 cells on $50Al_2O_3$ blasted surface

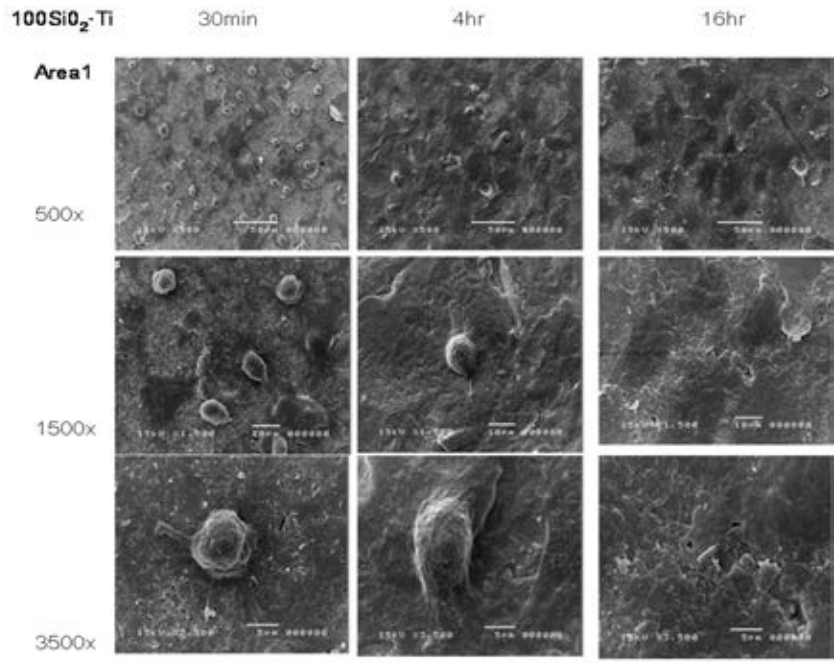


Figure A5 SEM, morphology and attachment of MC3T3-E1 cells on 100SiO_2 blasted surface

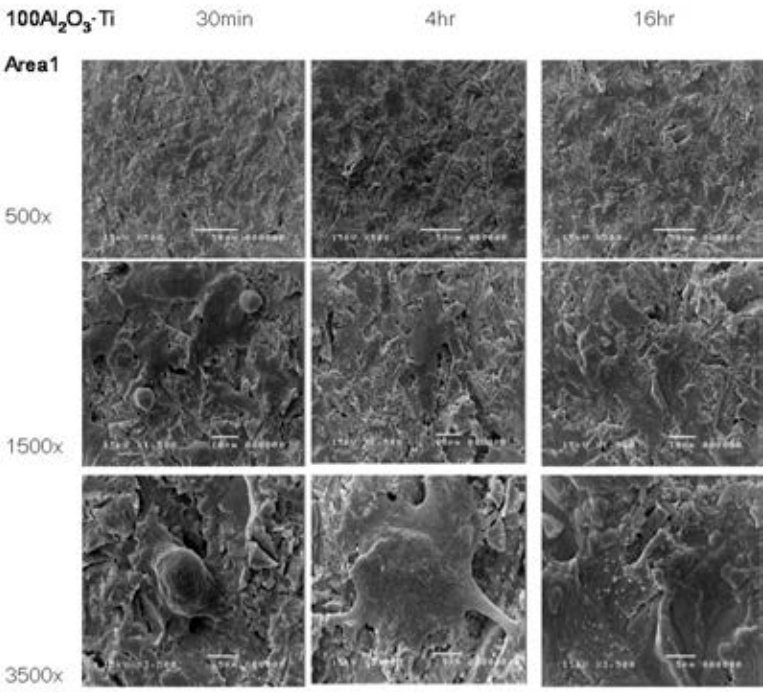


Figure A6 SEM, morphology and attachment of MC3T3-E1 cells on $100Al_2O_3$ blasted surface

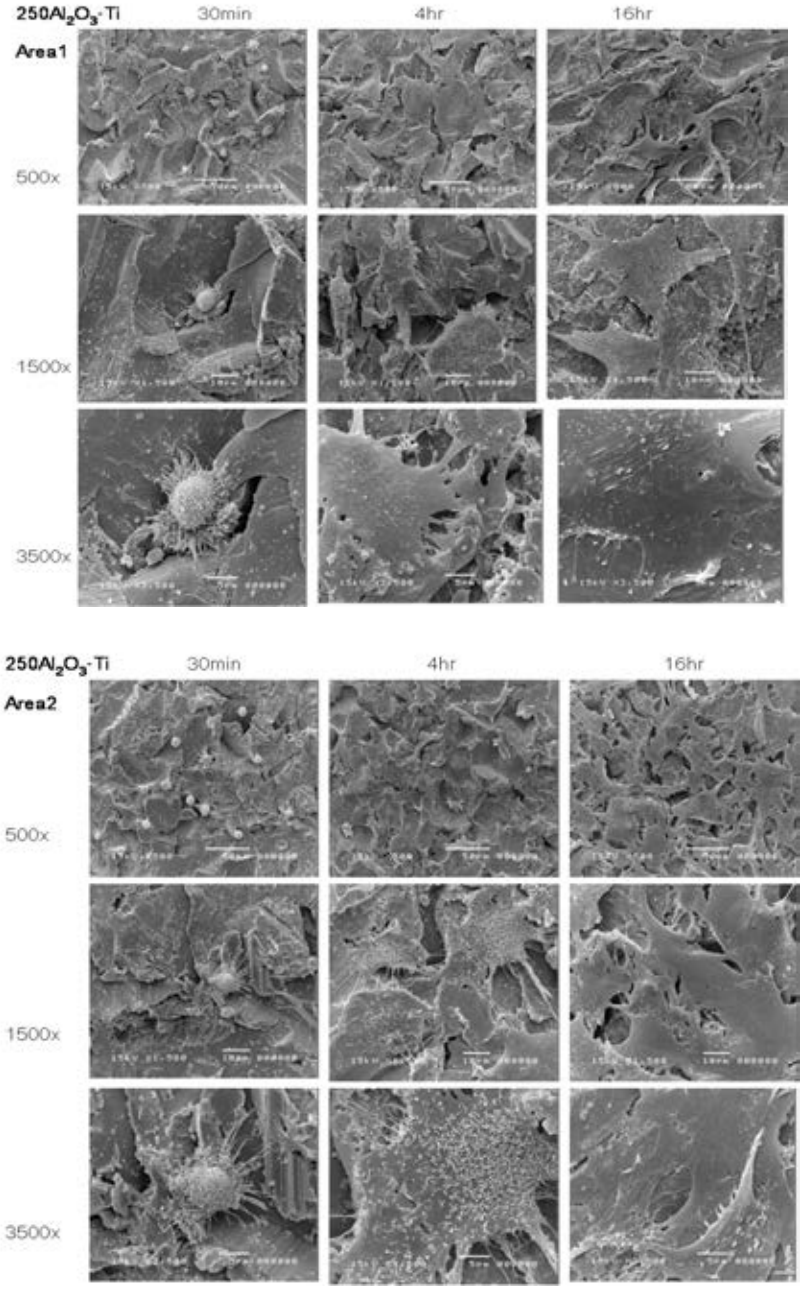


Figure A7 SEM, morphology and attachment of MC3T3-E1 cells on $250Al_2O_3$ blasted surface

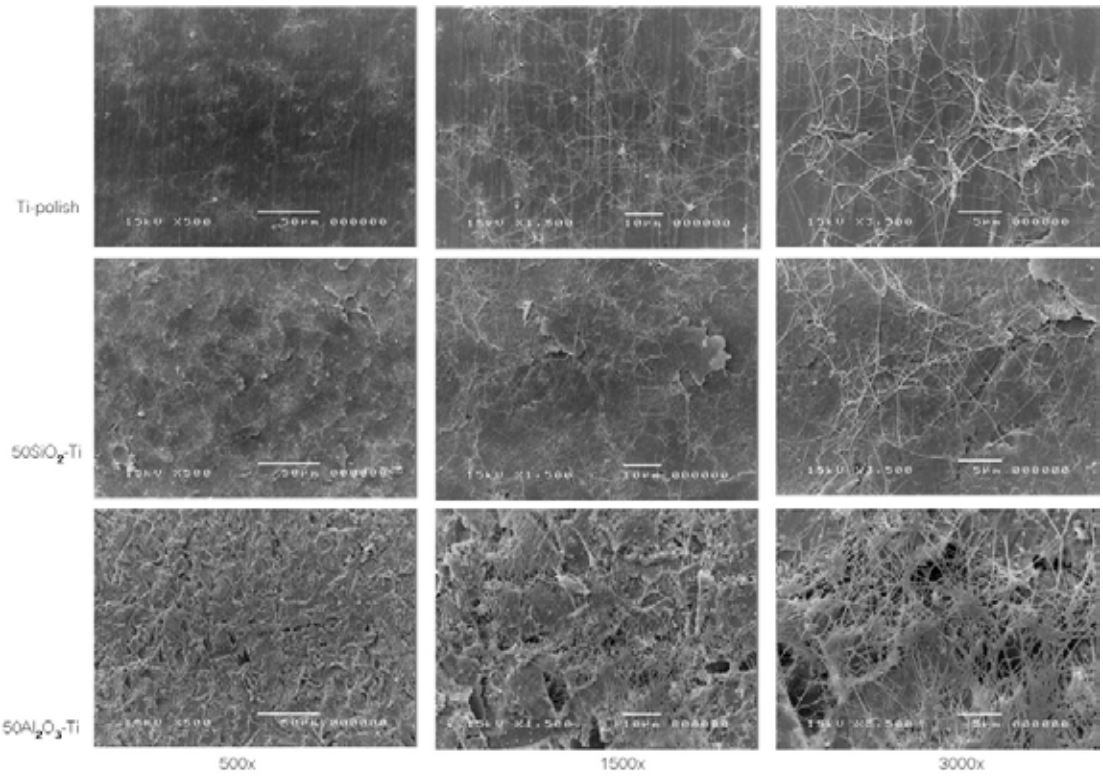


Figure A8 SEM, morphology of fibrin formation on different titanium surfaces

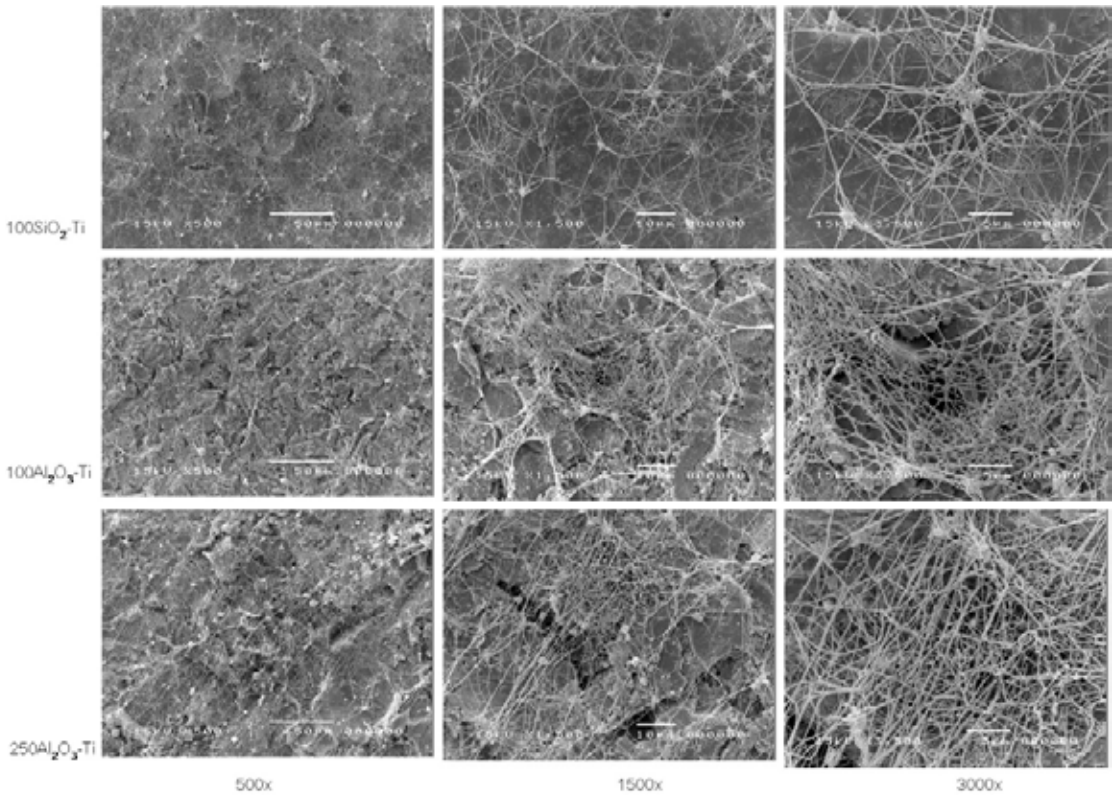


Figure A9 SEM, morphology of fibrin formation on different titanium surfaces

APPENDIC B: Data and statistical analysis of roughness parameters

type	sample	Sa	Ra(1)	St	Sds	Rt	RSm
Ti-polish	1	0.1261	0.06	2.639	1716	0.4143	0.03886
	2	0.1335	0.06009	1.711	1526	0.5018	0.03879
	3	0.1168	0.05239	1.1695	1411	0.4746	0.0394
	4	0.1422	0.0674	1.894	1588	0.4858	0.03881
	5	0.168	0.08164	1.687	1319	0.5264	0.03959
	6	0.1206	0.04439	0.9084	1533	0.2743	0.04243
	7	0.1262	0.06231	1.238	1584	0.5097	0.03589
	8	0.1345	0.0723	1.345	1672	0.5321	0.03698
	9	0.1254	0.0632	2.061	1753	0.4698	0.03888
	10	0.1477	0.0666	1.732	1532	0.4212	0.03913
	mean	0.1341	0.063032	1.63849	1563.4	0.461	0.038876
	SD	0.015217	0.010237	0.501186	132.6333	0.076643	0.0017
50SiO2	1	0.5393	0.2242	6.8684	920.86	1.6682	0.045504
	2	0.5353	0.2106	6.4958	926.76	1.6498	0.042208
	3	0.5587	0.2206	6.9612	895.56	1.5876	0.044258
	4	0.5093	0.2182	7.3992	951.68	1.4274	0.040644
	5	0.5524	0.2392	7.2462	932.34	1.7732	0.043628
	6	0.5222	0.2213	6.5914	1003.86	1.6656	0.041812
	7	0.5495	0.2371	6.8246	939.28	1.6968	0.04343
	8	0.5185	0.2375	7.5062	940.48	1.6798	0.044326
	9	0.5575	0.2389	7.1374	920.76	1.6456	0.043098
	10	0.4978	0.2176	5.859	960.22	1.5256	0.044526
	mean	0.5340	0.2265	6.8889	939.1800	1.6320	0.0433
	SD	0.0213	0.0106	0.4881	28.9881	0.0969	0.0015
50Al2O3	1	0.5133	0.2115	8.3352	1209.8	1.8168	0.039244
	2	0.5443	0.2344	13.566	1244.6	2.2126	0.040628
	3	0.5218	0.2327	10.8778	1215.8	1.8528	0.039608
	4	0.5229	0.2333	13.23	1216.8	1.8954	0.038946
	5	0.5221	0.2223	10.304	1199	1.9946	0.03893
	6	0.5504	0.2452	11.664	1246.2	2.0918	0.038818
	7	0.5544	0.2506	12.289	1188.8	2.1372	0.041558
	8	0.5329	0.2407	10.2514	1225.8	1.955	0.040434
	9	0.5149	0.2327	10.3832	1220.4	2.434	0.039358
	10	0.5109	0.2359	9.265	1220	2.2652	0.032812
	mean	0.5288	0.2339	11.0166	1218.720	2.0655	0.0390
	SD	0.0159	0.0110	1.6736	17.8119	0.1990	0.0024

Data and statistical analysis of roughness parameters

type	sample	Sa	Ra(1)	St	Sds	Rt	RSm
100SiO2	1	0.60702	0.23356	10.3634	808.4	1.7862	0.047656
	2	0.60326	0.2413	10.4786	781.22	2.2612	0.047054
	3	0.6787	0.277	12.162	738.68	2.163	0.046178
	4	0.62792	0.24872	9.5314	751.08	1.951	0.045818
	5	0.62546	0.24816	10.903	750.68	1.7654	0.045694
	6	0.62876	0.25228	9.6918	736.14	1.7876	0.047122
	7	0.6392	0.25208	10.2592	771.12	1.7286	0.04632
	8	0.63832	0.2517	11.9986	770.72	2.2446	0.047856
	9	0.61062	0.2457	9.6266	755.44	1.8874	0.047376
	10	0.66414	0.26294	11.1308	741.56	2.1244	0.046594
	mean	0.63234	0.251344	10.61454	760.504	1.96994	0.046767
	SD	0.024225	0.011833	0.935027	22.55851	0.209672	0.000759
100Al2O3	1	0.65062	0.26982	12.886	1121.8	2.5892	0.040124
	2	0.65414	0.2677	12.522	1094.6	2.49	0.042114
	3	0.62278	0.26348	15.892	1080.4	2.474	0.041418
	4	0.6308	0.25578	13.744	1074.8	2.0856	0.041226
	5	0.6462	0.26666	18.312	1063.2	2.0416	0.042732
	6	0.62984	0.2449	16.508	1081.6	2.2112	0.039348
	7	0.6299	0.25136	13.88	1082.2	2.2748	0.04101
	8	0.6143	0.24006	12.414	1105.8	2.1916	0.040842
	9	0.64748	0.26992	13.776	1102.6	2.6154	0.041118
	10	0.61752	0.24366	14.354	1083	2.0154	0.038866
	mean	0.634358	0.259333	14.4288	1089	2.29888	0.04088
	SD	0.014302	0.011712	1.909645	17.16793	0.226932	0.001174
250Al2O3	1	1.5442	0.59786	38.338	783.32	4.9578	0.047686
	2	1.5428	0.6424	28.26	777.94	4.8312	0.049512
	3	1.5578	0.56874	36.24	789.04	4.9512	0.04771
	4	1.5448	0.58066	32.28	776.92	4.658	0.0474
	5	1.431	0.59222	32.37	795.92	5.1844	0.047598
	6	1.4596	0.53538	41.486	788.76	4.6658	0.046672
	7	1.5768	0.58936	26.396	784.66	4.4836	0.048286
	8	1.5582	0.59786	38.338	784.32	4.9628	0.047686
	9	1.5134	0.60994	39.372	805.54	5.0618	0.04722
	10	1.44	0.56758	36.4	792.42	4.992	0.045416
	mean	1.51686	0.5882	34.948	787.884	4.87486	0.047519
	SD	0.053488	0.028412	4.952097	8.576515	0.213606	0.001049

One-Sample Kolmogorov-Smirnov Test

titanium			sa	ra
Ti-polish	N		10	10
	Normal Parameters[a,,b]	Mean	.134100	.063032
		Std. Deviation	.0152167	.0102368
	Most Extreme Differences	Absolute	.198	.184
		Positive	.198	.135
		Negative	-.128	-.184
	Kolmogorov-Smirnov Z		.627	.580
	Asymp. Sig. (2-tailed)		.827	.889
50SiO2-ti	N		10	10
	Normal Parameters[a,,b]	Mean	.534050	.226520
		Std. Deviation	.0212679	.0106273
	Most Extreme Differences	Absolute	.166	.240
		Positive	.123	.188
		Negative	-.166	-.240
	Kolmogorov-Smirnov Z		.526	.760
	Asymp. Sig. (2-tailed)		.945	.611
50Al2O3-ti	N		10	10
	Normal Parameters[a,,b]	Mean	.528790	.233930
		Std. Deviation	.0158519	.0110384
	Most Extreme Differences	Absolute	.245	.256
		Positive	.245	.129
		Negative	-.136	-.256
	Kolmogorov-Smirnov Z		.774	.808
	Asymp. Sig. (2-tailed)		.586	.531

One-Sample Kolmogorov-Smirnov Test

titanium			sa	ra
100SiO2-ti	N		10	10
	Normal Parameters[a,,b]	Mean	.632340	.251344
		Std. Deviation	.0242247	.0118334
	Most Extreme Differences	Absolute	.189	.268
		Positive	.189	.268
		Negative	-.115	-.117
	Kolmogorov-Smirnov Z		.596	.849
	Asymp. Sig. (2-tailed)		.869	.467
100Al2O3-ti	N		10	10
	Normal Parameters[a,,b]	Mean	.634358	.257334
		Std. Deviation	.0143024	.0116647
	Most Extreme Differences	Absolute	.198	.201
		Positive	.198	.157
		Negative	-.196	-.201
	Kolmogorov-Smirnov Z		.627	.635
	Asymp. Sig. (2-tailed)		.827	.815
250Al2O3-ti	N		10	10
	Normal Parameters[a,,b]	Mean	1.516860	.588200
		Std. Deviation	.0534883	.0284118
	Most Extreme Differences	Absolute	.286	.167
		Positive	.158	.167
		Negative	-.286	-.134
	Kolmogorov-Smirnov Z		.905	.528
	Asymp. Sig. (2-tailed)		.386	.943

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
sa	8.340	5	54	.000
ra	2.516	5	54	.041

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
sa	Between Groups	10.452	5	2.090	2734.042	.000
	Within Groups	.041	54	.001		
	Total	10.493	59			
ra	Between Groups	1.478	5	.296	1246.353	.000
	Within Groups	.013	54	.000		
	Total	1.491	59			

Multiple Comparisons

Dunnett T3

Dependent Variable	(I) titanium	(J) titanium	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
sa	Ti-polish	50SiO2-ti	-.3999500*	.0082697	.000	-.427801	-.372099
		50Al2O3-ti	-.3946900*	.0069486	.000	-.417791	-.371589
		100SiO2-ti	-.4982400*	.0090465	.000	-.529034	-.467446
		100Al2O3-ti	-.5002580*	.0066039	.000	-.522219	-.478297
		250Al2O3-ti	-1.3827600*	.0175856	.000	-1.446793	-1.318727
	50SiO2-ti	Ti-polish	.3999500*	.0082697	.000	.372099	.427801
		50Al2O3-ti	.0052600	.0083881	1.000	-.022910	.033430
		100SiO2-ti	-.0982900*	.0101939	.000	-.132245	-.064335
		100Al2O3-ti	-.1003080*	.0081048	.000	-.127736	-.072880
		250Al2O3-ti	-.9828100*	.0182025	.000	-1.047486	-.918134
	50Al2O3-ti	Ti-polish	.3946900*	.0069486	.000	.371589	.417791
		50SiO2-ti	-.0052600	.0083881	1.000	-.033430	.022910
		100SiO2-ti	-.1035500*	.0091549	.000	-.134602	-.072498
		100Al2O3-ti	-.1055680*	.0067516	.000	-.128039	-.083097
		250Al2O3-ti	-.9880700*	.0176417	.000	-1.052147	-.923993

Multiple Comparisons

Dunnett T3

Dependent Variable	(I) titanium	(J) titanium	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
sa	100SiO2-ti	Ti-polish	.4982400*	.0090465	.000	.467446	.529034
		50SiO2-ti	.0982900*	.0101939	.000	.064335	.132245
		50Al2O3-ti	.1035500*	.0091549	.000	.072498	.134602
		100Al2O3-ti	-.0020180	.0088960	1.000	-.032472	.028436
		250Al2O3-ti	-.8845200*	.0185683	.000	-.949724	-.819316
	100Al2O3-ti	Ti-polish	.5002580*	.0066039	.000	.478297	.522219
		50SiO2-ti	.1003080*	.0081048	.000	.072880	.127736
		50Al2O3-ti	.1055680*	.0067516	.000	.083097	.128039
		100SiO2-ti	.0020180	.0088960	1.000	-.028436	.032472
		250Al2O3-ti	-.8825020*	.0175087	.000	-.946480	-.818524
	250Al2O3-ti	Ti-polish	1.3827600*	.0175856	.000	1.318727	1.446793
		50SiO2-ti	.9828100*	.0182025	.000	.918134	1.047486
		50Al2O3-ti	.9880700*	.0176417	.000	.923993	1.052147
		100SiO2-ti	.8845200*	.0185683	.000	.819316	.949724
		100Al2O3-ti	.8825020*	.0175087	.000	.818524	.946480

Multiple Comparisons

Dunnett T3

Dependent Variable	(I) titanium	(J) titanium	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
ra	Ti-polish	50SiO2-ti	-.1634880*	.0046662	.000	-.179000	-.147976
		50Al2O3-ti	-.1708980*	.0047607	.000	-.186733	-.155063
		100SiO2-ti	-.1883120*	.0049480	.000	-.204801	-.171823
		100Al2O3-ti	-.1943020*	.0049077	.000	-.210649	-.177955
		250Al2O3-ti	-.5251680*	.0095500	.000	-.559378	-.490958
	50SiO2-ti	Ti-polish	.1634880*	.0046662	.000	.147976	.179000
		50Al2O3-ti	-.0074100	.0048455	.843	-.023518	.008698
		100SiO2-ti	-.0248240*	.0050296	.002	-.041566	-.008082
		100Al2O3-ti	-.0308140*	.0049900	.000	-.047418	-.014210
		250Al2O3-ti	-.3616800*	.0095926	.000	-.395940	-.327420
	50Al2O3-ti	Ti-polish	.1708980*	.0047607	.000	.155063	.186733
		50SiO2-ti	.0074100	.0048455	.843	-.008698	.023518
		100SiO2-ti	-.0174140*	.0051174	.043	-.034434	-.000394
		100Al2O3-ti	-.0234040*	.0050785	.003	-.040290	-.006518
		250Al2O3-ti	-.3542700*	.0096389	.000	-.388586	-.319954

Multiple Comparisons

Dunnett T3

Dependent Variable	(I) titanium	(J) titanium	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
ra	100SiO2-ti	Ti-polish	.1883120*	.0049480	.000	.171823	.204801
		50SiO2-ti	.0248240*	.0050296	.002	.008082	.041566
		50Al2O3-ti	.0174140*	.0051174	.043	.000394	.034434
		100Al2O3-ti	-.0059900	.0052545	.977	-.023455	.011475
		250Al2O3-ti	-.3368560*	.0097327	.000	-.371297	-.302415
	100Al2O3-ti	Ti-polish	.1943020*	.0049077	.000	.177955	.210649
		50SiO2-ti	.0308140*	.0049900	.000	.014210	.047418
		50Al2O3-ti	.0234040*	.0050785	.003	.006518	.040290
		100SiO2-ti	.0059900	.0052545	.977	-.011475	.023455
		250Al2O3-ti	-.3308660*	.0097123	.000	-.365279	-.296453
	250Al2O3-ti	Ti-polish	.5251680*	.0095500	.000	.490958	.559378
		50SiO2-ti	.3616800*	.0095926	.000	.327420	.395940
		50Al2O3-ti	.3542700*	.0096389	.000	.319954	.388586
		100SiO2-ti	.3368560*	.0097327	.000	.302415	.371297
		100Al2O3-ti	.3308660*	.0097123	.000	.296453	.365279

Statistical analysis of cell number on titanium samples

One-Sample Kolmogorov-Smirnov Test

type			mtt30min	mtt4hr	mtt16hr
Ti-polish	N		3	3	3
	Normal Parameters[a,,b]	Mean	.15933	.18500	.2030
		Std. Deviation	.009609	.014107	.01082
	Most Extreme Differences	Absolute	.236	.223	.276
		Positive	.192	.223	.203
		Negative	-.236	-.190	-.276
	Kolmogorov-Smirnov Z		.408	.386	.478
	Asymp. Sig. (2-tailed)		.996	.998	.976
50SiO2-ti	N		3	3	3
	Normal Parameters[a,,b]	Mean	.17367	.19167	.1923
		Std. Deviation	.009815	.007024	.00802
	Most Extreme Differences	Absolute	.385	.204	.200
		Positive	.385	.204	.184
		Negative	-.282	-.185	-.200
	Kolmogorov-Smirnov Z		.667	.354	.346
	Asymp. Sig. (2-tailed)		.766	1.000	1.000
50Al2O3-ti	N		3	3	3
	Normal Parameters[a,,b]	Mean	.21600	.21800	.2300
		Std. Deviation	.012490	.013077	.01082
	Most Extreme Differences	Absolute	.292	.343	.276
		Positive	.212	.343	.276
		Negative	-.292	-.246	-.203
	Kolmogorov-Smirnov Z		.506	.595	.478
	Asymp. Sig. (2-tailed)		.960	.871	.976

Statistical analysis of cell number on titanium samples

Test of Homogeneity of Variances

mtt30min

Levene Statistic	df1	df2	Sig.
1.147	6	14	.387

ANOVA

mtt30min

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.017	6	.003	29.447	.000
Within Groups	.001	14	.000		
Total	.018	20			

Statistical analysis of cell number on titanium samples

Multiple Comparisons

Dependent Variable:mtt30min

Scheffe

(I) type	(J) type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ti-polish	50SiO2-ti	-.014333	.007990	.772	-.04736	.01869
	50Al2O3-ti	-.056667*	.007990	.001	-.08969	-.02364
	100SiO2-ti	-.018333	.007990	.536	-.05136	.01469
	100Al2O3-ti	-.055000*	.007990	.001	-.08803	-.02197
	250Al2O3-ti	-.060667*	.007990	.000	-.09369	-.02764
50SiO2-ti	Ti-polish	.014333	.007990	.772	-.01869	.04736
	50Al2O3-ti	-.042333*	.007990	.008	-.07536	-.00931
	100SiO2-ti	-.004000	.007990	1.000	-.03703	.02903
	100Al2O3-ti	-.040667*	.007990	.011	-.07369	-.00764
	250Al2O3-ti	-.046333*	.007990	.004	-.07936	-.01331
50Al2O3-ti	Ti-polish	.056667*	.007990	.001	.02364	.08969
	50SiO2-ti	.042333*	.007990	.008	.00931	.07536
	100SiO2-ti	.038333*	.007990	.018	.00531	.07136
	100Al2O3-ti	.001667	.007990	1.000	-.03136	.03469
	250Al2O3-ti	-.004000	.007990	1.000	-.03703	.02903

Statistical analysis of cell number on titanium samples

Multiple Comparisons

Dependent Variable:mtt30min

Scheffe

(I) type	(J) type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100SiO2-ti	Ti-polish	.018333	.007990	.536	-.01469	.05136
	50SiO2-ti	.004000	.007990	1.000	-.02903	.03703
	50Al2O3-ti	-.038333*	.007990	.018	-.07136	-.00531
	100Al2O3-ti	-.036667*	.007990	.025	-.06969	-.00364
	250Al2O3-ti	-.042333*	.007990	.008	-.07536	-.00931
100Al2O3-ti	Ti-polish	.055000*	.007990	.001	.02197	.08803
	50SiO2-ti	.040667*	.007990	.011	.00764	.07369
	50Al2O3-ti	-.001667	.007990	1.000	-.03469	.03136
	100SiO2-ti	.036667*	.007990	.025	.00364	.06969
	250Al2O3-ti	-.005667	.007990	.997	-.03869	.02736
250Al2O3-ti	Ti-polish	.060667*	.007990	.000	.02764	.09369
	50SiO2-ti	.046333*	.007990	.004	.01331	.07936
	50Al2O3-ti	.004000	.007990	1.000	-.02903	.03703
	100SiO2-ti	.042333*	.007990	.008	.00931	.07536
	100Al2O3-ti	.005667	.007990	.997	-.02736	.03869

Statistical analysis of cell number on titanium samples

Test of Homogeneity of Variances

mtt4hr

Levene Statistic	df1	df2	Sig.
2.167	6	14	.109

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.010	6	.002	19.871	.000
Within Groups	.001	14	.000		
Total	.011	20			

Statistical analysis of cell number on titanium samples

Multiple Comparisons

Dependent Variable:mtt 4 hr

Scheffe

(I) type	(J) type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ti-polish	50SiO2-ti	-.006667	.007372	.989	-.03714	.02381
	50Al2O3-ti	-.033000*	.007372	.030	-.06347	-.00253
	100SiO2-ti	.000667	.007372	1.000	-.02981	.03114
	100Al2O3-ti	-.032333*	.007372	.034	-.06281	-.00186
	250Al2O3-ti	-.041667*	.007372	.005	-.07214	-.01119
50SiO2-ti	Ti-polish	.006667	.007372	.989	-.02381	.03714
	50Al2O3-ti	-.026333	.007372	.115	-.05681	.00414
	100SiO2-ti	.007333	.007372	.982	-.02314	.03781
	100Al2O3-ti	-.025667	.007372	.131	-.05614	.00481
	250Al2O3-ti	-.035000*	.007372	.019	-.06547	-.00453
50Al2O3-ti	Ti-polish	.033000*	.007372	.030	.00253	.06347
	50SiO2-ti	.026333	.007372	.115	-.00414	.05681
	100SiO2-ti	.033667*	.007372	.026	.00319	.06414
	100Al2O3-ti	.000667	.007372	1.000	-.02981	.03114
	250Al2O3-ti	-.008667	.007372	.960	-.03914	.02181

Statistical analysis of cell number on titanium samples

Multiple Comparisons

Dependent Variable:mtt 4 hr

Scheffe

(I) type	(J) type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100SiO2-ti	Ti-polish	-.000667	.007372	1.000	-.03114	.02981
	50SiO2-ti	-.007333	.007372	.982	-.03781	.02314
	50Al2O3-ti	-.033667*	.007372	.026	-.06414	-.00319
	100Al2O3-ti	-.033000*	.007372	.030	-.06347	-.00253
	250Al2O3-ti	-.042333*	.007372	.004	-.07281	-.01186
100Al2O3-ti	Ti-polish	.032333*	.007372	.034	.00186	.06281
	50SiO2-ti	.025667	.007372	.131	-.00481	.05614
	50Al2O3-ti	-.000667	.007372	1.000	-.03114	.02981
	100SiO2-ti	.033000*	.007372	.030	.00253	.06347
	250Al2O3-ti	-.009333	.007372	.943	-.03981	.02114
250Al2O3-ti	Ti-polish	.041667*	.007372	.005	.01119	.07214
	50SiO2-ti	.035000*	.007372	.019	.00453	.06547
	50Al2O3-ti	.008667	.007372	.960	-.02181	.03914
	100SiO2-ti	.042333*	.007372	.004	.01186	.07281
	100Al2O3-ti	.009333	.007372	.943	-.02114	.03981

Statistical analysis of cell number on titanium samples

Test of Homogeneity of Variances

mtt16hr

Levene Statistic	df1	df2	Sig.
1.070	6	14	.425

ANOVA

mtt16hr

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.009	6	.002	10.651	.000
Within Groups	.002	14	.000		
Total	.011	20			

Statistical analysis of cell number on titanium samples

Multiple Comparisons

Dependent Variable:mtt 16 hr

Scheffe

(I) type	(J) type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ti-polish	50SiO2-ti	.01067	.00982	.972	-.0299	.0512
	50Al2O3-ti	-.02700	.00982	.335	-.0676	.0136
	100SiO2-ti	-.00500	.00982	1.000	-.0456	.0356
	100Al2O3-ti	-.02300	.00982	.512	-.0636	.0176
	250Al2O3-ti	-.04300*	.00982	.034	-.0836	-.0024
50SiO2-ti	Ti-polish	-.01067	.00982	.972	-.0512	.0299
	50Al2O3-ti	-.03767	.00982	.078	-.0782	.0029
	100SiO2-ti	-.01567	.00982	.851	-.0562	.0249
	100Al2O3-ti	-.03367	.00982	.140	-.0742	.0069
	250Al2O3-ti	-.05367*	.00982	.006	-.0942	-.0131
50Al2O3-ti	Ti-polish	.02700	.00982	.335	-.0136	.0676
	50SiO2-ti	.03767	.00982	.078	-.0029	.0782
	100SiO2-ti	.02200	.00982	.561	-.0186	.0626
	100Al2O3-ti	.00400	.00982	1.000	-.0366	.0446
	250Al2O3-ti	-.01600	.00982	.838	-.0566	.0246

Statistical analysis of cell number on titanium samples

Multiple Comparisons

Dependent Variable:mtt 16 hr

Scheffe

(I) type	(J) type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100SiO2-ti	Ti-polish	.00500	.00982	1.000	-.0356	.0456
	50SiO2-ti	.01567	.00982	.851	-.0249	.0562
	50Al2O3-ti	-.02200	.00982	.561	-.0626	.0186
	100Al2O3-ti	-.01800	.00982	.755	-.0586	.0226
	250Al2O3-ti	-.03800	.00982	.074	-.0786	.0026
100Al2O3-ti	Ti-polish	.02300	.00982	.512	-.0176	.0636
	50SiO2-ti	.03367	.00982	.140	-.0069	.0742
	50Al2O3-ti	-.00400	.00982	1.000	-.0446	.0366
	100SiO2-ti	.01800	.00982	.755	-.0226	.0586
	250Al2O3-ti	-.02000	.00982	.660	-.0606	.0206
250Al2O3-ti	Ti-polish	.04300*	.00982	.034	.0024	.0836
	50SiO2-ti	.05367*	.00982	.006	.0131	.0942
	50Al2O3-ti	.01600	.00982	.838	-.0246	.0566
	100SiO2-ti	.03800	.00982	.074	-.0026	.0786
	100Al2O3-ti	.02000	.00982	.660	-.0206	.0606

Statistical analysis of gene expression

One-Sample Kolmogorov-Smirnov Test

Gene-titanium			col7	oc7	oc14
Ti-polish	N		3	3	3
	Normal Parameters[a,,b]	Mean	.65033	.98633	1.31833
		Std. Deviation	.224099	.122839	.045490
	Most Extreme Differences	Absolute	.276	.315	.282
		Positive	.276	.225	.282
		Negative	-.203	-.315	-.206
	Kolmogorov-Smirnov Z		.478	.545	.488
	Asymp. Sig. (2-tailed)		.976	.928	.971
50SiO2-ti	N		3	3	3
	Normal Parameters[a,,b]	Mean	.66067	.42800	.87600
		Std. Deviation	.165558	.285154	.269735
	Most Extreme Differences	Absolute	.359	.245	.186
		Positive	.258	.194	.186
		Negative	-.359	-.245	-.180
	Kolmogorov-Smirnov Z		.622	.424	.322
	Asymp. Sig. (2-tailed)		.833	.994	1.000
50Al2O3-ti	N		3	3	3
	Normal Parameters[a,,b]	Mean	.96333	1.15433	1.45167
		Std. Deviation	.133422	.181009	.058398
	Most Extreme Differences	Absolute	.315	.344	.318
		Positive	.225	.344	.318
		Negative	-.315	-.246	-.227
	Kolmogorov-Smirnov Z		.545	.596	.550
	Asymp. Sig. (2-tailed)		.928	.870	.923

Statistical analysis of gene expression

One-Sample Kolmogorov-Smirnov Test

Gene-titanium			col7	oc7	oc14
100SiO2-ti	N		3	3	3
	Normal Parameters[a,,b]	Mean	.75867	.20267	.88667
		Std. Deviation	.081794	.054308	.166061
	Most Extreme Differences	Absolute	.338	.372	.288
		Positive	.242	.270	.209
		Negative	-.338	-.372	-.288
	Kolmogorov-Smirnov Z		.586	.645	.499
	Asymp. Sig. (2-tailed)		.882	.800	.965
100Al2O3-ti	N		3	3	3
	Normal Parameters[a,,b]	Mean	1.32133	1.41867	1.13300
		Std. Deviation	.309975	.498177	.204421
	Most Extreme Differences	Absolute	.269	.343	.378
		Positive	.199	.343	.378
		Negative	-.269	-.245	-.275
	Kolmogorov-Smirnov Z		.466	.594	.655
	Asymp. Sig. (2-tailed)		.981	.872	.784
250Al2O3-ti	N		3	3	3
	Normal Parameters[a,,b]	Mean	1.66533	1.78967	1.25567
		Std. Deviation	.303869	.086408	.052013
	Most Extreme Differences	Absolute	.270	.301	.178
		Positive	.270	.301	.178
		Negative	-.199	-.217	-.177
	Kolmogorov-Smirnov Z		.467	.521	.308
	Asymp. Sig. (2-tailed)		.981	.949	1.000

Statistical analysis of gene expression

Test of Homogeneity of Variances

col7

Levene Statistic	df1	df2	Sig.
1.590	5	12	.236

ANOVA

col7

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.529	5	.506	10.444	.000
Within Groups	.581	12	.048		
Total	3.110	17			

Statistical analysis of gene expression

Multiple Comparisons

col7

Scheffe

(I) gene-7day	(J) gene-7day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ti-polish	50SiO2-ti	-.010333	.179673	1.000	-.71838	.69771
	50Al2O3-ti	-.313000	.179673	.696	-1.02104	.39504
	100SiO2-ti	-.108333	.179673	.995	-.81638	.59971
	100Al2O3-ti	-.671000	.179673	.068	-1.37904	.03704
	250Al2O3-ti	-1.015000*	.179673	.004	-1.72304	-.30696
50SiO2-ti	Ti-polish	.010333	.179673	1.000	-.69771	.71838
	50Al2O3-ti	-.302667	.179673	.724	-1.01071	.40538
	100SiO2-ti	-.098000	.179673	.997	-.80604	.61004
	100Al2O3-ti	-.660667	.179673	.073	-1.36871	.04738
	250Al2O3-ti	-1.004667*	.179673	.004	-1.71271	-.29662
50Al2O3-ti	Ti-polish	.313000	.179673	.696	-.39504	1.02104
	50SiO2-ti	.302667	.179673	.724	-.40538	1.01071
	100SiO2-ti	.204667	.179673	.927	-.50338	.91271
	100Al2O3-ti	-.358000	.179673	.574	-1.06604	.35004
	250Al2O3-ti	-.702000	.179673	.053	-1.41004	.00604

Statistical analysis of gene expression

Multiple Comparisons

col7

Scheffe

(I) gene-7day	(J) gene-7day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100SiO2-ti	Ti-polish	.108333	.179673	.995	-.59971	.81638
	50SiO2-ti	.098000	.179673	.997	-.61004	.80604
	50Al2O3-ti	-.204667	.179673	.927	-.91271	.50338
	100Al2O3-ti	-.562667	.179673	.157	-1.27071	.14538
	250Al2O3-ti	-.906667*	.179673	.010	-1.61471	-.19862
100Al2O3-ti	Ti-polish	.671000	.179673	.068	-.03704	1.37904
	50SiO2-ti	.660667	.179673	.073	-.04738	1.36871
	50Al2O3-ti	.358000	.179673	.574	-.35004	1.06604
	100SiO2-ti	.562667	.179673	.157	-.14538	1.27071
	250Al2O3-ti	-.344000	.179673	.612	-1.05204	.36404
250Al2O3-ti	Ti-polish	1.015000*	.179673	.004	.30696	1.72304
	50SiO2-ti	1.004667*	.179673	.004	.29662	1.71271
	50Al2O3-ti	.702000	.179673	.053	-.00604	1.41004
	100SiO2-ti	.906667*	.179673	.010	.19862	1.61471
	100Al2O3-ti	.344000	.179673	.612	-.36404	1.05204

Statistical analysis of gene expression

Test of Homogeneity of Variances

oc7

Levene Statistic	df1	df2	Sig.
5.071	5	12	.010

ANOVA

oc7

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.357	5	1.071	16.579	.000
Within Groups	.776	12	.065		
Total	6.133	17			

Statistical analysis of gene expression

Multiple Comparisons

oc7

Dunnett T3

(I) gene-7day	(J) gene-7day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ti-polish	50SiO2-ti	.558333	.179260	.298	-.65787	1.77454
	50Al2O3-ti	-.168000	.126298	.884	-.87351	.53751
	100SiO2-ti	.783667*	.077543	.017	.26337	1.30396
	100Al2O3-ti	-.432333	.296237	.823	-2.84803	1.98337
	250Al2O3-ti	-.803333*	.086710	.009	-1.28156	-.32510
50SiO2-ti	Ti-polish	-.558333	.179260	.298	-1.77454	.65787
	50Al2O3-ti	-.726333	.195002	.168	-1.84399	.39133
	100SiO2-ti	.225333	.167593	.863	-1.20893	1.65960
	100Al2O3-ti	-.990667	.331408	.296	-2.97453	.99320
	250Al2O3-ti	-1.361667*	.172026	.047	-2.68998	-.03335
50Al2O3-ti	Ti-polish	.168000	.126298	.884	-.53751	.87351
	50SiO2-ti	.726333	.195002	.168	-.39133	1.84399
	100SiO2-ti	.951667*	.109108	.038	.10665	1.79668
	100Al2O3-ti	-.264333	.306020	.985	-2.48670	1.95804
	250Al2O3-ti	-.635333	.115803	.077	-1.38672	.11606

Statistical analysis of gene expression

Multiple Comparisons

oc 7

Dunnett T3

(I) gene-7day	(J) gene-7day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100SiO2-ti	Ti-polish	-.783667*	.077543	.017	-1.30396	-.26337
	50SiO2-ti	-.225333	.167593	.863	-1.65960	1.20893
	50Al2O3-ti	-.951667*	.109108	.038	-1.79668	-.10665
	100Al2O3-ti	-1.216000	.289327	.212	-3.82709	1.39509
	250Al2O3-ti	-1.587000*	.058923	.000	-1.92607	-1.24793
100Al2O3-ti	Ti-polish	.432333	.296237	.823	-1.98337	2.84803
	50SiO2-ti	.990667	.331408	.296	-.99320	2.97453
	50Al2O3-ti	.264333	.306020	.985	-1.95804	2.48670
	100SiO2-ti	1.216000	.289327	.212	-1.39509	3.82709
	250Al2O3-ti	-.371000	.291917	.887	-2.90184	2.15984
250Al2O3-ti	Ti-polish	.803333*	.086710	.009	.32510	1.28156
	50SiO2-ti	1.361667*	.172026	.047	.03335	2.68998
	50Al2O3-ti	.635333	.115803	.077	-.11606	1.38672
	100SiO2-ti	1.587000*	.058923	.000	1.24793	1.92607
	100Al2O3-ti	.371000	.291917	.887	-2.15984	2.90184

Statistical analysis of gene expression

Test of Homogeneity of Variances

oc14

Levene Statistic	df1	df2	Sig.
2.465	5	12	.093

ANOVA

oc14

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.825	5	.165	6.590	.004
Within Groups	.301	12	.025		
Total	1.126	17			

Statistical analysis of gene expression

Multiple Comparisons

oc14

Scheffe

(I) gene-14day	(J) gene-14day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ti-polish	50SiO2-ti	.442333	.129231	.105	-.06693	.95160
	50Al2O3-ti	-.133333	.129231	.950	-.64260	.37593
	100SiO2-ti	.431667	.129231	.118	-.07760	.94093
	100Al2O3-ti	.185333	.129231	.832	-.32393	.69460
	250Al2O3-ti	.062667	.129231	.998	-.44660	.57193
50SiO2-ti	Ti-polish	-.442333	.129231	.105	-.95160	.06693
	50Al2O3-ti	-.575667*	.129231	.023	-1.08493	-.06640
	100SiO2-ti	-.010667	.129231	1.000	-.51993	.49860
	100Al2O3-ti	-.257000	.129231	.576	-.76627	.25227
	250Al2O3-ti	-.379667	.129231	.203	-.88893	.12960
50Al2O3-ti	Ti-polish	.133333	.129231	.950	-.37593	.64260
	50SiO2-ti	.575667*	.129231	.023	.06640	1.08493
	100SiO2-ti	.565000*	.129231	.026	.05573	1.07427
	100Al2O3-ti	.318667	.129231	.359	-.19060	.82793
	250Al2O3-ti	.196000	.129231	.799	-.31327	.70527

Statistical analysis of gene expression

Multiple Comparisons

oc14

Scheffe

(I) gene-14day	(J) gene-14day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100SiO2-ti	Ti-polish	-.431667	.129231	.118	-.94093	.07760
	50SiO2-ti	.010667	.129231	1.000	-.49860	.51993
	50Al2O3-ti	-.565000*	.129231	.026	-1.07427	-.05573
	100Al2O3-ti	-.246333	.129231	.617	-.75560	.26293
	250Al2O3-ti	-.369000	.129231	.226	-.87827	.14027
100Al2O3-ti	Ti-polish	-.185333	.129231	.832	-.69460	.32393
	50SiO2-ti	.257000	.129231	.576	-.25227	.76627
	50Al2O3-ti	-.318667	.129231	.359	-.82793	.19060
	100SiO2-ti	.246333	.129231	.617	-.26293	.75560
	250Al2O3-ti	-.122667	.129231	.965	-.63193	.38660
250Al2O3-ti	Ti-polish	-.062667	.129231	.998	-.57193	.44660
	50SiO2-ti	.379667	.129231	.203	-.12960	.88893
	50Al2O3-ti	-.196000	.129231	.799	-.70527	.31327
	100SiO2-ti	.369000	.129231	.226	-.14027	.87827
	100Al2O3-ti	.122667	.129231	.965	-.38660	.63193

Data and statistical analysis of *in vitro* mineralization

Alizarin	Ti-polish	50SiO2-Ti	50Al2O3-Ti	100SiO2-Ti	100Al2O3-Ti	250Al2O3-Ti
test1	0.676879	0.774566	1.034682	0.453757	0.904046	1.132948
test2	0.7407	0.58361	0.981952	0.486004	1.128361	0.927993
test3	0.553125	0.597917	0.723958	0.466667	0.740625	0.638542
test4	0.553261	0.46413	0.728261	0.368478	0.748913	0.78913
test5	0.653493	0.432904	0.674632	0.416912	0.855699	0.648162
mean	0.635491	0.570626	0.828697	0.438364	0.875529	0.827355
sd	0.081627	0.13492	0.166366	0.046507	0.157608	0.207808

Data and statistical analysis of *in vitro* mineralization

One-Sample Kolmogorov-Smirnov Test

Alizarin test			alizarin
Ti-polish	N		5
	Normal Parameters[a,,b]	Mean	.635491
		Std. Deviation	.0816270
	Most Extreme Differences	Absolute	.243
		Positive	.243
		Negative	-.187
	Kolmogorov-Smirnov Z		.544
	Asymp. Sig. (2-tailed)		.929
50SiO2-ti	N		5
	Normal Parameters[a,,b]	Mean	.570626
		Std. Deviation	.1349200
	Most Extreme Differences	Absolute	.220
		Positive	.220
		Negative	-.154
	Kolmogorov-Smirnov Z		.492
	Asymp. Sig. (2-tailed)		.969
50Al2O3-ti	N		5
	Normal Parameters[a,,b]	Mean	.828697
		Std. Deviation	.1663659
	Most Extreme Differences	Absolute	.327
		Positive	.327
		Negative	-.222
	Kolmogorov-Smirnov Z		.731
	Asymp. Sig. (2-tailed)		.659

Data and statistical analysis of *in vitro* mineralization

One-Sample Kolmogorov-Smirnov Test

Alizarin test			alizarin
100SiO2-ti	N		5
	Normal Parameters[a,,b]	Mean	.438364
		Std. Deviation	.0465074
	Most Extreme Differences	Absolute	.230
		Positive	.153
		Negative	-.230
	Kolmogorov-Smirnov Z		.514
	Asymp. Sig. (2-tailed)		.955
100Al2O3-ti	N		5
	Normal Parameters[a,,b]	Mean	.875529
		Std. Deviation	.1576080
	Most Extreme Differences	Absolute	.228
		Positive	.228
		Negative	-.196
	Kolmogorov-Smirnov Z		.510
	Asymp. Sig. (2-tailed)		.957
250Al2O3-ti	N		5
	Normal Parameters[a,,b]	Mean	.827355
		Std. Deviation	.2078081
	Most Extreme Differences	Absolute	.206
		Positive	.206
		Negative	-.182
	Kolmogorov-Smirnov Z		.460
	Asymp. Sig. (2-tailed)		.984

Data and statistical analysis of *in vitro* mineralization

Test of Homogeneity of Variances

alizarin

Levene Statistic	df1	df2	Sig.
2.294	5	24	.077

ANOVA

alizarin

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.764	5	.153	7.472	.000
Within Groups	.491	24	.020		
Total	1.255	29			

Data and statistical analysis of *in vitro* mineralization

Multiple Comparisons

alizarin

Scheffe

(I) ali	(J) ali	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ti-polish	50SiO2-ti	.0648659	.0904549	.991	-.262567	.392299
	50Al2O3-ti	-.1932058	.0904549	.490	-.520639	.134227
	100SiO2-ti	.1971279	.0904549	.467	-.130305	.524561
	100Al2O3-ti	-.2400374	.0904549	.257	-.567470	.087395
	250Al2O3-ti	-.1918635	.0904549	.497	-.519296	.135569
50SiO2-ti	Ti-polish	-.0648659	.0904549	.991	-.392299	.262567
	50Al2O3-ti	-.2580716	.0904549	.191	-.585504	.069361
	100SiO2-ti	.1322620	.0904549	.825	-.195171	.459695
	100Al2O3-ti	-.3049032	.0904549	.080	-.632336	.022530
	250Al2O3-ti	-.2567294	.0904549	.195	-.584162	.070703
50Al2O3-ti	Ti-polish	.1932058	.0904549	.490	-.134227	.520639
	50SiO2-ti	.2580716	.0904549	.191	-.069361	.585504
	100SiO2-ti	.3903336*	.0904549	.012	.062901	.717766
	100Al2O3-ti	-.0468316	.0904549	.998	-.374264	.280601
	250Al2O3-ti	.0013423	.0904549	1.000	-.326090	.328775

Data and statistical analysis of *in vitro* mineralization

Multiple Comparisons

alizarin

Scheffe

(I) ali	(J) ali	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100SiO2-ti	Ti-polish	-.1971279	.0904549	.467	-.524561	.130305
	50SiO2-ti	-.1322620	.0904549	.825	-.459695	.195171
	50Al2O3-ti	-.3903336*	.0904549	.012	-.717766	-.062901
	100Al2O3-ti	-.4371652*	.0904549	.004	-.764598	-.109732
	250Al2O3-ti	-.3889914*	.0904549	.013	-.716424	-.061559
100Al2O3-ti	Ti-polish	.2400374	.0904549	.257	-.087395	.567470
	50SiO2-ti	.3049032	.0904549	.080	-.022530	.632336
	50Al2O3-ti	.0468316	.0904549	.998	-.280601	.374264
	100SiO2-ti	.4371652*	.0904549	.004	.109732	.764598
	250Al2O3-ti	.0481739	.0904549	.998	-.279259	.375607
250Al2O3-ti	Ti-polish	.1918635	.0904549	.497	-.135569	.519296
	50SiO2-ti	.2567294	.0904549	.195	-.070703	.584162
	50Al2O3-ti	-.0013423	.0904549	1.000	-.328775	.326090
	100SiO2-ti	.3889914*	.0904549	.013	.061559	.716424
	100Al2O3-ti	-.0481739	.0904549	.998	-.375607	.279259

VITA

Miss Jiratchaya Panthachai was born in Yasothon, Thailand on September 24, 1981. In 2007, she graduated the Degree of Doctor of Dental Surgery (D.D.S.) from Faculty of Dentistry, Khon Kaen University. After graduation, she worked as a dentist in Chiang Yuen Hospital, Mahasarakam in 2007-2009 After that she started her study for the Master degree of prosthodontics at the Faculty of Dentistry, Chulalongkorn University in 2009. The research component of this degree was performed at the Research Unit of Mineralized Tissue, Faculty of Dentistry, Chulalongkorn University. At present, she works at Chiang Yuen Hospital, Mahasarakam.