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Mineralization of Progenitor Cells with Different Implant Topographies

Wen-Cheng Chen[a,*], Chia-Ling Ko[b], Hsien-Nan Kuo[c], Dan-Jae Lin[d,*], Hui-Yu Wu[e], Lo Yang[e], Ching-Wen Lou[f], Jia-Horng Lin[a,h*]

[a] *Department of Fiber and Composite Materials, Feng Chia University, Taichung City 407, Taiwan, ROC.*
[b] *School of Dentistry, College of Dental Medicine, Kaohsiung Medical University, Kaohsiung 807, Taiwan, ROC.*
[c] *Medical Device Development Division, Metal Industries Research & Development Centre, Kaohsiung 82151, Taiwan, ROC*
[d] *Department of Dental Hygiene, China Medical University, Taichung 40402, Taiwan, ROC.*
[e] *Department of Oral Hygiene, Kaohsiung Medical University, Kaohsiung 807, Taiwan, ROC.*
[f] *Institute of Biomedical Engineering and Material Science, Central Taiwan University of Science and Technology, Taichung 40601, Taiwan, ROC.*
[h] *School of Chinese Medicine, China Medical University, Taichung 40402 , Taiwan, ROC.*

Abstract

The major challenge for dental implants is achieving an optimal osteoregeneration. Different levels of roughness processed through sand-blasting/ acid-etching (SLA) then further treated with silane and peptide were measured. Peptide bonded with silane on the SLA and machine ground titanium (Ti) surface were used as a culture substitute. The sample properties on the osteogenic abilities were compared by testing the interaction with mesenchymal stem cells (MSCs, D1). When comparing to the SLA only group, the silane treated Ti surface with peptide bonded had smaller wetting angle and the cell proliferative ability did differ with statistical significance ($p<0.05$). A rougher surface binding with peptide provided higher hydrophilic ability and had the potential ability to enhance the proliferation and mineralization of the progenitor cell D1. Accordingly, a novel implant surface treatment method having tissues integrated was obtained through the supplement of peptide on the surfaces through SLA treatment of titanium.

Keywords: Implant; surface treatment; silane; peptide

* Corresponding author. Tel.: +886-4-24517250x3431(W.-C. Chen); x3405(J.-H. Lin); Fax: +886-4-24514625
E-mail address: wencchen@mail.fcu.edu.tw (W.-C. Chen); jhlin@fcu.edu.tw (J.-H. Lin); djlin@mail.cmu.edu.tw (D.-J. Lin)

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1. Introduction

Despite advances in dentistry, that number is expected to grow as the population ages. As a consequence, demand for dentures is expected to increase through next ten year. People who lose teeth might be due to the reasons of tooth decay, root canal failure, periodontitis (gum disease), trauma to the mouth, excessive wear, and tear. Dental implants are artificial tooth replacements that are used to counter tooth loss. In many cases, dental implants may be a better logical choice for the restoration of all considerable and desirable functionalities of the teeth and its supporting structure.

There are several choices with dental implant materials. Among those dental implant materials, titanium (Ti) metal or Ti alloy has better mechanical strength, chemical stability and biological compatibility. Therefore, they are widely used in dental implantation for their good corrosion resistance abilities. For osseointegrated implants, metallic, ceramic and polymeric materials have been used, which in particular, the titanium. To be termed osseointegration, the connection between osteoid and implant does not need to be hundred percent. The essence of osseointegration derives more from the stability of the fixation and anchor effect than the degree of contact in histologic terms. Since the biological compatibility of Ti metal is strongly related to oxidized surface structure, surface type, chemical composition and different metal surface treatments are developed for better surface characteristics and stronger binding between implant and bone tissues [1]. At present, the bonding of additives or bio-bonding to titanium and its alloy is still a problem and unclear. That is the reason why the surface conditions, such as the roughness, hardness, macrostructure, microstructure, oxidation thickness and phases are so important [2]. The present study is focusing on the surface treatments such as the surface after sandblasting, acid-etching and ions-supplying to produce a surface zone of bone tissue regeneration at early stage after implantation [3, 4].

Based on our previous studies, the topography and roughness of the implant surface could regulate the cell morphology and direction [5]. The purpose of this study was to achieve an active implant's surface, which could elimination the complication of fibrous encapsulation and enhance healing process on bone regeneration through the process of binding with peptides. We hope the results would provide a better technique, reduce the cost of surface treatment of titanium implants and extend into the practical application.

2. Experimental details

Commercially pure, grade IV titanium (c.p. Ti) plate samples (Buehler Ltd, USA) of cylinder measuring 9 x 2 mm in respective diameter and thickness of constant surface areas were used. The samples were embedded into an epoxy resin to be polished by sandpaper of decreasing grain sizes: #400, #800, and #1500. Samples were further polished with the aluminum oxide powder size of 1.0, 0.3 and 0.05 μm. They were then washed with ethanol, acetone and distilled water using ultrasonic oscillation for 5 min. The surfaces of the machine ground samples were sand-blasted with aluminum (Al_2O_3) particles (mean size ~50.0 μm). The sand-blasting was performed by using an air compressor with 4 kg/m^2 of powder blasted for 10 sec. After sand-blasting, the samples were acid-etched for 15 min [5]. An etching solution of the HCl-to-H_2SO_4-to-H_2O volume ratio of 1:1:1 was used. Prior to the silanization process, all specimens were immersed into deoxidization solution of H_2SO_4-to-H_2O_2 (30%) volume ratio of 1:1 for 2 h, then the specimens were treated with 10% 3-mercaptopropyltrimethoxysilane (MPTMS). The substrates were left to dry at room temperature overnight. After silanization, substrates were fixed with solution of 1% glutaraldehyde in phosphate buffered saline (PBS) and then rinsed several times with PBS. After fixation process, samples were sterilized in an autoclave. With the purpose of finding a peptide preparation procedure on the surfaces of silanization substrates, some of the substrates were dipped

separately in a vertical position into a vial containing 100 μg GRGDS (Gly-Arg-Gly-Asp-Ser)/ 1.0 ml PBS of the peptide colloidal suspension for 15 h. Before testing, they were rinsed again with water.

Nomenclature

G	the sample surfaces were treated with machine ground
G+Si	the sample surfaces were treated with machine ground and silane treatment
G+Si+P	the sample surfaces were treated with machine ground, silane and peptide bonded treatment
SLA+Si+P	the sample surfaces were treated with SLA, silane and peptide bonded treatment

D1 cells, bone marrow mesenchymal stem cells (MSCs) line cloned from Balb/C mice, were purchased from American Type Culture Collection (ATCC). D1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in 37 °C incubators with 5% CO_2. After a confluent cell layer was formed, the cells were detached using 0.25% trypsin in PBS and were resuspended in a DMEM medium to be used for the experiments. Cells were used before the tenth passage. An XTT Cell Viability Assay Kit provided a simple method to count live cells using an absorbance reader. The cells' adhesive and repopulate abilities were measured at time points of 1, 4, 7 and 14 days. After the cultured time, the cells on the samples' surface were washed with phosphate buffered saline (PBS) and transferred to a 200 μL culture medium with a 100 μL XTT kit and were incubated for another 4 h. The reaction medium was then measured spectrophotometrically at 490 nm using an ELISA microplate reader UVM-340 (ASYS Hitech GmbH, Eugendorf, Austria). Finally, the cell numbers were determined from a plot of absorbance (OD values) versus the respective D1 cells after adjustment via XTT assays. Each experiment was performed five times (n=5). The production of ALP, an early marker of osteogenesis, was determined using ALP T1016 kit (Applied Biosystems, Foster city, USA). Testing was performed at time intervals of 1, 4, 7 and 14 days after initial seeding of 5 x 10^4 D1 cells on the surface of the samples. The cell proliferation, ALP activities and morphologies would be tested. The statistical analysis was performed using JMP 6.0 software (SAS Institute Inc., Cary, NC, US) with statistical significance set at $p< 0.05$.

3. Results and Discussion

Titanium metal and Ti alloys have good mechanical strength, chemical stability and biological compatibility. Therefore, they are widely used in dental implantation for their good corrosion resistance abilities. Since the biological behavior of Ti metal is strongly related to the surface conditions, such as oxidized structure, surface topographies and chemical compositions. The sample groups and the respective roughness that were created using various processes were shown in Table 1.

Table 1. Surface conditions of roughness in different sample groups. (n=5).

Sample groups	Mean Ra value (μ m)	Standard deviation (μ m)
G	0.16	0.03
G+Si	0.26	0.06
G+Si+P	0.21	0.04
SLA+Si+P	1.23	0.13

The samples through SLA related process had largest roughness on their surfaces and samples through the machine ground process had smoothest surfaces. The groups of G+Si and G+Si+P showed no

statistical significance (p>0.05). Different surface treatments and binding with proteins are developed for better surface characteristics and stronger binding between implant and bone tissue. Ti- SLA treatment creates three dimensional roughness implant surface for improving bone anchorage. However, this type of surface treatment might leave some harmful metal ions or particles on the surface of dental implants, such as aluminum. Those remains would eventually lead to local or systemic toxic effects. In this study, machine ground smooth surfaces, SLA roughen surfaces and further treated by silane and peptide samples have been studied and compared. Based on the result obtained from FTIR-ATR spectrum, it clearly showed that the binding efficiency of MPTMS with Ti had the highest score [6, 7]. When comparing the experimental results obtained from FTIR-ATR spectrum, there was no significant difference between SLA+Si+P group and G+Si+P group. However, a noticeable reduction in contact angle was detected in G+Si group than SLA+Si+P group and G+Si+P group. The contact angle of different surface treatment samples were ranging from high to low in the following order: G+Si> G> G+Si+P> SLA+Si+P groups. When comparing the results of contact angle and surface wetting tension in different surface treatment groups, the SLA/MPTMS/peptide surface treatment of the SLA+Si+P group had the largest wetting tension and smallest contact angle among all groups. This implies that the MPTMS and peptide process could improve the hydrophilic performance of Ti specimen through the SLA treatment.

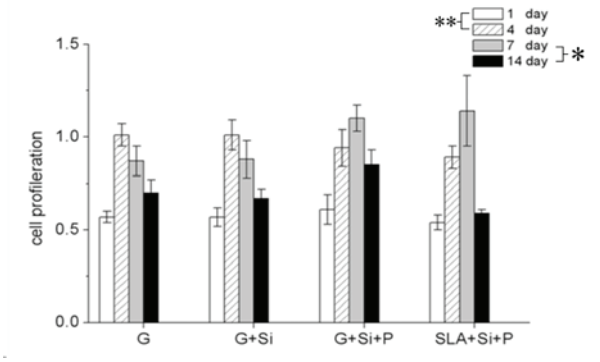

Fig. 1. Alkaline phosphatase activity of D1 cells seeded on different Ti surface conditions after 1, 4, 7, and 14 days of incubation. (*: significance p<0.05; **non-significance p>0.05, n=5).

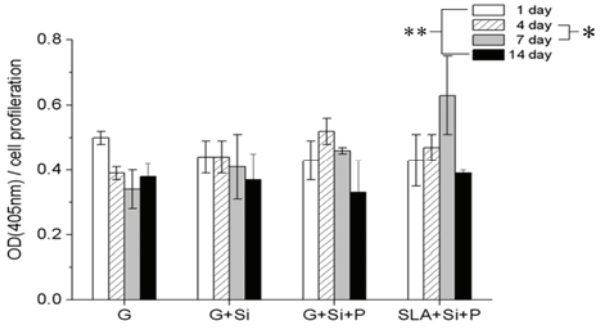

Fig. 2. Cell viability of D1 cells seeded on different Ti surface conditions after 1, 4, 7, and 14 days of incubation. (*: significance p<0.05; **non-significance p>0.05, n=5).

Cell number of viability was determined by the MTT assay, accordingly to the OD values (Fig. 1). Smooth and non-treated group of machine ground surface had the lowest OD values especial at day 7. However, cell numbers at day 1 were unaffected in all measurements. One-way ANOVA analysis of variance showed significant differences ($p<0.05$) in cell viability with the silane and silane/peptide treated Ti surfaces at the 7 and 14 days incubation and had no significantly differences ($p>0.05$) within 4 days incubation. ALP activity could be the evidence of D1 cell differentiation [8, 9]. It nearly reached a maximum on 7th day and started decreasing after 14 days of cell cultures. It is noteworthy that the tendency of cell viabilities and ALP activities was the same for all four samples tested. Additionally, the organosilane group with further peptide impregnation specimens showed higher D1 cells proliferation values and ALP activities than those without peptide impregnation at day 7 cell cultures (Fig. 1 and Fig. 2). It implies that the procedure of peptide impregnation could also improve the osteogenic performance of Ti dental implant. MSCs cell were able to proliferate and the cell numbers were on the ranging from high to low in the following order: SLA+Si+P> G+Si+P> G+Si> G. In the early stages of cell adhesion, proliferation and differentiation, surface modification with silane and peptide are clearly more important for MSCs cells proliferation than the effects of topographies. Accordingly, a SLA modified surface treatment having potential tissues integrated is obtained through the supplement of peptides in the implant part contacting with bone tissues.

4. Conclusions

As we know, the different titanium topographies were correlated to the cell adhesion, viability, and differentiation. Some specific growth factors and proteins could enhance the cell differentiation at the early stage of bone regeneration. In this study, cells were (MSCs, D1) adhered to and spread onto four test surfaces. The wetting abilities of silane and peptide treated surfaces were higher than machine ground and SLA processed samples. MSCs cell were able to proliferate and the cell numbers ranging from high to low were in the following order: SLA+Si+P> G+Si+P> G+Si> G within 1-7 days of culture. The same tendency was also shown on the ALP activities of MSCs cells within 4-7 days of culture. The results clearly indicated that silane and peptide did significantly affect D1 cell's behavior at the early stage of proliferation and differentiation. Further studies are required for better realizations of cell and tissue response in *in vivo* implantations.

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