Journal of Dental Sciences (2018) xx, 1–7

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

Original Article

The effect of plasma treatment on the osseointegration of rough titanium implant: A histo-morphometric study in rabbits

Wan-Ching Chou [a], Rex C.-C. Wang [a], Chih-Ling Huang [b], Tzer-Min Lee [c,d*]

[a] *Institute of Manufacturing Information and Systems, National Cheng Kung University, Tainan, Taiwan*
[b] *Center for Fundamental Science, Kaohsiung Medical University, Kaohsiung, Taiwan*
[c] *Institute of Oral Medicine, National Cheng Kung University Medical College, Tainan, Taiwan*
[d] *School of Dentistry, Kaohsiung Medical University, Kaohsiung, Taiwan*

Received 21 April 2018; Final revision received 28 May 2018
Available online ■ ■ ■

KEYWORDS
Hydrophilicity;
Functional OH groups;
Plasma treatment;
Bone-to-implant contact

Abstract *Background/purpose:* The surface properties, such as hydrophilicity and functional OH groups, play an important role in bone fixation *in vivo*. In our previous study, the plasma treatments of large grit and acid etching (SLA) method produce functional OH groups on the rough surface. There is no report in discussing the integration between basic Ti–OH groups and bone-to-implant contact (BIC). The aim of this study was to evaluate the effect of the functional OH groups on the rough surface both *in vitro* and *in vivo*.
Materials and methods: Functional hydroxyl groups were produced on a SLA-treated surface. The surface topography, roughness, wettability, and chemical composition were examined using various techniques. Twenty-four implants were inserted into the proximal tibia of four New Zealand white rabbits. The biological responses were measured in terms of histomorphometric analysis 4 and 8 weeks post-implantation.
Results: The surface morphology and roughness were similar among all groups. However, the concentration of OH groups and hydrophilicity were found increased in the plasma treatment. The cell morphology in RF-plasma treated groups had more polygonal type and higher expression of actin and vinculin. The bone-to-implant contact (BIC) ratios of RF-200W were significantly higher than other groups ($P < 0.05$). The relationship between basic OH groups and BIC showed linear correspondence.
Conclusion: The Ti–OH groups introduced on the rough surface by plasma treatments can trigger cell adhesion which further initiate new bone apposition. We propose that RF-plasma treatment can help to enhance bone healing at 4 and 8 weeks.

* Corresponding author. 1 University Road, Tainan, 701, Taiwan. Fax: +886 6 2095845.
E-mail address: tmlee@mail.ncku.edu.tw (T.-M. Lee).

https://doi.org/10.1016/j.jds.2018.06.002

Introduction

Artificial implants with good osseointegration is an important factor in the success of implantation. Osseointegration refers to bone-implant interface bonding. Direct contact between an implant and surrounding bone often depends on the implant surface properties.[1–3] Sand-blasting with large grit and acid etching (SLA) is commonly used as a surface treatment because it produce a high contact ratio with micron and submicron roughness surface.[4] SLA is often used in commercial implant surface treatment; however, since bone healing time takes about 8 weeks, the surface properties need to be further optimized. Compared with SLA treated implants, those with chemically modified SLA (modSLA) can reduces healing time to 3–4 weeks because the surface becomes more hydrophilic.[5] Therefore, to speed up osseointegration, plasma treatment can be considered SLA-implant surfaces to introduce functional groups and enhance hydrophilicity.

Study have shown that hydrophilic SLA surface influence osteoblast behavior by altering protein absorption and focal adhesion, which can directly induce cell differentiation through a subsequent intracellular signaling cascade.[6] Additional surface plasma treatment showed many advantages like introduce functional groups, change wettability, and eliminate contamination, making it an ideal and effective modification method for improving physicochemical properties.[7] The amount of surface hydroxyl $(OH)_s$ groups that contains acidic OH groups and basic OH groups increased by plasma treatment; such groups are a key factor in osteoblast–titanium interaction.[8] After plasma treatment, the surface becomes hydrophilic and contains abundant hydroxyl OH groups. Cells live on the OH-rich surface exhibit excellent growth and differentiation.[9–11] Canullo et al. reported that plasma treatment can improve protein adsorption and cell adhesion on a rough titanium substrate.[12] MacDonald et al. used radio-frequency plasma treatment to increase shear strength (determined using a pull-out test) and increase implant-femur bonding.[13]

The success of a clinical implant is mainly determined by the bonding strength and osseointegration between the implant and new surrounding bone tissues.[14] In our previous study, the plasma treatments of large grit and acid etching (SLA) method produced functional OH groups on the rough surface. Since there is no report in discussing the integration between basic Ti–OH groups and bone-to-implant contact (BIC). The present study uses simple and quick plasma treatment to introduce functional OH groups onto an implant surface and examines the cell cytoskeleton *in vitro* and bone-to-implant contact *in vivo*. The surface properties of plasma- and SLA-treated samples were also examined. Cell adhesion was investigated *in vitro* using immunofluorescence staining. Finally, bone-to-implant contact was measured to evaluate bone healing in a rabbit model.

Materials and methods

Sample preparation

Commercially pure Ti discs (cpTi, ASTM F67, Grade II) measuring 12.7 mm in diameter and 2 mm in thickness were used *in vitro*. A pure titanium implant measuring 11.52 mm in length and 2.48 mm in diameter and with a small head was used *in vivo*. The discs and implants were sand-blasted with large grit Al_2O_3 (particle size: 355–425 μm) at a pressure of 4 kg/cm^2. Then, a mixed acid (HCl/H_2SO_4 in ratio of 1:3) was used to treat the samples for 30 min at 80 °C. The samples were then washed with acetone, ethanol, and distilled water in sequence.

Plasma treatments

The SLA-treated samples (flat specimens and implants) were separately modified using plasma treatments under an atmosphere of oxygen. The DC and RF plasma treatment procedures followed those reported in a previous study.[11] The plasma-treated specimens were placed in the oxygen atmosphere for more than 10 min and then used absolute alcohol to keep its original properties before being analyzed.

The samples are denoted as follows: control (only SLA), DC-50W (SLA-treated titanium treated using DC plasma at 50 W), RF-50W (SLA-treated titanium treated using RF plasma at 50 W), and RF-200W (SLA-treated titanium treated using RF plasma at 200 W). The wettability, functional OH groups, and protein adsorption values are listed in Table 1.[11] The surface roughness was evaluated using a surfcorder (SE 1200; Kosaka Laboratory Ltd., Japan). The surface morphology was examined using scanning electron microscopy (SEM, JSM-6390LV, JEOL, Japan).

Cell cytoskeleton development

A fluorescence microscope (LSM780, Zeiss, Germany) was used to observe the cell morphology and cytoskeletal distribution. Cells were cultured at a seeding density of 5×10^3 cells/cm^2 on the specimens (four groups). Vinculin was stained using a mouse monoclonal antibody (Sigma, USA) and anti-mouse IgG conjugated with Alexa fluor 594 (Molecular Probes, USA). F-actin was labeled with Alexa fluor 488-labled phalloidin (Molecular Probes, USA). The samples were stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) nuclear dye and finally fixed using ProLong® Gold antifade reagents (Molecular Probes, USA) before fluorescence microscopy analysis.

Please cite this article in press as: Chou W-C, et al., The effect of plasma treatment on the osseointegration of rough titanium implant: A histo-morphometric study in rabbits, Journal of Dental Sciences (2018), https://doi.org/10.1016/j.jds.2018.06.002