Accelerated and Enhanced Bone Formation on Novel Simvastatin-Loaded Porous Titanium Oxide Surfaces

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ABSTRACT

Background: With increasing application of dental implants in poor-quality bones, the need for implant surfaces ensuring accelerated osseointegration and enhanced peri-implant bone regeneration is increased.

Purpose: A study was performed to evaluate the osseointegration and bone formation on novel simvastatin-loaded porous titanium oxide surface.

Materials and Methods: Titanium screws were treated by micro-arc oxidation to form porous oxide surface and 25 or 50 μg of simvastatin was loaded. The nontreated control, micro-arc oxidized, and simvastatin-loaded titanium screws were surgically implanted into the proximal tibia of 16-week-old male Wistar rats ($n = 36$). Peri-implant bone volume, bone-implant contact, and mineral apposition rates were measured at 2 and 4 weeks. Data were analyzed by one-way analysis of variance followed by Tukey's post hoc test.

Results: New bone was formed directly on the implant surface in the bone marrow cavity in simvastatin-loaded groups since 2 weeks. Bone-implant contact values were significantly higher in simvastatin-loaded groups than control and micro-arc oxidized groups at both time points ($p < .05$). Peri-implant bone volume and mineral apposition rate of simvastatin-loaded groups were significantly higher than control and micro-arc oxidized groups at 2 weeks ($p < .05$).

Conclusions: These data suggested that simvastatin-loaded porous titanium oxide surface provides faster osseointegration and peri-implant bone formation and it would be potentially applicable in poor-quality bones.

KEY WORDS: bone regeneration, micro-arc oxidation, osseointegration, simvastatin, titanium oxide

INTRODUCTION

The success of endosseous dental implants is directly related to osseointegration, a process of bone-implant surface interaction that ultimately leads to bone-to-implant anchorage. As the surface topography of implant has a major impact on osseointegration, various physical and chemical surface modifications have been developed to promote early osseointegration thus to shorten overall treatment time.[1,2] Among them, increased surface roughness has been shown to improve bone apposition on the implant.[3,4] Chemical treatment

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DOI 10.1111/cid.12045

of implant surfaces such as acid etching or fluoride treatment has also been applied for increased osseointegration.[5–7] In order to enhance osseointegration and osteogenesis around peri-implant region, protein growth factors such as recombinant human bone morphogenetic protein-2 (BMP-2) are being tested applying on the implant surfaces.[8,9] However, protein growth factors require a carrier that must be resorbed when osseointegration is about to take place, which may be a disadvantage. Moreover, chemical stability of proteins until implant placement is a problem to overcome. Cost-effectiveness ratio is also questionable with recombinant growth factors. If bioactive substances can be loaded directly on the implant surface without carrier, it would be more attractive strategy for promoting osteogenesis around the titanium (Ti) implants. Micro-arc oxidation (MAO) technique for modifying the Ti surface has been reported in which a positive voltage is applied to a Ti specimen immersed in an electrolyte.[10] A few micrometer-thick newly formed titanium oxide layer is porous with interconnected pores and firmly adhered to the substrate, which is beneficial for the biological performance of the implants. Another advantage of this MAO process is the possibility of incorporating Mg, Ca, and P ions into the surface layer, which are shown to enhance bioactivity of implant surface.[11–13] In addition, bone stimulating drugs or molecules may be incorporated directly into the porous titanium oxide layer, from which they would be released efficiently at the implant installation site. Statin, one of the most commonly prescribed lipid-lowering agents, has been shown to upregulate BMP-2 in osteoblastic cells.[14] Our previous works and many other in vivo studies have validated that statins stimulate bone healing when applied locally.[14–29] The objective of this study was to evaluate the effect of simvastatin loaded onto the porous titanium oxide surface by MAO on osseointegration and bone regeneration around the implant.

MATERIALS AND METHODS

Preparation of Drug-Loaded Titanium Screw Implants

The surfaces of titanium screws (1.8 mm in diameter and 5 mm in length at threaded region) were treated by MAO technique using an electrolyte solution consisting of 0.1 M of calcium glycerophosphate and 0.15 M of magnesium acetate tetrahydrate. The voltage of 420 V was applied from a pulsed electrical source for 7 minutes. After the MAO process, the treated screws were cleaned and sterilized. Simvastatin (OHARA Pharmaceutical Co. Ltd., Koka, Shiga, Japan) was dissolved in ethanol and applied on the oxidized surface by wetting homogenously. The ethanol was evaporated out completely in a laminar air flow in a clean bench. Titanium screw implants loaded with 25 or 50 μg of simvastatin/implant were prepared.

Characterization of Implant Surfaces

The titanium screw implants after MAO treatment were observed by scanning electron microscopy.

In Vitro Release of Simvastatin from Implant

The release of simvastatin was measured using an ultraviolet-visible spectrophotometer, NanoDrop, ND-1000 (NanoDrop Technologies, Wilmington, NC, USA). The spectrometer was calibrated using six standards of simvastatin solution at 37°C. The absorbance was measured at 238 nm and working curve for calculation of simvastatin concentrations was established from the absorbance values. The samples were placed in 500 μL of 0.1 M of tris buffer solution (pH 7.4) and positioned in a Taitec Personal 11 Shaker (Taitec Corp., Tokyo, Japan) set at 100 rpm and 37°C. The amount of the drug released into the tris buffer was measured 24 hours after the initial immersion, then everyday for 14 days. The cumulative concentration was calculated using the previously determined working curve.

Anesthesia and Surgical Procedures

This study was approved by the institutional committee for animal experiments. Sixteen-week-old male Wistar rats were used. The animals were anesthetized with a combination of ketamine and xylazine (40 mg/kg; 5 mg/kg). The proximal part of tibia, about 1 cm from the condyle, was exposed and drilled with a series of burs under continuous saline coolant. Ti screw was then inserted into the drill hole with the torque value of 10 to 15 N. Each animal received control and drug-load implants or micro-arc oxidized and drug-loaded implants randomly at left and right tibiae so that each implant type was inserted in six separate animals ($n = 6$

for each group). The soft tissue flap was repositioned and sutured. Ten days before and 3 days before sacrificing, calcein and tetracycline were injected, respectively. At 2 and 4 weeks after surgery, the animals were sacrificed and the tibiae were harvested.

Radiological Analysis by Microcomputerized Tomography (Micro-CT)

Directly after retrieval of the samples, x-ray imaging was performed by a micro-CT scanner (InspeXio; Shimadzu Science East Corporation, Tokyo, Japan) with a voxel size of 25 μm/pixel. Tri/3D-Bon software (RATOC System Engineering Co., Ltd., Tokyo, Japan) was used to make a three-dimensional reconstruction from the obtained set of scans and to convert the micro-CT image data into bone mineral density values and accordingly create color-labeled images. Out of the entire three-dimensional data set, a cylindrical region of interest (ROI) with a diameter of 2.3 mm and a height that covered the entire length of the implant inserted in the bone was selected for analysis. ROI was placed around the implant and the implant in the ROI was selected and extracted by means of binarization, leaving 250-μm-wide ROI ring surrounding the implant. The volume of the newly formed bone around the titanium screw was measured three dimensionally. The volume measurements were performed by one experienced examiner being blinded for the identity of the specimens.

Measurement of Mineral Apposition Rate (MAR)

After harvesting and fixation procedures, the specimens were dehydrated in graded alcohol and embedded in the Rigolac resin (Nisshin EM, Tokyo, Japan). The samples were cut with the rotary diamond saw and polished until 50-μm-thick sections were obtained. The unstained sections were observed under a fluorescent microscope (BZ-8000, Keyence Corporation, Tokyo, Japan) for fluorochrome labeling. For MAR, interlabel distances were measured and the values were divided by the time interval between administrations of two vital markers.

Measurement of Bone-Implant Contact (BIC)

The sections were stained with 0.1% toluidine blue for microscopic observation. Microscopic images were taken with the resolution (1 pixel equals 2.83 μm) (BZ-8000, Keyence Corporation), and by using ImageJ software (National Institutes of Health, Bethesda, MD, USA) the implant surface in contact with mineralized bone, referred to as the "BIC," was marked and calculated as a percentage.

Statistical Analysis

Data were first analyzed by one-way analysis of variance and if significant difference was detected ($p < .05$), Tukey's post hoc multiple comparison tests were performed.

RESULTS

Characterization of Implant Surfaces

Titanium implant surfaces after MAO had characteristic porous configuration with irregular raised areas. The pore size ranged from 0.5 to 2 μm (Figure 1).

Release of Simvastatin from Implant Surface In Vitro

Approximately 80% of adsorbed simvastatin was released after 24 hours. This initial burst release was followed by the gradual and stable release of the drug that was maintained until 2 weeks (Figure 2).

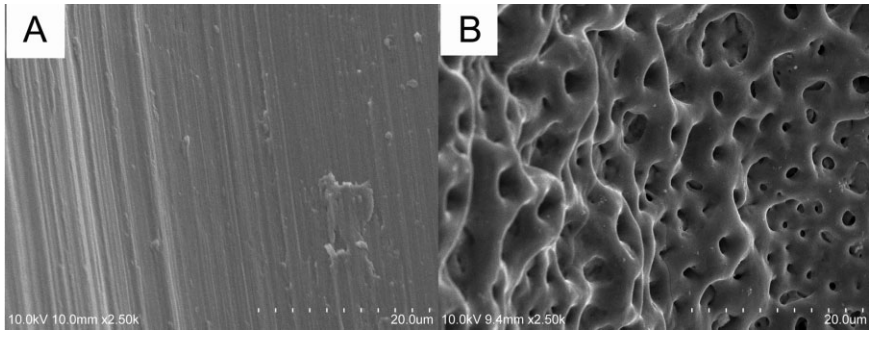

Figure 1 Scanning electron microscopy showing the surfaces of (A) control and (B) micro-arc oxidized Ti screws.

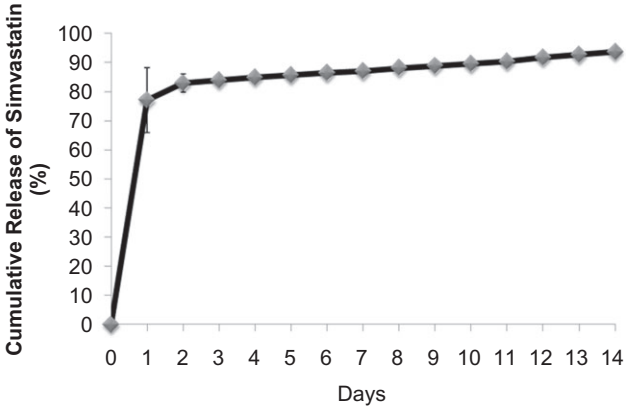

Figure 2 Release profile of simvastatin from micro-arc oxidized Ti screws.

Peri-Implant Bone Volume

Figure 3, A and B demonstrated peri-implant bone formation in micro-CT images in transverse and cross-sectional views. At 2 weeks, peri-implant bone volume measurements in micro-CT revealed significantly higher bone volumes in simvastatin-loaded implant groups than control and micro-arc oxidized groups ($p < .05$) (Figure 3C). There was no significant difference between two different simvastatin-loaded groups. At 4 weeks, all groups showed similar amount of peri-implant bone volumes (Figure 3D).

HISTOLOGICAL FINDINGS

At 2 weeks, control implants showed minimum bone formation in the threads located at cortical bone and marrow cavity. Micro-arc oxidized implants formed more bone than control group both at cortical and marrow cavity areas. On the other hand, both simvastatin-loaded implants revealed obviously more bone deposition directly on the implant surfaces at cortical bone as well as in the marrow cavity compared with those of control and micro-arc oxidized implants at 2 weeks. At the cortical bone region, active bone formation was observed with new bone already occupied the whole thread. In the marrow cavity, apparent amount of new bone was formed on the implant surface extending toward the periphery. Histological findings suggest that contact osteogenesis predominated on oxidized and simvastatin-loaded implant surfaces (Figure 4, B–D). At 4 weeks, bone formation around the implants in all groups appeared to be increased to the similar

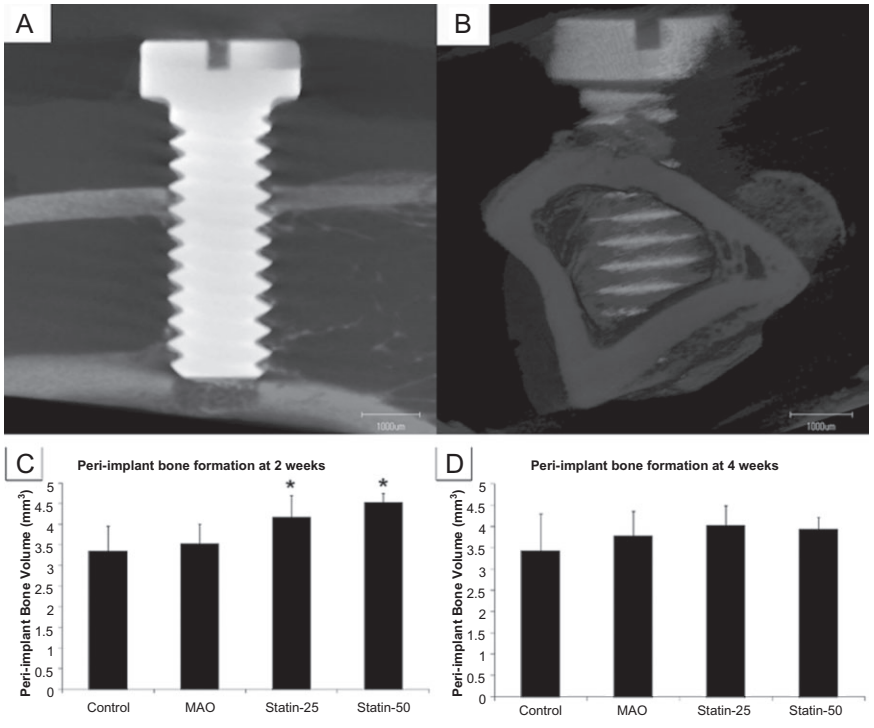

Figure 3 Upper panel shows microcomputerized tomography images of control Ti screw implant surgically inserted into rat tibia, (A) longitudinal section and (B) cross section. Lower panel shows peri-implant bone volume of control, micro-arc oxidized (MAO), 25-μg simvastatin-loaded (statin-25), and 50-μg simvastatin-loaded (statin-50) groups at (C) 2 weeks and (D) 4 weeks. *$p < .05$ compared with control and MAO groups.

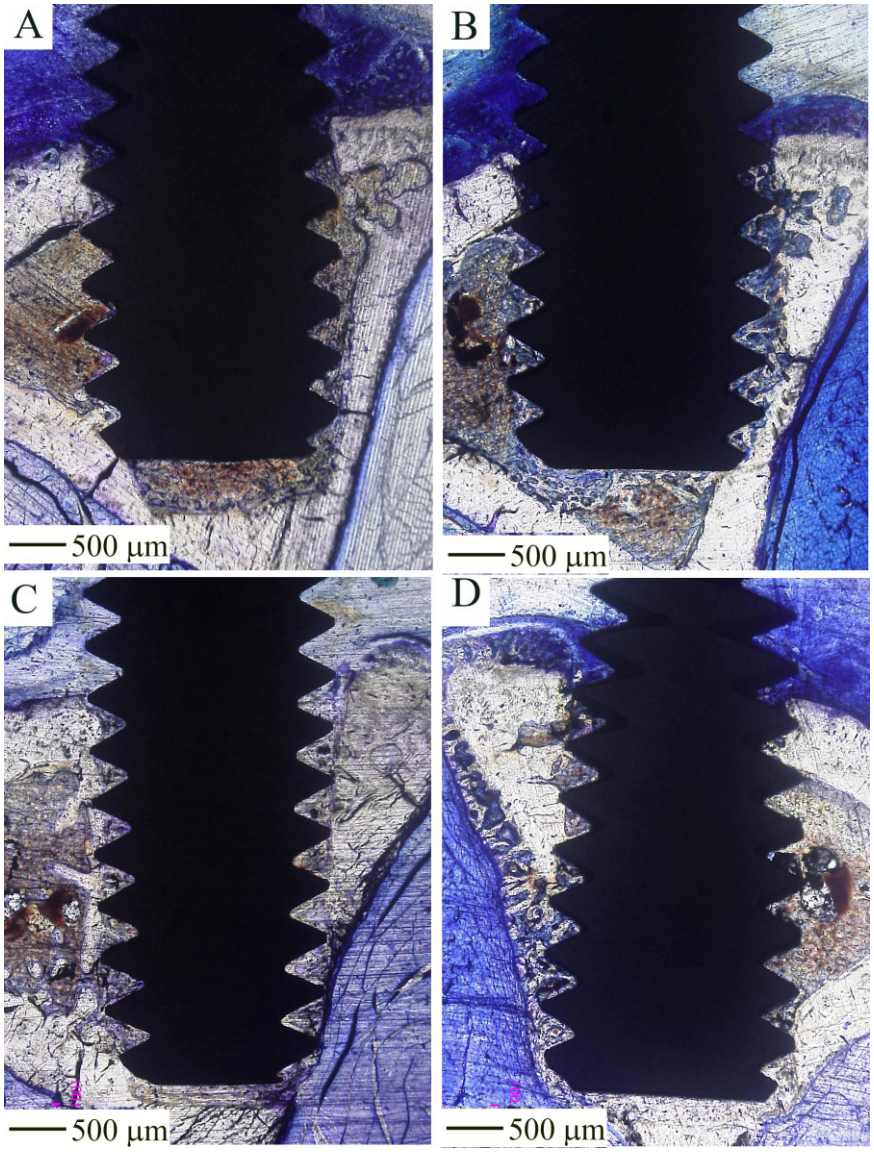

Figure 4 Photomicrographs showing bone deposition around (A) control, (B) micro-arc oxidized, (C) 25-µg simvastatin-loaded, and (D) 50-µg simvastatin-loaded Ti screws at 2 weeks, stained with toluidine blue.

extent especially in the bone marrow cavity, although amount of new bone formed directly on the implant surfaces remained less in control and micro-arc oxidized implants (Figure 5).

BIC

BIC values were significantly higher in simvastatin-loaded implant groups than control and micro-arc oxidized groups at both time points ($p < .05$). There was no significant difference between 25- and 50-µg simvastatin-loaded groups (Figure 6).

Mineral Appositional Rate

At 2 weeks, the mineral appositional rates were significantly higher in simvastatin-loaded implant groups than control and micro-arc oxidized groups ($p < .05$). At 4 weeks, although the rates appeared to be higher in the drug-loaded implant groups, the differences were not statistically significant (Figure 7, C and D).

DISCUSSION

With increasing application of early loading protocols and placement of implants in poor-quality bones,

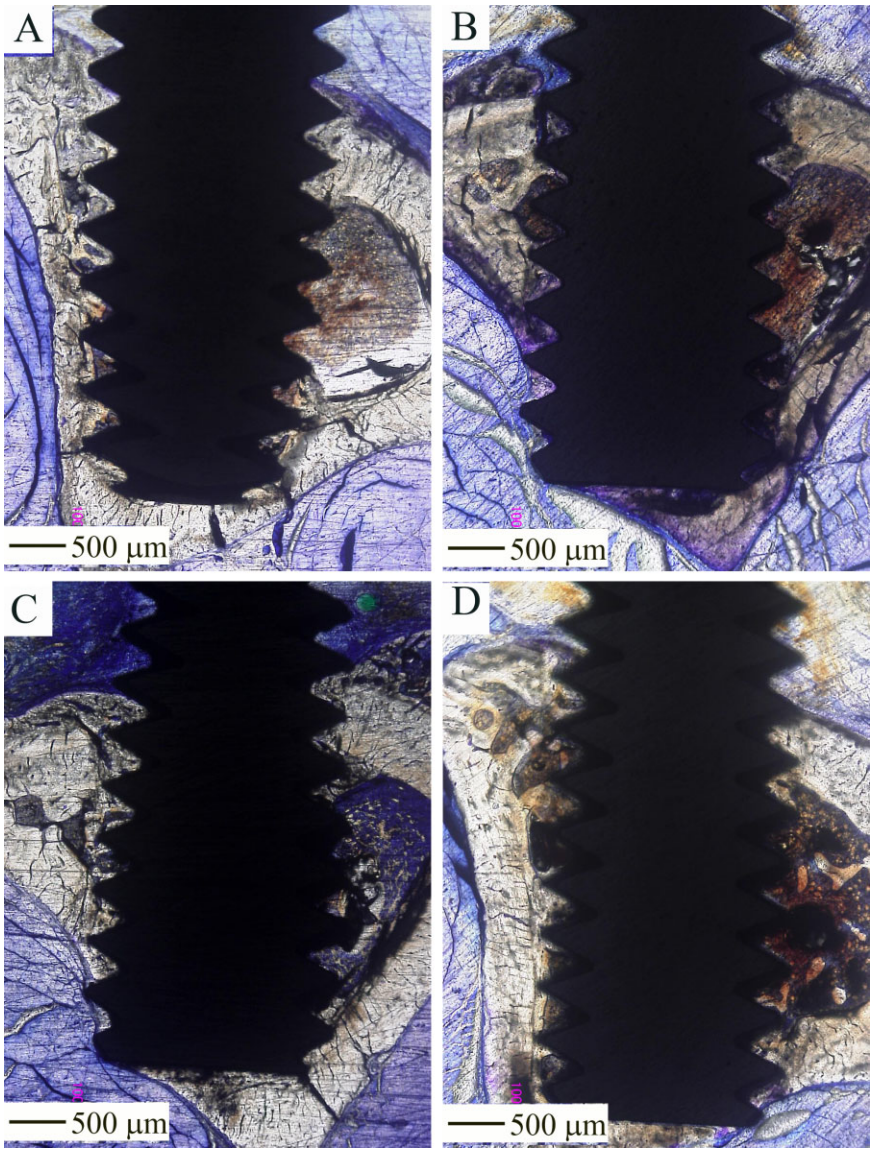

Figure 5 Photomicrographs showing bone deposition around (A) control, (B) micro-arc oxidized, (C) 25-μg simvastatin-loaded, and (D) 50-μg simvastatin-loaded Ti screws at 4 weeks, stained with toluidine blue.

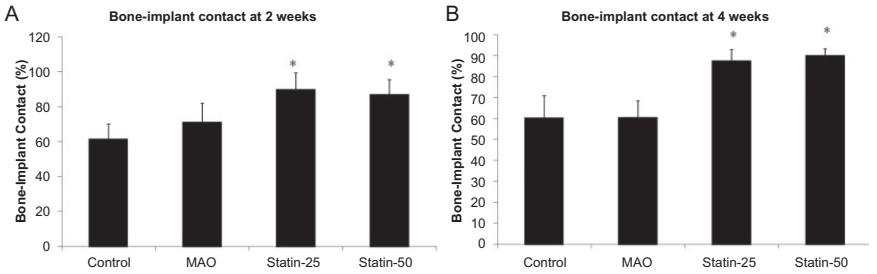

Figure 6 Bone-implant contact percentages of control, micro-arc oxidized (MAO), 25-μg simvastatin-loaded (statin-25), and 50-μg simvastatin-loaded (statin-50) groups at (A) 2 weeks and (B) 4 weeks. *$p < .05$ compared with control and MAO groups.

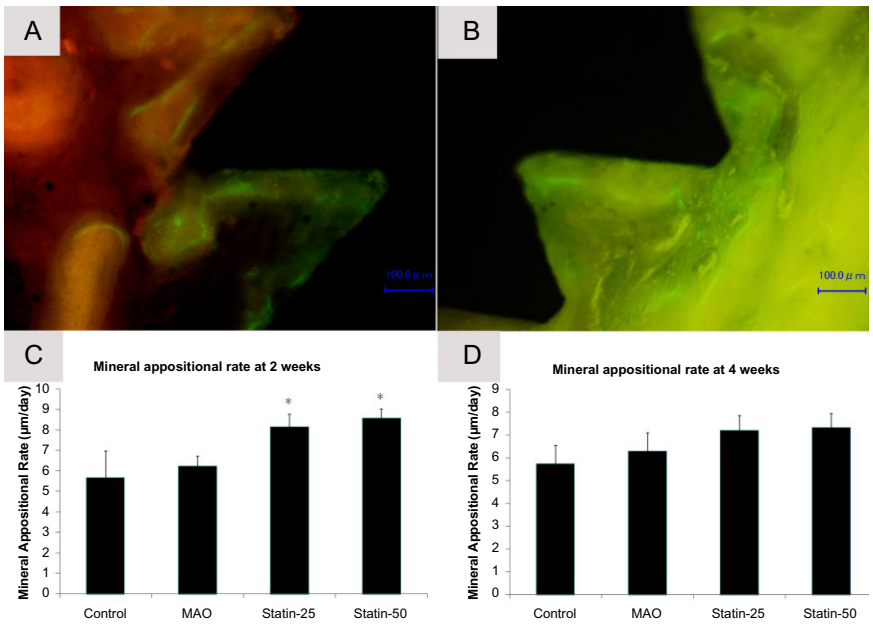

Figure 7 Upper panel demonstrates photomicrographs showing fluorochrome labels representing bone deposition around titanium screw implant loaded with 25 μg of simvastatin at (A) 2 weeks and (B) 4 weeks. Lower panel demonstrates mineral appositional rates of control, micro-arc oxidized (MAO), 25-μg simvastatin-loaded (statin-25), and 50-μg simvastatin-loaded (statin-50) groups at (C) 2 weeks and (D) 4 weeks. $^{*}p < .05$ compared with control and MAO groups.

there is a need for accelerated osseointegration and enhanced peri-implant bone regeneration especially in poor-quality bones. More promising implant surfaces are warranted in order to achieve the advantages of early implant loading such as decreasing treatment time and expenses, and increasing patient satisfaction.

In this study, we tested novel simvastatin-loaded porous titanium oxide surfaces. The titanium surface was oxidized by micro-arc technique producing porous titanium oxide surface. Recent evidences have shown the increased osseointegration and bone conduction by similar surfaces produced by anodizing and MAO processes.[30–39] This porous implant surface provides not only an excellent condition for bone cells but also interconnected pores to retain the drug efficiently and release effectively. Our study demonstrated that micro-arc oxidized porous titanium oxide surface can retain simvastatin efficiently. In vitro release pattern of simvastatin indicated that considerable amount of drug was released immediately after immersion into solution. Such burst release may be attributed to direct attachment of simvastatin on the oxidized titanium surface as no carrier material was used. It is speculated that similar immediate release of simvastatin would have been achieved at the local area around titanium screw implant, affecting the early cellular responses immediately after surgical implant placement. It is clear from the results that simvastatin-loaded implants showed more BIC as early as 2 weeks. The difference was more prominent in the region of marrow cavity, suggesting accelerated contact osteogenesis in simvastatin-loaded groups. Moreover, the mineral appositional rates of drug-loaded groups were faster and peri-implant bone volumes were greater in drug-loaded groups than control and nondrug-loaded groups especially at early time point. These findings suggest the accelerated and enhanced osseointegration and bone regeneration around the implant by simvastatin-loaded implants. In simvastatin-loaded groups, considerable amount of new bone was formed even on and around the implant threads located in the marrow cavity. However, the bone volume did not further increased by 4 weeks. All of the loaded simvastatin might have been released during the first 2 weeks and disappeared completely thereafter. At 4 weeks, peri-implant bone volume in all groups appeared to be similar especially in the marrow space areas. It may be due to increasing new bone formation by distance osteogenesis originating from inner aspect of cortical bone in all groups. Nevertheless, more matured peri-implant bone was observed in simvastatin-loaded groups at 4 weeks, suggesting faster peri-implant bone regeneration at 2 weeks and earlier remodeling by 4 weeks. It would be interesting to compare the effects of simvastatin with or without drug delivery carrier. More prolonged effect

of simvastatin may be achieved with the use of drug delivery carrier.

We tested 25 or 50 μg of simvastatin per implants. We chose these doses according to previous experiments with simvastatin affecting in certain area of bone. Both doses were effective to stimulate osseointegration and peri-implant bone regeneration without showing any untoward effects in our experiment.

Simvastatin is one of the lipid soluble statins that effectively stimulates bone formation when applied locally. Moreover, it is chemically stable and inexpensive. No carrier is needed to deliver the drug at the implant installation site when it is loaded directly onto the porous oxide layer on the implant surface. Osteogenic cells can attach and lay down bone matrix directly on the implant surface because there is no intervening layer of drug carrier on implant surface. We have recently reported that simvastatin upregulates BMP-2 and transforming growth factor-β with subsequent stimulation of osteogenic cell proliferation, migration, recruitment, and differentiation in the early phase of bone healing, leading to increased bone formation.[40] In the present study, it is speculated that simvastatin, which was released immediately from the porous Ti surface, enhanced growth factor expression, osteogenic cell proliferation, migration, recruitment, and differentiation, subsequently stimulating de novo bone formation directly on the implant surface (contact osteogenesis) in the similar manner. More bone was observed directly on the surfaces in simvastatin-loaded implants especially in the bone marrow cavity of tibia, whereas control surfaces showed less bone on the Ti surface. Bone formation seems to originate from the inner cortical bone surface extending toward the implant (distance osteogenesis) in control groups. Taken together, simvastatin-loaded titanium oxide surfaces would be potentially applicable in poor-quality bones to accelerate osseointegration and bone formation around implants.

CONCLUSIONS

The simvastatin loaded onto porous titanium oxide surface accelerated and enhanced osseointegration and peri-implant bone regeneration at early time point in our study model and it has potential to apply clinically in poor-quality bones. Further studies are necessary to validate the effects of the simvastatin-loaded implants in alveolar bones of the bigger animal models.

REFERENCES

1. Le Gu'ehennec L, Soueidan A, Layrolle P, Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. Dent Mater 2007; 23:844–854.
2. Shibli JA, Grassi S, de Figueiredo LC, et al. Influence of implant surface topography on early osseointegration: a histological study in human jaws. J Biomed Mater Res B Appl Biomater 2007; 80:377–385.
3. Buser D, Schenk RK, Steinemann S, Fiorellini JP, Fox CH, Stich H. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. J Biomed Mater Res 1991; 25:889–902.
4. Wong M, Eulenberger J, Schenk R, Hunziker E. Effect of surface topology on the osseointegration of implant materials in trabecular bone. J Biomed Mater Res 1995; 29:1567–1575.
5. Buser D, Nydegger T, Oxland T, 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 1999; 45:75–83.
6. Ellingsen JE, Johansson CB, Wennerberg A, Holmén A. Improved retention and bone-to-implant contact with fluoride modified titanium implants. Int J Oral Maxillofac Implants 2004; 19:659–666.
7. Cooper LF, Zhou Y, Takebe J, et al. Fluoride modification effects on osteoblast behavior and bone formation at TiO2 gritblasted c.p. titanium endosseous implants. Biomaterials 2006; 27:926–936.
8. Boyne P, Jones SD. Demonstration of the osseoinductive effect of bone morphogenetic protein within endosseous dental implants. Implant Dent 2004; 13:180–184.
9. Liu Y, de Groot K, Hunziker EB. BMP-2 liberated from biomimetic implant coatings induces and sustains direct ossification in an ectopic rat model. Bone 2005; 36:745–757.
10. Han Y, Hong SH, Xu K. Synthesis of nanocrystalline titania films by micro-arc oxidation. Mater Lett 2002; 56:744–747.
11. Li LH, Kong YM, Kim HW, et al. Improved biological performance of Ti implants due to surface modification by micro-arc oxidation. Biomaterials 2004; 25:2867–2875.
12. Wei D, Zhou Y, Jia D, Wang Y. Effect of heat treatment on the structure and in vitro bioactivity of microarc-oxidized (MAO) titania coatings containing Ca and P ions. Surf Coat Technol 2007; 201:8723–8729.
13. Han Y, Xu K. Photoexcited formation of bone apatite-like coatings on micro-arc oxidized titanium. J Biomed Mater Res 2004; 71A:608–614.
14. Mundy G, Garrett R, Harris S, et al. Stimulation of bone formation in vitro and in rodents by statins. Science 1999; 18:53–57.

15. Thylin MR, McConnell JC, Schmid MJ, et al. Effects of statin gels on murine calvarial bone. J Periodontol 2002; 73:1141–1148.
16. Wong RWK, Rabie ABM. Statin collagen grafts used to repair bone defects in the parietal bone of rabbits. Br J Oral Maxillofac Surg 2003; 41:244–248.
17. Stein D, Lee Y, Schmid MJ, et al. Local simvastatin effects on mandibular bone growth and inflammation. J Periodontol 2005; 76:1861–1870.
18. Sato D, Nishimura K, Ishioka T, Kondo H, Kuroda S, Kasugai S. Local application of simvastatin to rat incisor socket: carrier-dependent effect on bone augmentation. J Oral Tissue Eng 2005; 2:81–85.
19. Nyan M, Sato D, Oda M, et al. Bone formation with the combination of simvastatin and calcium sulfate in critical-sized rat calvarial defect. J Pharmacol Sci 2007; 104:384–386.
20. Garrett IR, Gutierrez GE, Rossini G, et al. Locally delivered lovastatin nanoparticles enhance fracture healing in rats. J Orthop Res 2007; 25:1351–1357.
21. Ozec I, Kilic E, Gumus C, Goze F. Effect of local simvastatin application on mandibular defects. J Craniofac Surg 2007; 18:546–550.
22. Wang JW, Xu SW, Yang DS, Lv RK. Locally applied simvastatin promotes fracture healing in ovariectomized rat. Osteoporos Int 2007; 18:1641–1650.
23. Wu Z, Liu C, Zang G, Sun H. The effect of simvastatin on remodeling of the alveolar bone following tooth extraction. Int J Oral Maxillofac Surg 2008; 37:170–176.
24. Lee Y, Schmid MJ, Marx DB, et al. The effect of local simvastatin delivery strategies on mandibular bone formation in vivo. Biomaterials 2008; 29:1940–1949.
25. Ma B, Clarke SA, Brooks RA, Rushton N. The effect of simvastatin on bone formation and ceramic resorption in a peri-implant defect model. Acta Biomater 2008; 4:149–155.
26. Moriyama Y, Ayukawa Y, Ogino Y, Atsuta I, Koyano K. Topical application of statin affects bone healing around implants. Clin Oral Implants Res 2008; 19:600–605.
27. Jeon JH, Piepgrass WT, Lin YL, Thomas MV, Puleo DA. Localized intermittent delivery of simvastatin hydroxyacid stimulates bone formation in rats. J Periodontol 2008; 79:1457–1464.
28. Ayukawa Y, Yasukawa E, Moriyama Y, et al. Local application of statin promotes bone repair through the suppression of osteoclasts and the enhancement of osteoblasts at bone-healing sites in rats. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 107:336–342.
29. Nyan M, Sato D, Kihara H, Machida T, Ohya K, Kasugai S. Effects of the combination with alpha-tricalcium phosphate and simvastatin on bone regeneration. Clin Oral Implants Res 2009; 20:280–287.
30. Sul YT, Johansson CB, Jeong Y, Roser K, Wennerberg A, Albrektsson T. Oxidized implants and their influence on the bone response. J Mater Sci Mater Med 2001; 12:1025–1031.
31. Sul YT, Johansson C, Wennerberg A, Cho LR, Chang BS, Albrektsson T. Optimum surface properties of oxidized implants for reinforcement of osseointegration: surface chemistry, oxide thickness, porosity, roughness, and crystal structure. Int J Oral Maxillofac Implants 2005; 20:349–359.
32. Xiropaidis AV, Qahash M, Lim WH, et al. Bone-implant contact at calcium phosphate-coated and porous titanium oxide (TiUnite)-modified oral implants. Clin Oral Implants Res 2005; 16:532–539.
33. Huang YH, Xiropaidis AV, Sorensen RG, Albandar JM, Hall J, Wikesjo UM. Bone formation at titanium porous oxide (TiUnite) oral implants in type IV bone. Clin Oral Implants Res 2005; 16:105–111.
34. Sul YT, Johansson CB, Roser K, Albrektsson T. Qualitative and quantitative observations of bone tissue reactions to anodised implants. Biomaterials 2002; 23:1809–1817.
35. Sul YT, Johansson CB, Jeong Y, Wennerberg A, Albrektsson T. Resonance frequency and removal torque analysis of implants with turned and anodized surface oxides. Clin Oral Implants Res 2002; 13:252–259.
36. Rocci A, Martignoni M, Gottlow J. Immediate loading of Branemark System TiUnite and machined-surface implants in the posterior mandible: a randomized open-ended clinical trial. Clin Implant Dent Relat Res 2003; 5:S57–S63.
37. Jungner M, Lundqvist P, Lundgren S. Oxidized titanium implants (Nobel Biocare TiUnite) compared with turned titanium implants (Nobel Biocare mark III) with respect to implant failure in a group of consecutive patients treated with early functional loading and two-stage protocol. Clin Oral Implants Res 2005; 16:308–312.
38. Schupbach P, Glauser R, Rocci A, et al. The human bone-oxidized titanium implant interface: a light microscopic, scanning electron microscopic, back-scatter scanning electron microscopic, and energy-dispersive x-ray study of clinically retrieved dental implants. Clin Implant Dent Relat Res 2005; 7:36–43.
39. Sul YT, Johansson CB, Albrektsson T. Oxidized titanium screws coated with calcium ions and their performance in rabbit bone. Int J Oral Maxillofac Implants 2002; 17: 625–634.
40. Nyan M, Miyahara T, Noritake K, et al. Molecular and cellular mechanisms in the healing of rat calvarial defects after local application of simvastatin combined with alpha tricalcium phosphate. J Biomed Mater Res B Appl Biomater 2009; 93B:65–73.