Bone Responses to Rough Titanium Implants Coated With Biomimetic Ca-P in Rabbit Tibia

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Abstract: A new technique to biomimetic deposition calcium phosphate (BDCaP) coating onto rough titanium substrates has been developed recently. This biomimetic deposition technique seems to be promising. It appears to have some advantages such as an inexpensive and simple set-up, and the possibility to synthesize layers with a defined surface morphology. The aim of this study was to examine the bone responses to BDCaP-coated implants in a rabbit model. Thirty one implants (16 BDCaP and 15 rough) were inserted into both tibia of 15 rabbits. After 2, 4, and 8 weeks following the implantation, the tibias were retrieved and prepared for histological evaluation. After 2 weeks, BDCaP-coated implant showed more bone ingrowth inside threads than the rough implants in medullary region (31.43% vs. 24.38%). Histological and quantitative histomorphometrical measurements demonstrated no more bone ingrowth and bone-implant contact for coated implant as compared with uncoated implant in cortical region at all experiment periods. From the histological viewpoint, the BDCaP coating did not have any positive effect on new bone formation. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 90B: 857–863, 2009

Keywords: implants; rabbits; biomimetic; calcium phosphate; coating; bone ingrowth

INTRODUCTION

Calcium phosphate coatings have been demonstrated to increase the bioactivity and success rate of titanium and titanium alloys for some special dental and orthopedic cases, such as dental implants, hip and knee joints, and fracture fixation.[1] There are many techniques to coat titanium implants with calcium phosphate, the most common being the plasma-spray technique. However, the very high temperatures used in the process may alter the chemical structure of the hydroxyapatite (HA), and the "line-of-sight" application does not permit uniform coating of complex surface geometries.[2] To overcome these drawbacks, several other deposition methods have been developed.

Among the methods, the biomimetic deposition calcium phosphate (BDCaP) coating is a promising technique, which allows a coating to be deposited on substrates in a simulated body fluid (SBF) under physiological conditions of temperature and pH. This biomimetic method, originally developed by Kokubo et al.,[3] has since undergone modification and refinement by several groups of researchers.[4–10] It is simple to perform, is cost-effective and can be applied even to heat-sensitive, nonconductive, and porous materials of large dimensions and with complex surface geometries.[11] It has reported that the biomimetic deposition of carbonate apatite (BCA) coating by using five times concentrated SBF within 48 h[4,9,10] or octacalcium phosphate (OCP) coating by using supersaturated Ca-P solution within 72 h.[7] This coating may, therefore, be useful in facilitating early bone ingrowth into porous surfaces without the potential for coating debris, macrophage infiltration, fibrous tissue encapsulation, and eventual coating failure as may occur with the plasma-sprayed hydroxapatite coating.[12] Furthermore, these coatings can be osteoconductive and osteoinductive (by incorporating osteogenetic agents).[11,13,14]

Some studies on various biomimetic calcium phosphate coatings have shown enhanced bone-implant contact and gap healing in a goat model,[15–17] increased fixation strength

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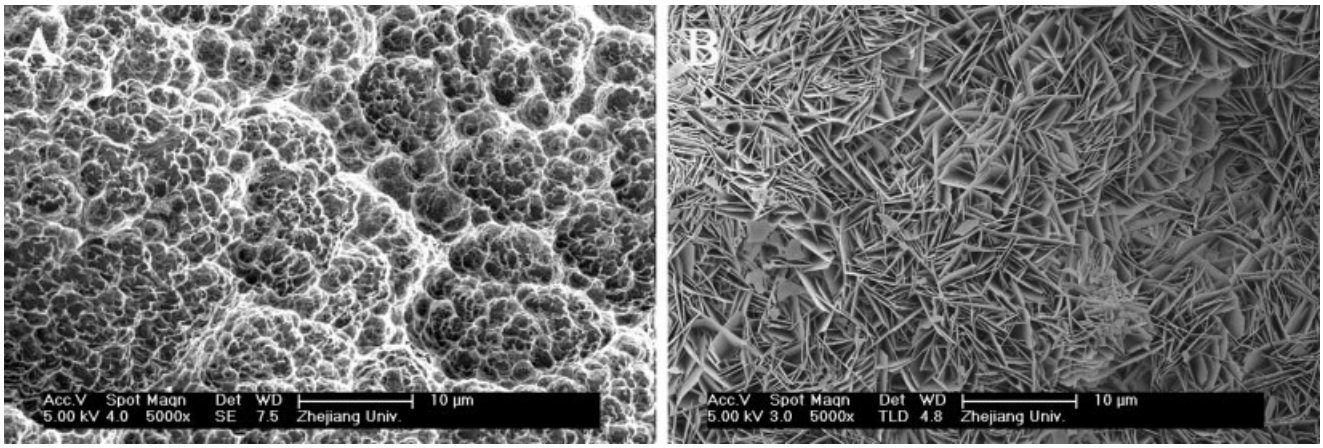

Figure 1. SEM images of the SAH and the coating: (A) SAH, (B) BDCaP coating.

in a rabbit model,[18,19] enhanced early implant fixation in rat model,[2] and stimulated early osseointegration with the metal surface in dog model.[12] In an *in vivo* study, the biomimetic coating appeared to be resorbed after 8 weeks and did stimulate early osseointegration with the metal surface with a reduction in fibrous tissue encapsulation.[12] OCP coating on porous implants could induce ectopic bone in muscles of goat after 12 weeks of implantation while the OCP-coated porous cylinders exhibited bone formation in the center of the implant in the femoral condyle.[15]

Recently, a modified biomimetic technique to deposition calcium phosphate coatings onto roughened titanium substrate has been developed.[20,21] The BCA coating[20] or the compound of HA and OCP coating[21] on the multilevel micropits pure titanium surface were attained. These new BDCaP coatings on a rough surface may promote a faster bone apposition in comparison to the uncoated rough surface. But in a recent animal study, the mean removal torque values of the BDCaP (compound of HA and OCP)-coated implants were not significantly higher than that of the uncoated rough implants after 2, 4 weeks of healing period ($p > 0.05$), and the mean removal torque values of BDCaP-coated implants revealed slightly lower than that of the uncoated rough implants after 6, 8, 12 weeks of healing.[22] However, it is not clear whether this coating increases bone formation around implant at early stage.

The aim of this study was, therefore, to investigate whether this new biomimetic coating is favorable for implant osseointegration at the early stage of implantation in a rabbit model.

MATERIALS AND METHODS

The implant materials, the surface topography, and Ca-P characterization have been described in detail elsewhere.[22] In summary, the roughened surface (SAH) was prepared by sandblasting, acid etching, and treatment with a solution containing H_2O_2 and HCl, and then heat treating. The SEM images show a level of macropits in the dimension of about 10–30 μm, each of which embracing an arrangement of smaller, round-shaped micropits with diameters of about 0.5–3 μm [Figure 1(A)]. The coating procedure was performed using a biomimetic deposition method.[21] A thin plate-like calcium-phosphate coating was deposited on the rough substrate surface that was proved to be the mixture of hydroxycarbonated apatite (HA) and OCP, the coating's Ca/P ratio was 1.51.[21] An excellent coverage of the surface was detected by SEM [Figure 1(B)].

Experimental Design and Implantation Procedure

Thirty-one screw designed commercially pure titanium implants with an external diameter of 3.0 mm and a length of 8 mm (Xihu biomaterial research institute, Hangzhou, China) were placed in the proximal tibias of the left and right hind limbs of 16 adult rabbits. The diameter of the implant neck was 3.5 mm. The pitch of the thread was 0.7 mm and the depth of thread was 0.35 mm. By intermittent drilling with a low rotary speed and profuse saline irrigation, one hole was drilled in the medial surface of each tibia and sequentially enlarged to 3.0 mm in diameter. The implant was gently screwed into place until the implant neck wedged tightly into the cortical bone. All implants penetrated the first cortical bone through bone marrow into the opposite cortical bone. Each rabbit received one SAH implant and one BDCaP-coated implant.

To testify whether the coating still remained on the implant surface after implantation, one BDCaP-coated implant was placed in the last rabbit in order to prepare for backscattered SEM analysis.

Histological and Histomorphometric Evaluation

Rabbits were euthanized by using an overdose of SuMian-Xin (1.0 mL, i.m. Quartermaster University of PLA, Chang Chun, People's Republic of china, The Military Veterinary Institute) at 2, 4, and 8 weeks following the implantation. The section preparation process has been described in detail elsewhere.[23] In summary, tibia blocks including the

implant and surrounding tissue were dissected from all rabbits. Specimens were stored in 10% neutral buffered formalin for 5–7 days. Undecalcified cut and ground sections were prepared with the EXAKT system. One section was taken from the central part of each specimen. Section was cut to a thickness of 200 μm and ground and polished to a final thickness of $\sim$30 μm. Section was stained with Stevenel's blue and van Gieson's picro fuchsin for histomorphometric evaluation.

The animal with one BDCaP-coated implant was immediately euthanized after implantation. One undecalcified section with a thickness of 200 μm was achieved. This section and the others remained undecalcified blocks after cutting was prepared and polished, gold sputtered, and was analyzed by a field-emission scanning electron microscope equipped with a solid-state, four diode backscattering electron (BSE) detector plus an auxiliary EDS microanalytical system (EDX, EDAX, GENENIS4000). So the fate of the BDCaP coating versus implantation time was observed.

Incandescent light microscopy (BX51, Olympus, Japan) was used to observe the histological behavior. One experienced examiner performed the histomorphometric analysis by using light microscopy and a PC-based image analysis system (Image-Pro Plus®, Media Cybernetics, Silver Springs, MD). The histological and histomorphometrical analysis consisted of: (1) bone to implant contact percentage (BIC) in cortical region, (2) bone area percentage inside the implant threads in cortical region, (3) bone to implant contact percentage and bone area percentage inside the implant threads in medullary region at 2 weeks.[24]

Statistics Analysis

Histomorphometric data were statistically analyzed. Group means and standard deviations were used to calculate each parameter. Differences between experimental samples were analyzed by using a one-way ANOVA with *post-hoc* tests. A p-value <0.05 was required for statistical significance. The software of SPSS 12.0 (SPSS, Chicago, IL) was used for all statistical analysis.

RESULTS

During the test periods, the experimental animals remained in good health. At sacrifice, neither clinical signs of inflammation nor adverse tissue reactions were observed. All implants were still *in situ* at sacrifice.

Histological Observation

Implantation Time 2 Weeks. The overall bone response to the two different surfaces was similar. The original drill cavity could always be recognized (Figure 2). Newly formed woven bone tissue was filled in the drill cavity and also could be observed in the periosteal and endosteal region as indicated by deep red immature bone, while the bone tissue structures in the original cortex were light red. These phenomena also called periosteal reaction.[25] Osteoblasts were lining the woven bone trabeculae, and some lamellar segments were presented. All implants in the control and experimental groups were histologically in direct contact with the surrounding woven bone in cortical region along their threads and neck. The BDCaP-coated implants showed more active bone formation in the medullary cavity compared with that of the control implants. In the medullary cavity, the woven bone was in direct contact with roughened substrate of the implant without any intervening fibrous tissue layers (Figure 3). Only in some region, the BDCaP coating remained, and the thickness of the remaining coating was about 17 μm (Figure 4).

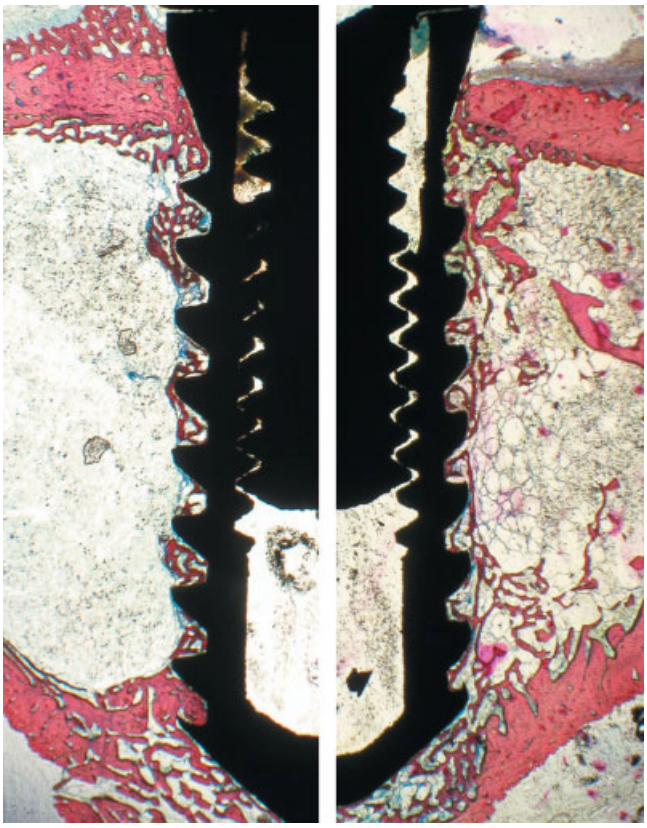

Figure 2. Histological section of SAH (left, control) and BDCaP-coated (right) implants 2 weeks after implantation in rabbit tibiae. The BDCaP implant shows more woven bone around the medullary cavity, which is devoid of surrounding bone, compared with that of the SAH implant (original magnification, $\times$12.5). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Implantation Time 4 Weeks. In these specimens, the healing process had proceeded. In all sections, osteoblasts synthesized lamellar bone on woven bone surfaces and then built up tissue deposition circumferentially around and toward the central vessel. So the primary woven bone or osteons progressed by lamellar compaction to partially compacted (Figure 5). Because of the extensive bone formation, the original drill hole could not recognize easily.

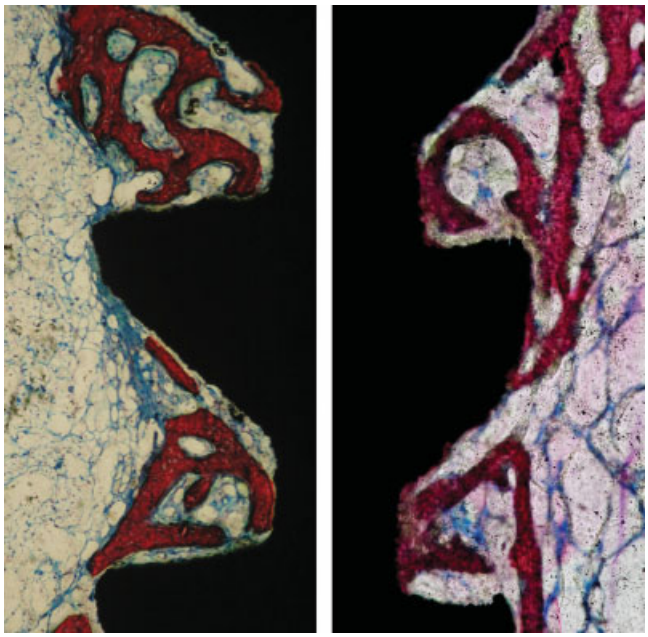

Figure 3. Histological section of SAH (left) and BDCaP-coated (right) implants 2 weeks after implantation in medullary cavity. The bone-implant contact of the BDCaP-coated implants was broader than that of the SAH implants (original magnification, ×100). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The primary woven bone around implant in the medullary region mostly disappeared and only few mineralized bone was in direct contact with the roughened substrate, especially inside the threads. Few BDCaP coating remained, and the thickness was about 8 μm (Figure 4).

Implantation Time 8 Weeks. The bony contact improved, and no marked histological differences were noted on both implant surfaces. Fully compacted bone reached the roughened titanium surface and a broad bone contact was observed around both implant surfaces. It was difficult to distinguish between the new bone and the old cortical bone. In some regions, signs of ongoing bone remodeling with resorption and apposition were observed. The endosteal tissue reaction on the control implant surface seemed to be more pronounced compared to the BDCaP-coated implant surface, namely more new bone growth is directed toward and downward the control implant surface. More medullary cavities were found among cortical region around the control implant surface than that around the BDCaP-coated implant surface (Figure 6). There was no remaining BDCaP coating observed on the interface between titanium implant and bone tissue both at cortical bone region and marrow cavity.

Histomorphometrical Evaluation

Histomorphometric results of BIC in the cortical bone region are shown in Table I. No significant differences were found between two surfaces during all periods ($p > 0.05$). Results of bone area percentage inside threads in the cortical bone region are shown in Table II, there were also no significant differences during all periods ($p > 0.05$).

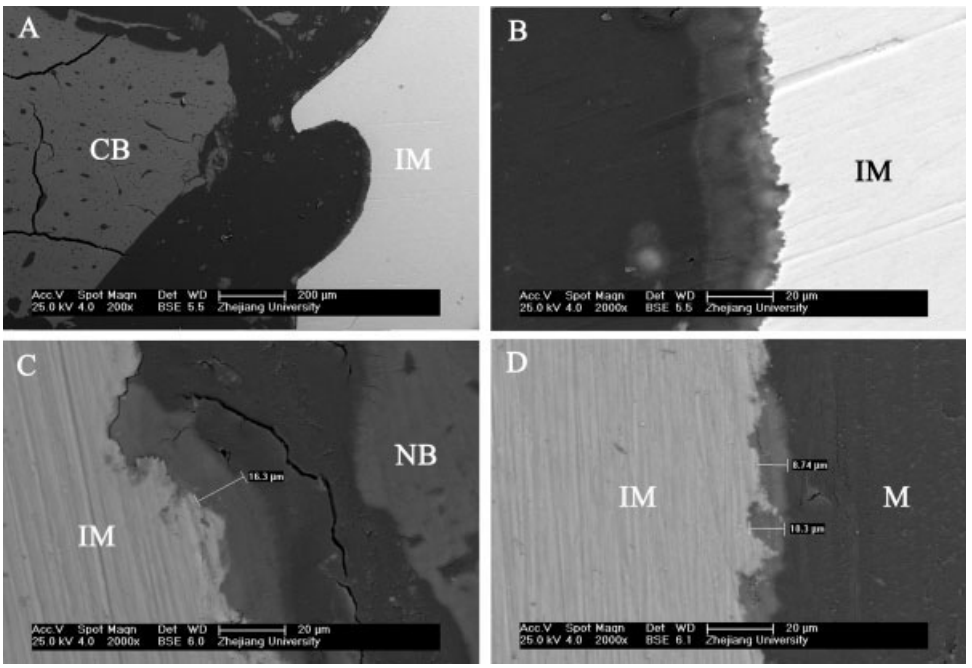

Figure 4. Backscattered SEM micrographs of the BDCaP-coated implant immediately after implantation (A, B), and after 2 (C) and 4 (D) weeks of healing. (A) At cortical region, the coating was observed obviously, only few coating was scrapped on the thread pitch. (B) The coating thickness was about 25 μm. (C, D) The coating was found on some areas of implant surface. CB, cortical bone; M, marrow cavity; IM, implant; NB, new bone.

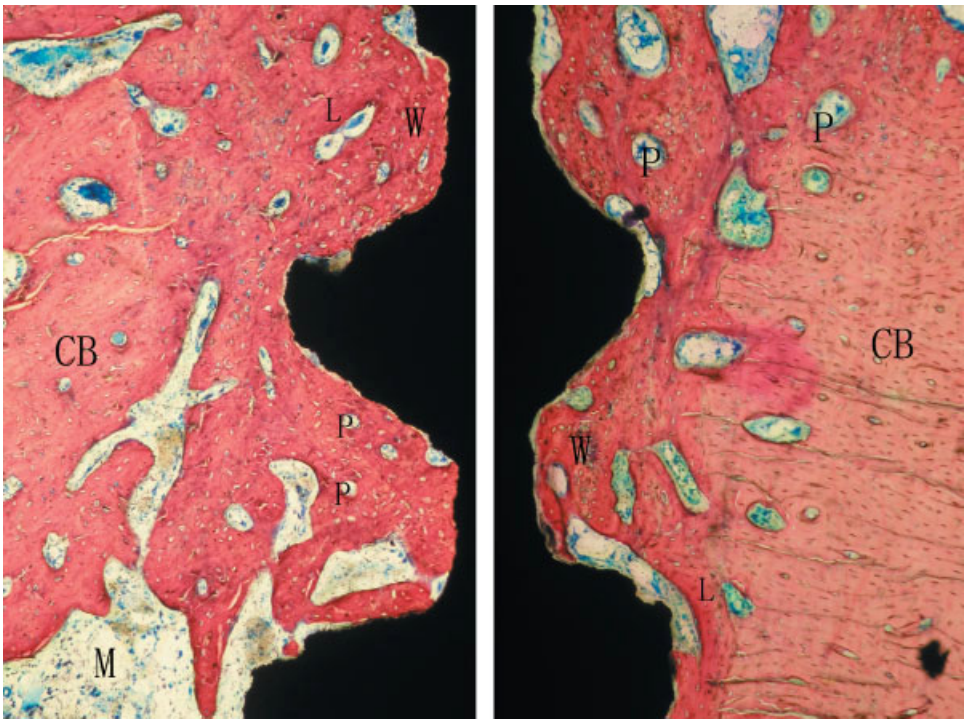

Figure 5. Histological section of SAH (left) and BDCaP-coated (right) implants 4 weeks after implantation in rabbit tibiae. Pictures have been taken in the cortical bone region and thus at approximately similar locations. Woven bone (W) has been covered with more recently synthesized lamellar bone (L). The lamellar bone was increasing and organizing to compact the tissue. CB, cortical bone; M, marrow cavity; P, primary osteon (original magnification, ×100). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

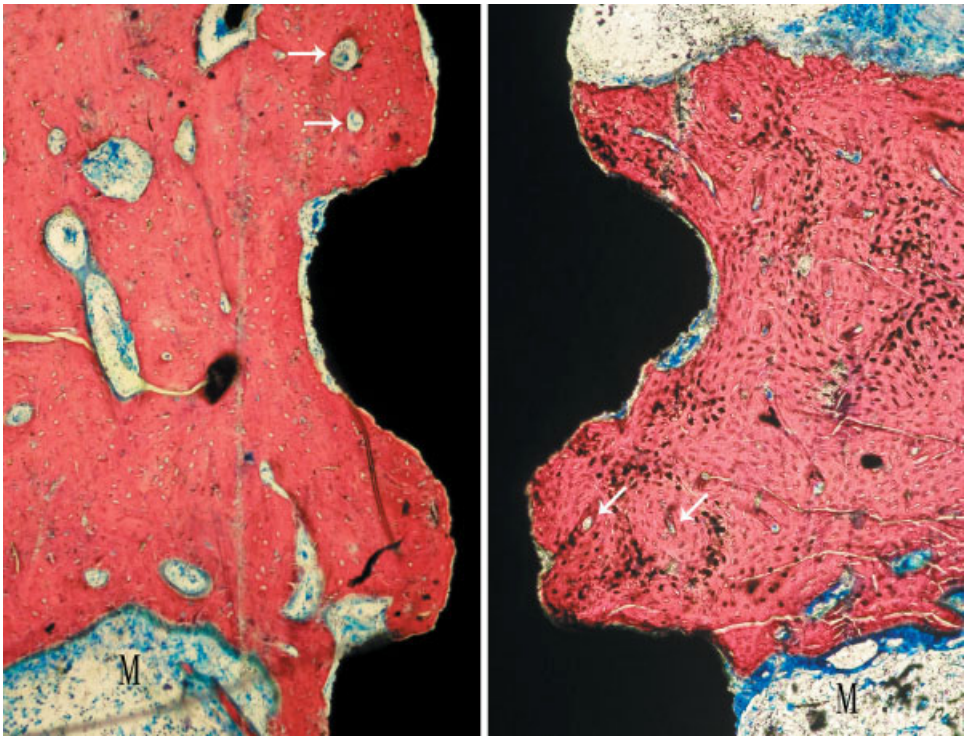

Figure 6. Histological section of SAH (left) and BDCaP-coated (right) implants 8 weeks after implantation in rabbit tibiae. Pictures have been taken in the cortical bone region and thus at approximately similar locations. The new bone near the BDCaP-coated surface seemed more compacted as compared with that of the control surface. White arrows, osteons; M, marrow cavity (original magnification, ×100). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I. Histomorphometric Results, Percentage of Direct Bone–Implant Contact (%) in Cortical Bone Region

	2 Weeks	4 Weeks	8 Weeks
SAH	52.44 ± 12.05	59.95 ± 9.56	69.72 ± 8.19
BDCaP	45.04 ± 5.13	66.09 ± 10.18	73.54 ± 6.36
p-Value	0.336	0.148	0.574

Histomorphometric results of BIC and bone area percentage inside threads in medullary cavity at 2 weeks are shown in Table III. No significant differences were seen in BIC and bone area between two surfaces ($p > 0.05$).

DISCUSSION

This study investigated whether the novel BDCaP coating on roughened implant surface promotes early bone apposition. The results have shown that the bone area percentage inside implant threads in medullary region on BDCaP group was 28.9% higher than that on SAH group after 2 weeks of implantation. The high BIC% and bone area percentage in the medullary canal of the rabbit tibia for both implant surfaces, which was characterized by an absence of cancellous bone, indicating the improved osteoconductive properties of both surfaces. Although there was no statistical difference, this suggested that the BDCaP coating may improve the osteoconductivity of the roughened SAH implant. But only rarely bone was observed inside implant threads in medullary region after 4 and 8 weeks of implantation, this indicated that bone formation was confined in time, namely, new bone did not form continuously with prolonging implantation time and on the contrary it was resorbed. This result was similar to Barrere's results[15] that bone induced by an OCP coating degraded with time.

The BIC and bone area inside threads in the cortical region did not show significant differences between two groups during all periods. The present results were accorded with our previous mechanical results.[22] But these results were different as compared to others studies. Bone contact has been found significantly higher for BCA-coated dense Ti6Al4V and porous Ta cylinders than the corresponding noncoated implants.[17] Results of that study show that the BCA coating enhances the bone integration as compared to the noncoated implants. It was also reported that *de novo* bone formation was observed on the cell-seeded crystalline OCP-coated discs that were implanted in the backs of nude mice 4 weeks, and thought the coating was more suitable for bone tissue engineering.[26] A 4-μm HA coating was prepared on Ce-TZP/Al_2O_3 nanocomposite plate surface by a biomimetic route, namely, plate was soaked in Kokubo's SBF[3] at 37°C for 5 days, which can improve the bone-bonding ability of plate after 4 weeks of implantation.[27] The differences between our results and other study results could be related to some sort of particular characteristic of the present BDCaP coating process, such as purity level, crystallinity, calcium/phosphate ratio, and the presence of other calcium-phosphate phases. The results may also be attributed to different animal models, implant patterns/surfaces, and biomimetic processes.

TABLE II. Histomorphometric Results, Bone Area Percentage Inside Implant Threads (%) in Cortical Portion

	2 Weeks	4 Weeks	8 Weeks
SAH	60.85 ± 7.69	72.41 ± 8.63	83.16 ± 9.35
BDCaP	59.09 ± 18.88	65.43 ± 4.25	80.94 ± 11.05
p-Value	0.882	0.137	0.744

TABLE III. Histomorphometric Results in Medullary Cavity at 2 Weeks

	Percentage of Direct Bone–Implant Contact (%)	Bone Area Percentage Inside Implant Threads (%)
SAH	18.71 ± 5.04	24.38 ± 1.99
BDCaP	20.27 ± 7.47	31.43 ± 13.48
p-Value	0.727	0.407

However, there were some reports that were similar to our results. The histomorphometric linear ingrowth percentages were not statistically significant between the apatite-coated samples and uncoated samples at 6, 8, 12 weeks after implantation, but were trending toward significance ($p = 0.065$, at 6 weeks).[12] Ekholm et al.[28] applied Kokubo's biomimetic process to coat cellulose sponges with HA and implanted the sponges into the femurs of rats, which generated a strong and highly cellular inflammatory reaction and less osteoid tissue. Recently Zagury et al.[29] have reported that the BIC of the biomimetic HA-coated implants showed no statistically significant differences as compared with titanium alloy samples (67.31% vs. 63.10%) inserted in rabbits tibiae after 3 months healing periods. The HA biomimetic coating that Zagury et al.[29] applied was performed according to the protocol described by Kokubo and coworkers[18] and modified by Andrade. The novel BDCaP coating maybe degraded too quickly to induce new bone formation at a relative long period. This point was agreed with Bernstein's conclusion that degradable calcium phosphate coatings have the potential to stimulate bone regeneration, but the less rapidly degrading material achieves better results.[30]

Although the novel BDCaP coating has little effect on bone formation, it has the osteoinductive property and may be as a carries of some growth factors, like bone morphogenetic proteins. Additionally, for osteoporosis patients with a need for implant therapy, some osteoporosis drugs, such as bisphosphonate, may attain the best efficacy in the implant sites by using the biomimetic coating. Future studies are needed to investigate these topics.

CONCLUSION

From the histological viewpoint, the BDCaP coating did not have any positive effect on new bone formation. However, further detailed studies are needed to better understand the effects of BDCaP-coated surface on osteoblast and osteoclast responses and bone responses.

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REFERENCES

1. Clemens JA, Klein CP, Vriesde RC, Rozing PM, de Groot K. Healing of large (2 mm) gaps around calcium phosphate-coated bone implants: A study in goats with a follow-up of 6 months. J Biomed Mater Res 1998;40:341–349.
2. Kuroda S, Virdi AS, Li P, Healy KE, Sumner DR. A low-temperature biomimetic calcium phosphate surface enhances early implant fixation in a rat model. J Biomed Mater Res A 2004;70:66–73.
3. Kokubo T, Kushitani H, Sakka S, Kitsugi T, Yamamuro T. Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramic A-W. J Biomed Mater Res 1990;24:721–734.
4. Barrere F, van Blitterswijk CA, de Groot K, Layrolle P. Influence of ionic strength and carbonate on the Ca-P coating formation from SBFx5 solution. Biomaterials 2002;23:1921–1930.
5. de Groot K, Wolke JG, Jansen JA. Calcium phosphate coatings for medical implants. Proc Inst Mech Eng [H] 1998;212: 137–147.
6. Barrere F, Layrolle P, van Blitterswijk CA, de Groot K. Biomimetic calcium phosphate coatings on Ti6AI4V: A crystal growth study of octacalcium phosphate and inhibition by Mg2+ and HCO3. Bone 1999;25(2 Suppl):107S–111S.
7. Barrere F, Layrolle P, Van Blitterswijk CA, De Groot K. Biomimetic coatings on titanium: A crystal growth study of octacalcium phosphate. J Mater Sci Mater Med 2001;12:529–534.
8. Liu Y, Layrolle P, de Bruijn J, van Blitterswijk C, de Groot K. Biomimetic coprecipitation of calcium phosphate and bovine serum albumin on titanium alloy. J Biomed Mater Res 2001;57:327–335.
9. Habibovic P, Barrere F, van Blitterswijk CA, de Groot K, Layrolle P. Biomimetic hydroxyapatite coating on metal implants. J Am Ceram Soc 2002;85:517–522.
10. Barrere F, van BC, de Groot K, Layrolle P. Nucleation of biomimetic Ca-P coatings on ti6A14V from a SBF x 5 solution: influence of magnesium. Biomaterials 2002;23:2211–2220.
11. Liu Y, Li JP, Hunziker EB, de Groot K. Incorporation of growth factors into medical devices via biomimetic coatings. Philos Trans A Math Phys Eng Sci 2006;364:233–248.
12. Vasudev DV, Ricci JL, Sabatino C, Li P, Parsons JR. In vivo evaluation of a biomimetic apatite coating grown on titanium surfaces. J Biomed Mater Res A 2004;69:629–636.
13. Liu Y, de Groot K, Hunziker EB. Osteoinductive implants: The mise-en-scene for drug-bearing biomimetic coatings. Ann Biomed Eng 2004;32:398–406.
14. 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.
15. Barrere F, van der Valk CM, Dalmeijer RA, Meijer G, van Blitterswijk CA, de Groot K, Layrolle P. Osteogenecity of octacalcium phosphate coatings applied on porous metal implants. J Biomed Mater Res A 2003;66:779–788.
16. Barrere F, van der Valk CM, Dalmeijer RA, van Blitterswijk CA, de Groot K, Layrolle P. In vitro and in vivo degradation of biomimetic octacalcium phosphate and carbonate apatite coatings on titanium implants. J Biomed Mater Res A 2003;64:378–387.
17. Barrere F, van der Valk CM, Meijer G, Dalmeijer RA, de Groot K, Layrolle P. Osteointegration of biomimetic apatite coating applied onto dense and porous metal implants in femurs of goats. J Biomed Mater Res B Appl Biomater 2003;67:655–665.
18. Yan WQ, Nakamura T, Kawanabe K, Nishigochi S, Oka M, Kokubo T. Apatite layer-coated titanium for use as bone bonding implants. Biomaterials 1997;18:1185–1190.
19. Yan WQ, Nakamura T, Kobayashi M, Kim HM, Miyaji F, Kokubo T. Bonding of chemically treated titanium implants to bone. J Biomed Mater Res 1997;37:267–275.
20. He FM, Chen S, Liu L, Zhao SS, Sheng ZL, Wang XX. Experimental study of fast formation of biomimetic apatite coatings on pure titanium surface. Zhonghua Kou Qiang Yi Xue Za Zhi 2006;41:240–241.
21. He F, Liu L, Zhao S, Zhao S, Chen S, Wang X. Fast formation of biomimetic apatite coatings on pure porous titanium implant's surface. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi 2007;24:806–811.
22. Fuming H, Guoli Y, Xiaoxiang W, Shifang Z. The removal torque of titanium implant inserted in rabbit femur coated with biomimetic deposited Ca-P coating. J Oral Rehabil 2008;35:754–765.
23. Yang GL, He FM, Zhao SS, Wang XX, Zhao SF. Effect of H_2O_2/HCl Heat Treatment of Implants on In Vivo Peri-implant Bone Formation. Int J Oral Maxillofac Implants 2008;23:1020–1028.
24. Lee JH, Ryu HS, Lee DS, Hong KS, Chang BS, Lee CK. Biomechanical and histomorphometric study on the bone-screw interface of bioactive ceramic-coated titanium screws. Biomaterials 2005;26:3249–3257.
25. Reigstad O, Franke-Stenport V, Johansson CB, Wennerberg A, Rokkum M, Reigstad A. Improved bone ingrowth and fixation with a thin calcium phosphate coating intended for complete resorption. J Biomed Mater Res B Appl Biomater 2007;83:9–15.
26. Dekker RJ, de Bruijn JD, Stigter M, Barrere F, Layrolle P, van Blitterswijk CA. Bone tissue engineering on amorphous carbonated apatite and crystalline octacalcium phosphate-coated titanium discs. Biomaterials 2005;26:5231–5239.
27. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Kokubo T, Nakamura T. Bone-bonding ability of a hydroxyapatite coated zirconia-alumina nanocomposite with a microporous surface. J Biomed Mater Res A 2006;78:693–701.
28. Ekholm E, Tommila M, Forsback AP, Martson M, Holmbom J, Aaritalo V, Finnberg C, Kuusilehto A, Salonen J, Yli-Urpo A, Penttinen R. Hydroxyapatite coating of cellulose sponge does not improve its osteogenic potency in rat bone. Acta Biomater 2005;1:535–544.
29. Zagury R, Harari ND, Conz MB, Soares Gde A, Vidigal GM Jr. Histomorphometric analyses of bone interface with titanium-aluminum-vanadium and hydroxyapatite-coated implants by biomimetic process. Implant Dent 2007;16:290–296.
30. Bernstein A, Nobel D, Mayr HO, Berger G, Gildenhaar R, Brandt J. Histological and histomorphometric investigations on bone integration of rapidly resorbable calcium phosphate ceramics. J Biomed Mater Res B Appl Biomater 2008;84: 452–462.