Biomechanical Measurements of Calcium-Incorporated Oxidized Implants in Rabbit Bone: Effect of Calcium Surface Chemistry of a Novel Implant

Young-Taeg Sul, DDS, PhD;* Eung-Sun Eung-Sun Byon, PhD;† Yongsoo Yongsoo Jeong, PhD†

ABSTRACT

Background: In oral implantology there has been a general trend away from machine-turned minimally rough and acid-etched and blasted implants toward intermediary roughened surfaces. Mechanical interlocking at micron resolution is claimed to be the dominant reason for the fixation of such implants in bone. However, clinical demands for stronger and faster bone bonding to the implant (eg, in immediately loaded and compromised bone cases) have motivated the development of novel surfaces capable of chemical bonding.

Purpose: The purpose of the present study is to investigate bone tissue reactions to a newly developed calcium-incorporated oxidized implant. The specific aim is to assess the effect of calcium surface chemistry on the bone response.

Materials and Methods: Calcium (Ca) ion–incorporated implants were prepared by micro arc oxidation methods. Surface oxide properties were characterized by using various surface analytic techniques involving scanning electron microscopy, x-ray diffractometry, x-ray photoelectron spectroscopy, and optical interferometry. Twenty screw-shaped commercially pure (CP) titanium implants (10 turned implants [controls] and 10 Ca-incorporated implants [tests]) were inserted in the femoral condyles of 10 New Zealand White rabbits.

Results: After a healing period of 6 weeks, resonance frequency analyses and removal torque measurements of the Ca-incorporated oxidized implants demonstrated statistically significant improvements of implant integration with bone in comparison to machine-turned control implants ($p = 0.013$ and $p = 0.005$, respectively).

Conclusions: The Ca-reinforced surface chemistry of the oxidized implants significantly improved bone responses in a rabbit model. The present study suggests that biochemical bonding at the bone-implant interface, in combination with mechanical interlocking, may play a dominant role in the fixation of Ca-incorporated oxidized implants in bone. The observed rapid and strong integration of test Ca implants may have clinical implications for immediate or early loading and improved performance in compromised bone.

KEY WORDS: biochemical bonding, biomechanical test, bone responses, calcium surface chemistry, oxidized titanium implant

Throughout the last decade surface innovation in osseointegrated implants has been focused on topographic change. Many clinical oral implants currently have topographically altered ''rougher'' surfaces, such as those of plasma-sprayed, acid-etched, and blasted rough implants. However, the surfaces of the implants have a wide potential to ensure clinically favorable performance, reflecting not only topographic alterations but also improved chemical and physical properties.

Only a few implants with modified surface chemistry (but not modified by a coating of ''bioactive bulk

*Department of Biomaterials/Handicap Research, University of Gothenburg, Gothenburg, Sweden; †senior researcher, Surface Engineering Department, Korea Institute of Machinery and Materials, Changwon, Korea.

Reprint requests: Young-Taeg Sul, DDS, PhD, Department of Biomaterials/Handicap Research, University of Gothenburg, Box 412, S-405 30 Gothenburg, Sweden; e-mail: young-taeg.sul@hkf.gu.se

materials'') have shown promising in vivo results. Ellingsen in 1995 found that fluoride-treated titanium implants improved the bone response by 3 to 4 times when compared to titanium controls in rabbit ulnae.[1] In 2000, Johansson and colleagues confirmed improved bone responses to fluoride-treated implants when compared to bone responses to titanium dioxide (TiO_2)–blasted control implants in rabbits.[2] These potentially bioactive Osseospeed® implants (Astra Tech AB, Mölndal, Sweden) demonstrated excellent clinical results in a prospective randomized clinical study on hip arthroplasties and are currently tested as oral implants.[3] Yan and colleagues in 1997 and Fujibayashi and colleagues in 2001 reported that sodium hydroxide heat-treated sodium-incorporated implants exhibited significantly higher failure loads in rabbit tibiae.[4,5] Hanawa and colleagues in 1997 reported that in rabbit bone, calcium ion–implanted titanium was superior to titanium alone for bone conduction.[6] In another study using ion implantation techniques, De Maeztu and colleagues in 2003 found different bone responses to implants implanted with various ions (positive ions of calcium, cobalt, nitrogen, and neon) in a rabbit model.[7] Sul and colleagues, reporting in 2002 and 2003, introduced implants with different surface chemistries (such as magnesium-, calcium-, sulfur-, and phosphorus-incorporated implants) in animal studies and reported significant improvements in bone response.[8–11] Sul investigated the significance of the surface chemistry of ion-incorporated implants to bone tissue reactions[12] and subsequently proposed two action mechanisms of osseointegration of his oxidized implants: (1) mechanical interlocking through bone growth in pores and (2) biochemical bonding.[13] The calcium (Ca)-incorporated oxidized implant used in the present study is a recent innovation. Major differences exist between both Ca-incorporated implants with respect to surface properties, exemplified by surface composition and crystal structure.

The general purpose of the present study is to investigate bone tissue reactions to a newly developed Ca-incorporated oxidized implant. The specific aim is to assess the role of calcium surface chemistry on bone response. Ca ion–incorporated implants were prepared by using micro-arc oxidation (MAO) methods. With various surface analysis techniques, a detailed surface characterization was drawn to allow verification of the role of specific surface oxide properties in bone response. Bone tissue reactions in the rabbit femur were evaluated by resonance frequency analysis and removal torque measurements after a follow-up of 6 weeks.

MATERIALS AND METHODS

Implant Design and Preparation

Twenty screw implants (American Society for Testing and Materials [ASTM] grade 1), 3.0 mm in diameter and 7.2 mm in length (5 mm insertion length), were manufactured from 5 mm rods of commercially pure titanium. Ten control implants had machine-turned surfaces whereas the other 10 test implants had a Ca ion–incorporated oxidized surface. These latter implants were prepared by MAO in a newly invented calcium electrolyte system. The test Ca implants were prepared with the MAO process in galvanostatic mode in a newly invented Ca-containing electrolyte system. The electrochemical oxidation method used in the present study has been described in our previous study.[14]

Surface Characteristics of Test Ca Implants and Controls

Surface chemical analysis was investigated by x-ray photoelectron spectroscopy (XPS) (ESCALAB 250, VG Scientific Ltd., West Sussex, UK). The XPS spectra were recorded by using normal Al K-alpha radiation (1,486.8 eV) with a probing beam size of 200 μm. The outermost surface of the implants was etched with argon (Ar) ions with an ion energy of 5 keV and a beam current of 0.3 μA for 150 seconds, corresponding to 2 nm in thickness and resulting in the removal of surface contaminants. The surface oxide of the test Ca implants and the controls was mainly TiO_2. The XPS survey spectrum of the received test Ca implant surfaces revealed the presence of the calcium elements, titanium, oxygen, carbon, and fluorine (Figure 1). The relative atomic concentration at the surfaces of test Ca implants as received was about 23.4% titanium, about 57.9% oxygen, about 9.1% carbon, about 7.4% Ca, and 2.3% fluorine. After a short Ar^+ sputter cleaning to a depth corresponding to about 2 nm, the calcium concentration was unchanged. Carbon rapidly decreased to 4.9%, and fluoride disappeared to noise levels of about 0.5%. The relative atomic concentrations of the as-received surfaces and the Ar^+ sputter-cleaned surfaces

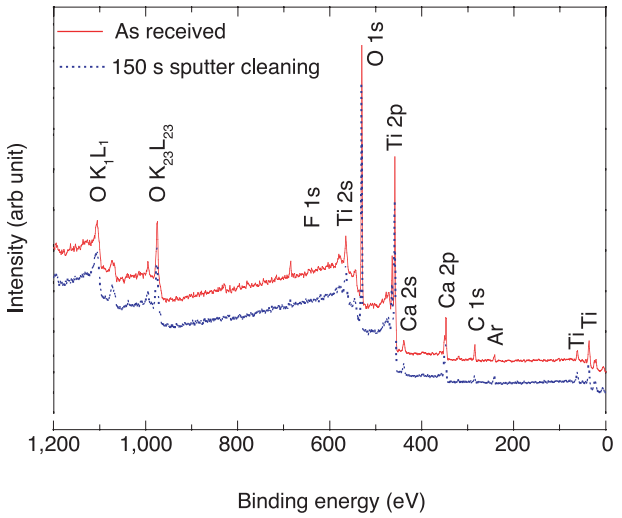


Figure 1 X-ray photoelectron spectroscopy survey spectra of as-received surfaces and Ar^+ sputter-cleaned surfaces of Ca-incorporated implants (probing beam size, 200 mm; sputtering ion energy, 5 keV; beam current, 0.3 mA). (Ar = argon; arb = arbitrary; C = carbon; Ca = calcium; O = oxygen; Ti = titanium)

are summarized in Table 1. High-resolution XPS revealed doublet peaks of Ca 2p (ie, Ca 2p1/2) at about 350.8 eV and Ca 2p3/2 at about 347.3 eV (Δ = 3.5) (Figure 2). Scanning electron microscopy (SEM) showed the porous surface structure of test Ca implants and characteristics of machine-turned orientation in the control implants (Figure 3). An image analysis system (Image Inside, Focus Co., Ltd., Daejeon, Korea) was used to determine the pore characteristics of the test implants. The mean surface porosity was 24% (n = 3, standard deviation [SD] = 0.1). The pores were generally 0.3 to 1.5 μm in diameter. A few pores about 3.0 μm in diameter were detected. The mean oxide thickness was about 4,070 nm (n = 15, SD = 600) in test Ca implants as measured with SEM on cross-sectional views (Figure 4) and < 17 nm in controls as measured with Auger electron spectroscopy in our previous study.[14] The crystal structure was measured with low-angle x-ray diffraction with a thin-film collimator (X'Pert PRO-MNR, Philips Ltd., Almelo, The Netherlands) on the plate type of specimen. The step size was 0.02° between 15° and 70° of measured scan. The spectra were recorded with Cu K-alpha radiation (0.154056 Å). X-ray diffraction patterns showed a mixture of anatase and rutile phases for test Ca implants and an amorphous phase for controls (Figure 5). The surface roughness as measured with an optical interferometer (MicroXAM™, ADE Phase Shift, Tucson, AZ) revealed an arithmetic average height deviation (Sa) of 0.64 μm (n = 9, SD = 0.2) and a developed surface ratio (Sdr) (ie, the ratio of the increment of the interfacial area of a surface over the sampling area) of 28.8% (n = 9, SD = 11.2) for test Ca implants; the number of summits of a unit sampling area (Sds) was 0.09 μm^{-2} (n = 9, SD = 0.03). Control implants were found to have an Sa of 0.55 μm (n = 9, SD = 0.21), an Sdr of 10.6% (n = 9, SD = 3.9), and an Sds of 0.09 μm^{-2} (n = 9, SD = 0.04).

Animals and Surgical Technique

A total of 10 mature (mean weight, 2.8 kg) New Zealand White rabbits were used in this study; this was approved by the local animal ethics committee of the University of Gothenburg (Gothenburg, Sweden). Each rabbit received one test implant and one control implant in the femur close to the condyle region. During surgery the animals were anesthetized with intramuscular injections of fentanyl and fluanison (Hypnorm Vet®, Janssen, Saunderton, England) at a dose of 0.5 mL per kilogram of body weight

TABLE 1 Atoms Detected by X-Ray Photoelectron Spectroscopy Measurements on Test Implants As Received and after Sputter Cleaning

	As Received			After 150 s of Sputter Cleaning		
Element	Center	FWHM	AT (%)	Center	FWHM	AT (%)
Ti 2p	459.00	1.460	23.36	459.15	1.775	25.16
O 1s	530.55	1.321	57.93	530.60	1.349	61.49
C 1s	284.90	1.264	9.05	284.95	1.231	4.90
Ca 2p	347.5	1.560	7.37	347.25	1.634	7.93
F 1s	685.10	1.086	2.30	685.85	0.100	0.52

AT = Atomic Concentration; FWHM = Full-Width Half-Maximum.

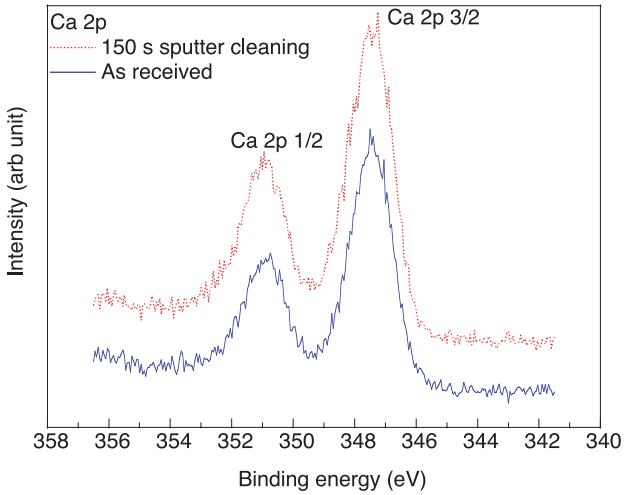


Figure 2 High resolution x-ray photoelectron spectroscopy of calcium (Ca) incorporated into oxidized implants, indicating the presence of calcium titanate. (arb = arbitrary)

and intraperitoneal injections of diazepam (Valium®, Roche, France) at a dose of 2.5 mg per animal. The skin and fascial layers were opened and closed separately. The periosteal layer was gently pulled away from the surgical area and was not resutured. During all surgical drilling sequences, low rotary drill speeds (not exceeding 2,000 rpm) and saline cooling were used. The animals were kept in separate cages, and immediately after surgery they were allowed full weight bearing. After a follow-up of 6 weeks the animals were sacrificed by intravenous injections of Pentobarbitalum® (Apoteksbolaget, Uppsala, Sweden).

Evaluations of Bone Response

All 20 implants were evaluated with resonance frequency and removal torque measurements.

Resonance frequency analysis. Resonance frequency analysis (RFA) is a nondestructive technique for demonstrating Implant Stability Quotient (ISQ) in terms of interfacial stiffness. The frequency response of the system was measured by attaching the transducer (an L-shaped cantilever beam) to the screw implant. The excitation signal was given over a range of frequencies (typically 5 to 15 ISQ, with a peak amplitude of 1.0 V), and the first flexural resonance measured.[15]

Removal torque tests. The removal torque instrument is an electronic instrument with a strain gauge transducer used for testing implant stability (as peak loosening torque, in newton-centimeters) in the bone bed; this process thus can be regarded as a three-dimensional test roughly reflecting the interfacial shear strength between bone tissue and the implant.[13,15] The device ensures a fixed rotation rate (in contrast to hand-controlled devices) to eliminate ''operator errors,'' and it has been shown to achieve high reproducibility and low operator sensitivity. The present study used a newly developed alignment table to ensure that the rotation axis is kept in a straight line between the transducer and the implant. This alignment table was designed to make a three-dimensional adjustment at the micrometer scale.

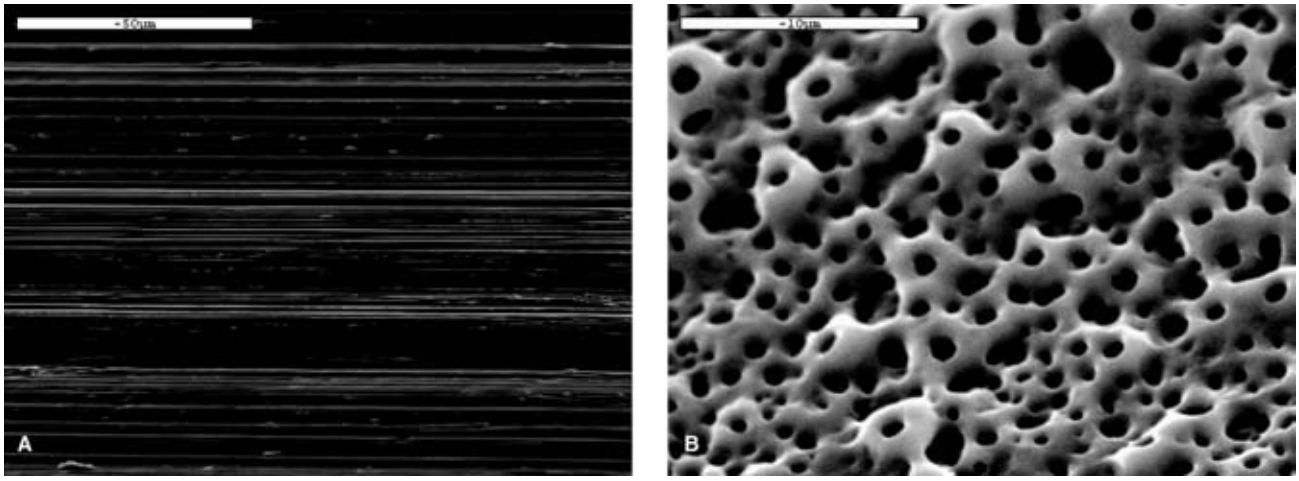
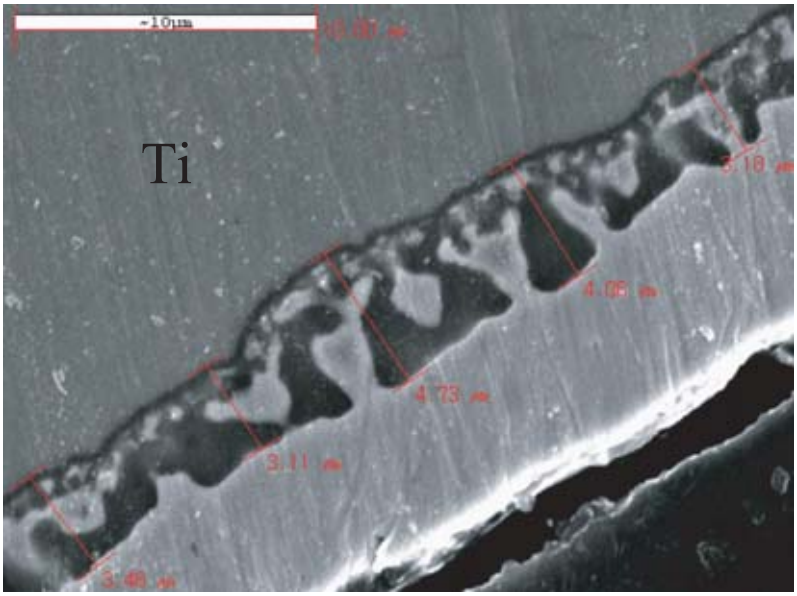


Figure 3 Scanning electron micrographs of a machine-turned control surface (A) and the porous surface structure of a test calcium implant (B).



Area	1	2	3	4	5	Average	SD	Total of Average
Top	3.67	4.28	4.38	4.9	5.1	4.47	0.56	—
Flank	3.48	3.11	4.73	4.05	3.18	3.71	0.68	—
Valley	4.23	3.56	4.64	3.95	3.79	4.03	0.42	4.07

Figure 4 Oxide thickness in a cross-sectional view of a test implant. Nickel plating was performed before resin mounting. A total of 15 areas were measured on five thread tops, five thread valleys, and five thread flanks. (Ti = titanium)

Statistics

Statistical analyses were performed by using the Wilcoxon signed rank test. Differences were considered statistically significant at $p < .05$, highly significant at $p < .01$, and not significant at $p > .05$.

RESULTS

The surface oxide properties of the implants used are described in "Materials and Methods" and are summarized in Table 2.

There were statistically significant differences of resonance frequency values between test Ca implants and control implants ($p = .013$). The mean values of the RFA were 69.4 ISQ (SD, 1.8; range, 67–72) for test implants and 66.1 ISQ (SD, 3.2; range, 63–73) for controls at 6 weeks of follow-up (Figure 6).

The test Ca implants revealed a significantly greater removal torque in comparison with control implants ($p = .005$). The mean values of removal torque were

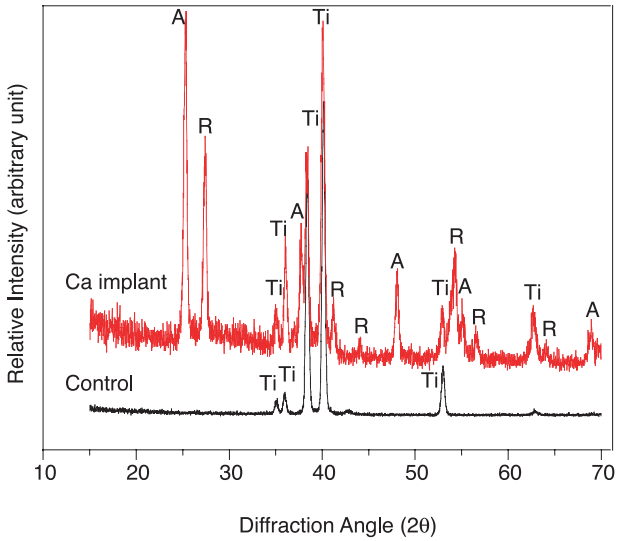


Figure 5 X-ray diffraction patterns on commercially pure titanium plates abraded by 800-grit silicon carbide paper and oxidized in the same manner as the test magnesium screw implant (with an acceleration voltage of 35 kV and a current of 25 mA). (A = anatase phase; Ca = calcium; R = rutile phase; Ti = titanium)

TABLE 2 Surface Oxide Characteristics of Test and Control Implants

Oxide Characteristic	Machine-Turned Implant (Control)	Ca-Incorporated Oxidized Implant (Test)
Chemical composition	Mainly TiO_2; contaminant, C.	Mainly TiO_2 and Ca (≤8%); contaminant, C; traces of F
Morphology	Nonporous structure with machine-turned grooves ≤ 10 μm	Porous structure with great number of craters
Pore size	—	≤ 1.5 μm
Porosity	—	24.0% (± 0.1)
Oxide thickness	17 ± 6 nm	4,000 ± 600 nm
Crystal structure	Amorphous	Anatase and rutile
Roughness	Sa = 0.55 ± 0.21 μm; Sdr = 10.6 ± 3.9%; Sds = 0.09 ± 0.04 μm^{-2}	Sa = 0.64 ± 0.2 μm; Sdr = 28.8 ± 11.2%; Sds = 0.09 ± 0.03 μm^{-2}

Sa = arithmetic average height deviation; Sdr = developed surface ratio; Sds = number of summits of unit sampling area; TiO_2 = titanium dioxide.

27.6 Ncm (SD, 6.8; range, 19–42 Ncm) for test implants and 8.8 Ncm (SD, 3.3; range, 4–15 Ncm) for controls at 6 weeks of follow-up (Figure 7).

DISCUSSION

The present study verifies our previous findings of significant improvements of the bone response to Ca surface chemistry of Ca-incorporated oxidized implants.[11,12] The same surgeon then used similar types of animals of a similar age and from the same supplier in other tests with an older type of Ca-reinforced implant. It is noteworthy (albeit not tested in a proper test/control fashion) that the mean removal torque values were 314% greater for the newly developed Ca implants in comparison to controls whereas previously used Ca implants showed only a 153% increase in comparison to the same types of controls. Major differences between the two types of Ca-incorporated implants exist in the surface properties, in particular, surface composition and crystal structure.[11,12] The currently tested Ca implant differs from the former in that the relative Ca concentration is 7% instead of 11%, and a relative fluorine concentration of 2.3% is additionally found in the newly developed implant. The

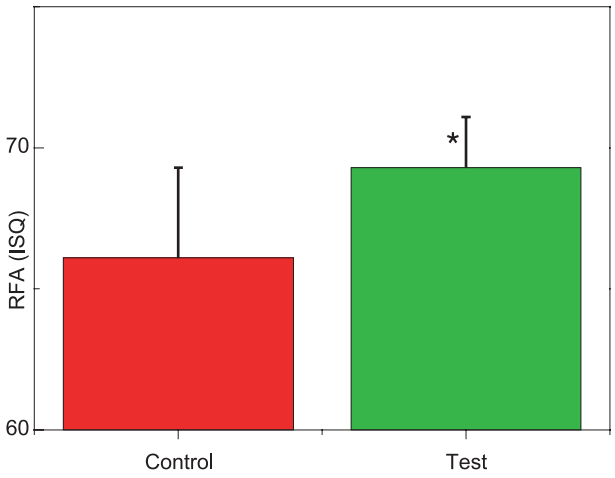


Figure 6 Mean resonance frequency (RFA) values (as Implant Stability Quotient [ISQ] units) after a follow-up of 6 weeks, indicating a statistically significant difference between machine-turned implants and calcium-incorporated oxidized implants (*p = .013).

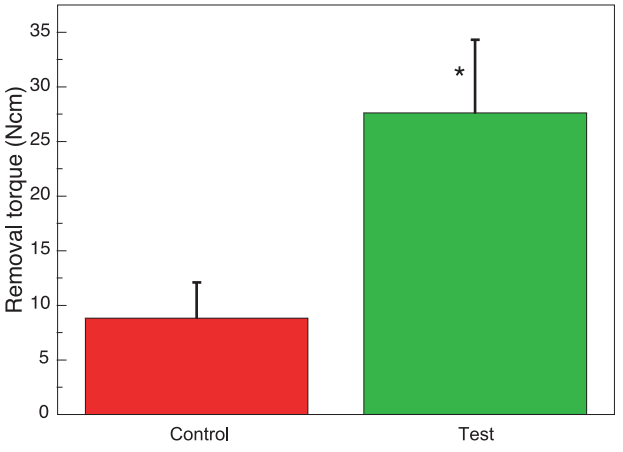


Figure 7 Mean removal torque values after a follow-up of 6 weeks, indicating a statistically highly significant difference between machine-turned implants and calcium-incorporated oxidized implants (*p = .005).

chemical element of fluorine in the newly developed Ca implants is regarded as a surface contaminant since it disappeared to noise levels of about 0.5% after Ar^+ sputter cleaning, corresponding to oxide 2 nm thicker. The crystal structure of the present Ca implant (ie, a mixture of anatase and rutile) differs from the anatase-alone structure of the previous Ca implants.

As suggested in our previous in vivo studies,[16] the crystal structure of mixed anatase and rutile forms may, at least in part, contribute to the bone response. The roughness values of Sa and Sds were rather similar between control and test implants. Therefore, Sa and Sds cannot be key parameters of the surface roughness to explain the improved bone response to Ca-incorporated implants whereas the differences in Sdr between test and control implants may influence the present results.

As far as mechanical interlocking through bone ingrowth into the pores of the test Ca implants is concerned, our previous observations (Figure 8) suggested that bone growth into pores $\leq$ 1.5 μm in diameter probably had not been completed at a follow-up of 6 weeks as indicated by SEM and energy dispersive spectrometer measurements.[10] Mechanical interlocking through bone growth is dependent on the size and shape of pores or pits or cavities and also may necessitate a healing time longer than 6 weeks.

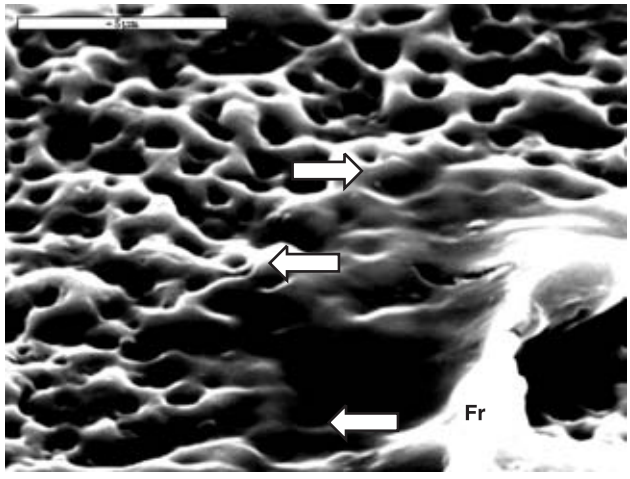


Figure 8 Scanning electron micrograph of a test implant prepared by means of a cryofracture technique. Pores are still visible, indicating that the pores were not completely filled with bone tissue. Bone tissue spreads further over the pores of oxidized implants but is unlikely to go into pores before bone formation commences around the marginal border of the pores (*arrows*). ''Fr'' represents the fracture line that occurred during the cryofracture process.

There have been few reported studies on the effects of calcium surface chemistry, but some conflicting data have been reported. Ellingsen investigated the hypothesis that Ca ions adsorb to TiO_2 and further to macromolecules with high affinity for Ca^{2+} and suggested that calcium binding may be one mechanism of protein adsorption to TiO_2.[17] Nayab and colleagues demonstrated in 2001 that alveolar bone cells on Ca ion–implanted surfaces were mostly highly flattened, with large cell bodies and many cytoplasmic processes extending onto the surfaces, in contrast to the Ar-implanted titanium and potassium-implanted surfaces.[18] Hanawa and colleagues reported that a larger amount of new bone was formed on the Ca ion–implanted surface inserted in rat tibia for 2, 8, and 18 days than was formed on the titanium surface.[6] However, Keller and colleagues in 1994 observed no indications of favorable osteoblast-like cell activity on the Ca ion–implanted and plasma immersion ion implantation surfaces in comparison to CP titanium.[19] Spector and colleagues also investigated bone response to Ca ion–implanted titanium in a canine model and reported in 1994 that bone response to Ca ion–implanted titanium was poorer than bone response to hydroxyapatite-coated titanium implants. One reason for these conflicting data may relate to the different Ca surface chemistry of the ion implantation techniques.[20] Hanawa and colleagues in 1997 claimed that the surface chemistry of Ca ion implantation was strongly dependent on the used energy exemplified by acceleration energy or ion beam current density.[21]

Reinforced Ca surface chemistry may most probably be the key property behind the enhancement of the bone response in the present study. However, the action mechanism of enhanced bone response to Ca surface chemistry has not yet been investigated. Sul in 2002 previously proposed the Ca surface chemistry–mediated chemical bonding mechanism of Ca-incorporated oxidized implants illustrated in Figure 9.[13] There are few available clues in the literature to the proposed action mechanism.

One explanation is that biochemical bonding via ''electrostatic/ionic'' bonds may be formed across the interface of bone tissue and the Ca chemistry of Ca-incorporated implants, as follows: Ca^{2+} cations of the calcium titanate (Ca implants) can be bound electrostatically to a great number of polyanions of proteoglycans that play a key role in binding bone cells to

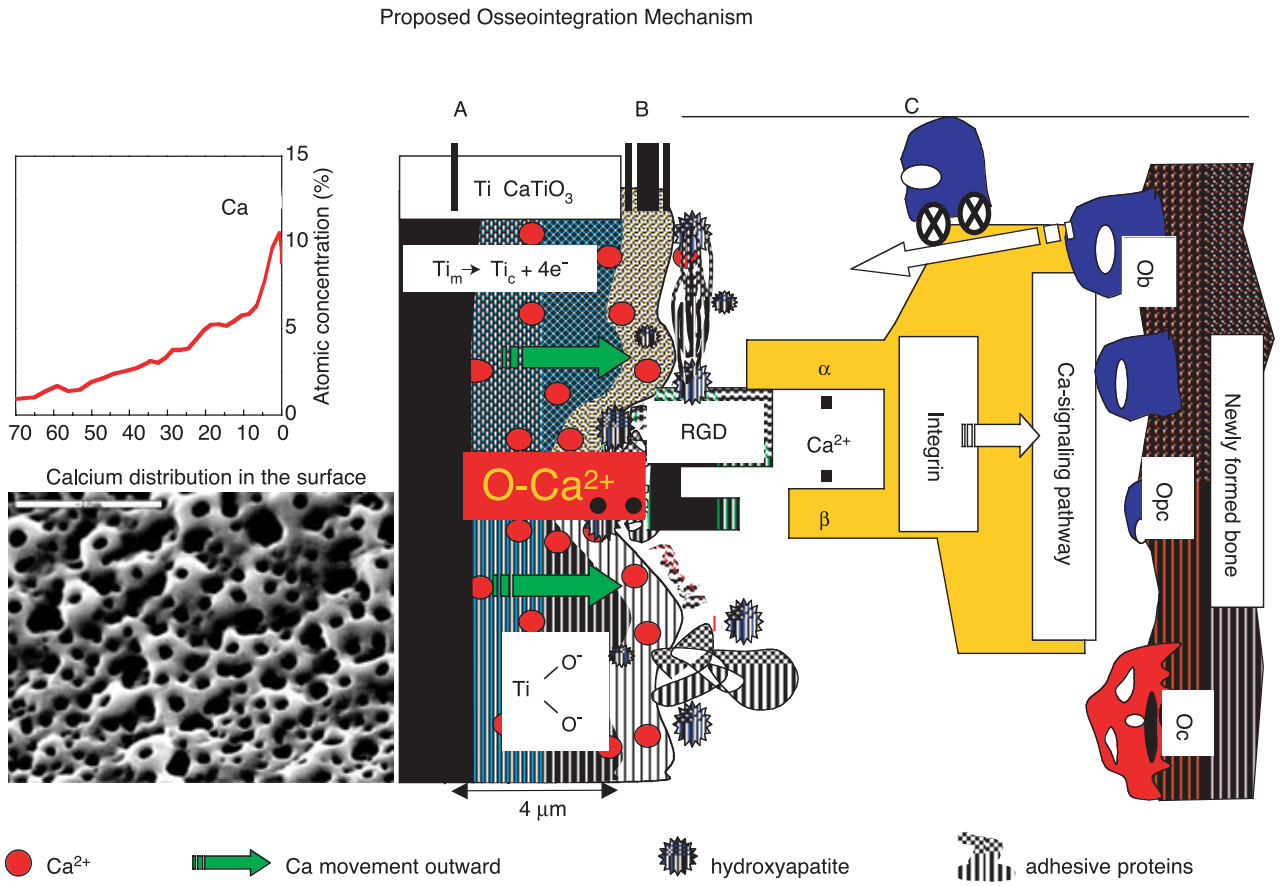


Figure 9 Proposed osseointegration mechanism. (1) Movement of calcium (Ca) cations in the Ca implant toward the extracellular body fluid may be the key driving force for the subsequent pathways. (2) Ca cations in the Ca implant bond electrostatically with polyanionic molecules of adhesive bone matrix proteins such as collagen type I, thrombospondin, fibronectin, vitronectin, fibrillin, osteoadherin, osteopontin, bone sialoprotein, osteocalcin, and osteonectin. (3) Ca cations stimulate Arg-Gly-Asp (RGD) surface receptors and also trigger further recruitment of osteoprogenitor cells and osteoblasts via Ca-signaling pathways. At top of figure, "A" represents the metal-oxide interface, "B" represents the oxide–bone matrix interface (biofilm formation with further localization of noncollagenous proteins; 50–500 nm of amorphous layer), and "C" indicates bone cell reactions at the conditioned film. ($CaTiO_3$ = calcium titanate; O = Oxygen; Ob = Osteoblast; Oc = Osteoclast; Opc = Osteoprogenitor cell; RGD = Arg-Gly-Asp; Ti = titanium) (Adapted from Sul YT.[13])

the implant surface. Proteoglycans in bone matrix are known to have highly polyanionic properties; glycosaminoglycan binds covalently to the protein core of proteoglycans in bone matrix and is coupled with a lot of hydroxyl, carboxyl, and sulfate groups, which in turn makes the proteoglycan highly polyanionic.[22,23] A number of in vitro protein adsorption studies have suggested that calcium possibly provides a "bridge" between the titanium and macromolecules.[24–26] In essence, many of the adhesive bone matrix proteins (including proteoglycans, collagen, thrombospondin, fibronectin, vitronectin, fibrillin, osteoadherin, osteopontin, and bone sialoproteins) have high affinity to calcium-binding sites and also contain Arg-Gly-Asp (RGD) sequences on the surface. On the other hand, surface Ca cations of Ca implants provide binding sites for the attachment of adhesive bone matrix proteins.

It has been shown that Ca ions in the implant materials moved to the outermost surface layer and further into the experimental electrolyte solution.[27] Calcium oxide (CaO)–containing titania gels immersed in simulated body fluid released larger amounts of the Ca ions with increasing CaO contents of the gels.[28] During physiologic functioning of the implant, movement of Ca ions in the Ca implant causes local saturation of the Ca concentration on the implant surface. This would result in precipitation of Ca^{2+} with phosphate (PO_4) ions in extracellular fluid.[29,30] Furthermore, movement of Ca cations of the Ca

implant into neighboring tissue preferentially facilitates the adhesion of osteoprogenic cells via activation of Ca-dependent cell surface adhesive molecules such as integrin and accelerates the Ca-signal pathway of such cells. As a result, electrostatic ion bonds may lead to the formation of a binding complex of the Ca implant (via Ca cations), the adhesive bone matrix proteins (via RGD sequence), and osteoblast cells (via cell membrane receptors such as integrin).

CONCLUSIONS

The present study implies that the quantitative and qualitative differences of the chemical composition (in particular, calcium) of the implant surface may be of great significance for bone tissue responses. Fast and strong integration of test Ca implants may have clinical implications for immediate/early loading and improved performance in compromised bone. Further investigations are needed for a better understanding of the biochemical bonding theory proposed as the action mechanism of the enhanced osseointegration in the present study.

ACKNOWLEDGMENTS

This study was supported by a research grant of the Biochallenge Project from the Ministry of Science and Technology of Korea and Osspol AB (Sweden), the Swedish Research Council, the Sylvan Foundation, and the Hjalmar Svensson Research Foundation.

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