A Histomorphometric Study of Nanothickness and Plasma-Sprayed Calcium–Phosphorous-Coated Implant Surfaces in Rabbit Bone

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Background: Novel implant surface treatments with a nanothickness bioactive ceramic deposition onto rough surfaces have been recently introduced. This study aims to evaluate histologically and histomorphometrically (bone-to-implant contact [BIC] and bone area fraction occupancy [BAFO]) the early bone response to plasma-sprayed calcium–phosphate (PSCaP)-coated versus a 300- to 500-nm thickness bioactive ceramic nano-coated plateau root form implants in a rabbit femur model.

Methods: A total of 48 plateau root form implants were bilaterally placed in the distal aspect of the femur of 12 white New Zealand rabbits, remaining for 20, 30, and 60 days in vivo (n = 4 animals per time in vivo, n = 2 implants per surface per animal). After sacrifice, the implants in bone were non-decalcified processed to slides of approximately 30 μm thickness, and were morphologically and morphometrically (BIC and BAFO) evaluated.

Results: Higher degrees of bone structural organization were temporally observed for the PSCaP surface compared to the nano surface over time. BIC and BAFO was significantly higher ($P<0.05$) for PSCaP at all implantation times evaluated.

Conclusions: Within the limits of this study it is possible to state that bioactive ceramic coatings of both thicknesses were biocompatible and osteoconductive. However, the early bone response was favored by the presence of the thicker PSCaP coating. *J Periodontol 2010;81:556-561.*

KEY WORDS

Animal research; biocompatible materials; ceramics; dental implants; histology.

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During preclinical testing, endosseous dental implant surfaces have been characterized with histologic and histomorphometric analysis examining the quality of the bone–implant interface and the amount of implant surface in contact with bone tissue.[1] In general, animal and human studies have shown that implants with moderately rough surfaces had a higher bone–implant contact (BIC) percentage and mechanical properties[2] compared to the smoother machined implants.[3-10] In addition, greater forces are required to remove implants with a rougher surface compared to implants with smoother surfaces.[1]

A variety of methods have been used over the last decade to change dental implant surface texture and chemistry to further improve the early bone-to-implant response.[11] Specifically to surface chemistry modifications, the incorporation of Ca- and P-based bioactive ceramics has received significant attention over the years, yielding the economically viable highly osteoconductive plasma-sprayed hydroxyapatite coatings (PSHA).[12,13] However, because of its inherent chemistry inhomogeneity and poor coating-implant adhesion,[14] controversy persists over the long-term clinical effectiveness of PSHA-coated

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implants despite clinical studies and case reports documenting high survival rates.[15,16]

To benefit from the highly osteoconductive properties and at the same time avoid the limitations in relation to chemistry and mechanical properties of PSHA-coated implants, incorporation of bioactive ceramics in substantially reduced (nanometer range) dimensions has been successfully introduced by a variety of methods.[11,13,14,17,18] However, whereas previous studies[11,13,14,17] have shown positive results for nanometer range coatings compared to uncoated moderately rough surfaces, reports regarding their effectiveness compared to thicker coatings are limited.

The objective of this study is to evaluate, histologically and histomorphometrically, (BIC and bone area fraction occupancy [BAFO]) the early bone response to plasma-sprayed calcium-phosphate (PSCaP) implants versus 300- to 500-nm thickness bioactive ceramic nano-coated implants in a rabbit femur model.

MATERIALS AND METHODS

This study used a total of 48 plateau root form implants‖ (4.5 mm in diameter by 6 mm in length), and two different surface groups were included. The first group, nano implants (n = 24), presented an alumina-blasted acid-etched Ti-6Al-4V substrate plus an ion beam-assisted deposition of nanothickness Ca- and P-based bioceramic-coated surface. The second group presented a PSCaP-coated surface (n = 24). Both surfaces have been previously physicochemically characterized.[13]

The experimental in vivo laboratory model was conducted on approval of the Ethical Committee for Human and Animal Studies of the School of Medicine, University of Chieti, Chieti, Italy. A total of 12 New Zealand rabbits were used. The animals were sedated with a dose of ketamine¶ (44 mg/kg) and xylazine# (6 to 8 mg/kg) prior to surgery. A local injection of 1.8 ml lidocaine** without vasoconstrictor was then performed.

A full-thickness incision was made to expose the distal portion of the femur, and osteotomies were prepared with a 2-mm pilot bur used on a specially designed electric machine operated at 1,100 rpm with saline irrigation. The subsequent drilling was completed with slow speed sequential drilling with burs of growing diameter (2.5 to 4.5 mm) used on a handpiece†† operated at 50 rpm without saline irrigation.

The implants were then inserted in a press-fit fashion into the osteotomy sites by manual pressure and by lightly tapping a mallet. The implants were placed 1 mm below the bone level according to the manufacturer's‡‡ instructions. Standard layer suture techniques were adopted with resorbable sutures for the internal layers§§ and non-resorbable suture for the skin.‖‖ After the surgery, a single dose of antibiotic was administered intramuscularly (0.25 cefazolin). Each group of four rabbits were sacrificed at 20, 30, and 60 days after surgery with an overdose¶¶ yielding an equal number of specimens per surfaces evaluated per time in vivo (n = 8). The implant distribution included two implants of each surface per animal, and proximal and distal sites were interpolated per surface. The limb and site distribution allowed the same number of implant surfaces per implantation sites for analysis.

Following sacrifice, the implants and surrounding tissues were immediately stored in 10% buffered formalin and processed to obtain thin ground sections. The specimens were processed using an automated system.## The specimens were dehydrated in a graded series of ethanol rinses and embedded in a glycolmethacrylate resin.*** After polymerization the specimens were sectioned, along the longitudinal axis of the implants, with a high-precision diamond disk at about 150 μm and ground down to about 30 μm with a specially designed grinding machine. Three slides were obtained from the central region, along the long axis of each implant. These slides were stained with acid fuchsin and toluidine blue and examined with transmitted light under a microscope.†††

Histomorphometry was carried out using a light microscope‡‡‡ connected to a high-resolution video camera§§§ and interfaced to a monitor and PC.‖‖‖ This optical system was associated with a digitizing pad¶¶¶ and a histometry software package with image-capturing capabilities.### The histomorphometric measurements involved mean percentage of BIC determined along the whole implant in bone perimeter, and mean percentage of BAFO between the implant plateaus. An experienced operator without knowledge of the sample labeling and specimen number performed all histomorphometric measurements. The BAFO acquisition included the middle three interplateau regions of each implant. The arithmetic average of the three slides per implant was used for BIC and BAFO statistical analyses.

Statistical analyses were performed by one-way analysis of variance (ANOVA). The Tukey post-hoc

‖ Bicon, Boston, MA.
¶ Ketalar, Parker Davis, Milan, Italy.
# Rompum, Bayer, Leverkusen, Germany.
** Lidocaine, Astra, Södertälje, Sweden.
†† Bicon.
‡‡ Bicon.
§§ Vicryl 4.0, Ethicon, Somerville, NJ.
‖‖ Monocryl, Ethicon.
¶¶ Tanax T-61, Teva, Milan, Italy.
Precise 1 Automated System, Assing, Rome, Italy.
*** Technovit 7200 VLC, Kulzer, Wehrheim, Germany.
††† Leitz, Wetzlar, Germany.
‡‡‡ Laborlux, Leitz.
§§§ 3CCD, JVC KY-F55B, JVC, Yokohama, Japan.
‖‖‖ Intel Pentium III 1200 MMX, Intel, Santa Clara, CA.
¶¶¶ Matrix Vision, Oppenweiler, Germany.
Image-Pro Plus 4.5, Media Cybernetics, Immagini and Computer, Milano, Italy.

Table 1.

Statistical Summary for BIC and BAFO Over In Vivo Time for the PSCaP and Nano Surfaces (SD)*

Time in vivo	BIC (%)		BAFO (%)	
	Nano	PSCaP	Nano	PSCaP
20 days	23.0 ± 0.2	27.1 ± 1.1	16.1 ± 1.6	20.1 ± 1.2
30 days	31.5 ± 2.4	43.0 ± 3.0	25.1 ± 1.2	31.1 ± 1.4
60 days	46.0 ± 4.1	61.0 ± 4.5	34.1 ± 1.8	46.1 ± 1.3

BIC = bone-to-implant contact; BAFO = bone area fraction occupancy.
* Significant differences were observed between all groups at each evaluation time (P <0.05). Statistically significant increases in BIC and BAFO were observed over time regardless of surface evaluated (P<0.05).

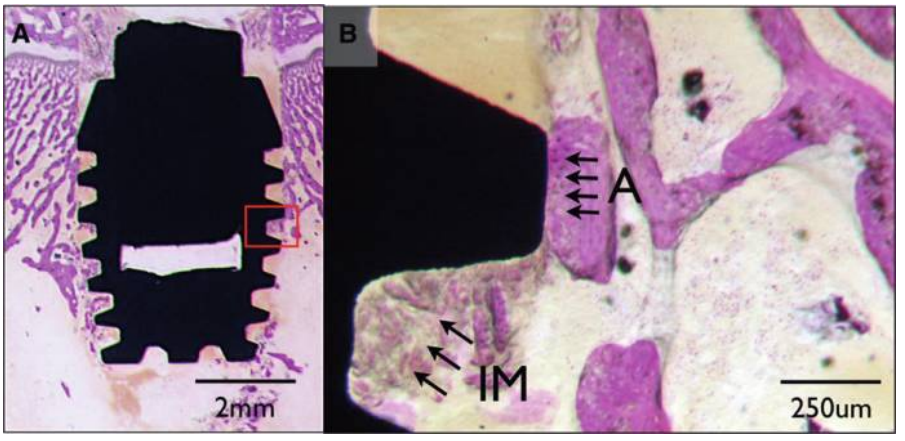

Figure 1.
A) *Optical micrograph showing the overall interaction between bone and plateau root form implant (acid fuchsin and toluidine blue; original magnification ×12.5).* ***B)*** *Irrespective of implant surface, bone formation occurred by woven bone filling between plateaus in an intramembranous-like (IM) fashion, and through appositional bone (A) at the plateau tips (acid fuchsin and toluidine blue; original magnification ×50).*

test was used for multiple comparisons. The level of significance was set to P <0.05.

RESULTS

All the animals remained in good health throughout the length of the experiment. At sacrifice, neither clinical signs of inflammation nor adverse tissue reaction were observed. All implants were stable.

Histomorphometry (BIC and BAFO)

The one-way ANOVA results showed a significant effect of implant surface (P <0.05) over all times evaluated for both BIC and BAFO measurements. For all time point measurements, significantly higher BIC and BAFO were observed for the PSCaP implants versus the nano surface (Table 1). Statistically significant increases in BIC and BAFO were observed over time regardless of surface evaluated (P <0.05) (Table 1).

Histomorphology

The wound healing pattern observed for all groups between the implant plateaus followed the intramembranous-type healing mode (Fig. 1), and appositional bone healing was observed at the plateau tips where direct contact existed between implant and bone immediately after placement (Fig. 1). In general, the healing chambers between plateaus were partially filled with woven bone at 20 days, and bone microstructural evolution with onset of remodeling occurred for all groups at 30 days, and further evolving at 60 days. However, temporal morphologic differences were observed between surface groups.

PSCaP: 20 Days. The osteoblasts produced osteoid matrix directly on the implant surface. Lines of cuboidal-shaped osteoblasts were visible around the implant perimeter (Fig. 2). Only a few multinucleated giant cells and inflammatory cells were present. The newly formed bone tissue and the pre-existing bone were vital and colonized by osteocytes. In a few areas, the pre-existing bone was being resorbed by osteoclasts that were remodeling the bone prepared during the surgical procedure. The BIC percentage was 27.1% ± 1.1% (mean ± SD), and the BAFO percentage was 20.1% ± 1.2% (mean ± SD) (Table 1).

PSCaP: 30 Days. A reduced number of osteoblasts were observed near the surface compared to 20 days. The peri-implant bone was more mature and, in some portions of the interface, in direct contact with the implant surface. Few marrow spaces were present (Fig. 3A). The BIC percentage was 43% ± 3% (mean ± SD), and the BAFO percentage was 34.1% ± 1.8% (mean ± SD) (Table 1).

PSCaP: 60 Days. The compact bone was present in the cortical area, whereas in the other regions the surface was lined in some areas by small trabeculae (Fig. 3B). The newly formed bone tended to grow into the small irregularities of the implant surface. A decreased osteoblastic presence was observed. The BIC percentage was 61% ± 4.5% (mean ± SD), and the BAFO percentage was 46.1% ± 1.3% (mean ± SD) (Table 1).

Nano: 20 Days. Histomorphologic analysis of the nano implant surface at 20 days revealed the formation of a thin layer of bone onto the surface of the implant.

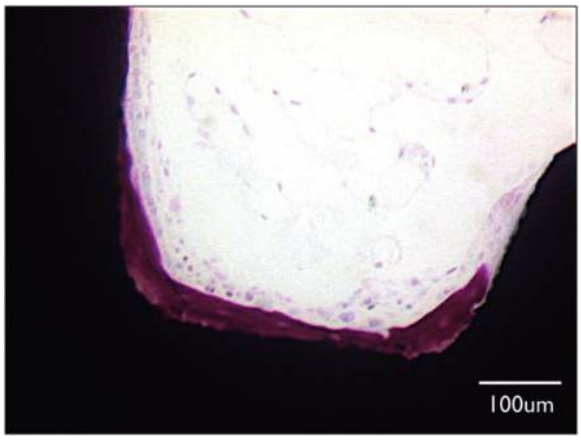

Figure 2.
At 20 days, lines of cuboidal-shaped osteoblasts and bone formation directly onto the surface were visible around the PSCaP implant surface perimeter (acid fuchsin; original magnification ×100).

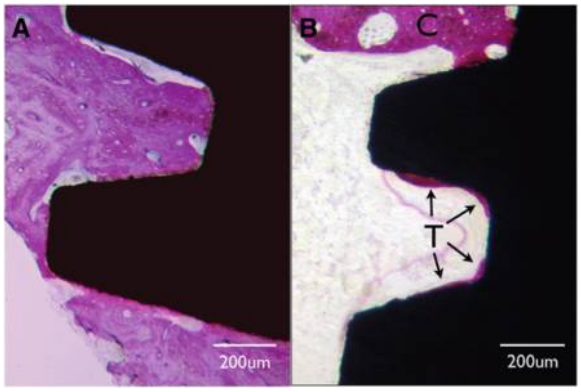

Figure 3.
A) *Intimate contact between bone–PSCaP surface interface at 30 days.* ***B)*** *Optical micrograph of a PSCaP at 60 days. Compact bone was present in the cortical area (C), whereas in the other regions the surface was lined by small trabeculae (T) (arrows) that tended to surround the whole extent of the surface perimeter (acid fuchsin and toluidine blue; original magnification ×50).*

Newly formed small bone trabeculae were observed growing toward the coronal implant surface (Fig. 4). A large number of osteoblasts producing osteoid matrix toward the implant surface were observed. Only a few inflammatory cells were present. No osteoblasts were observed on the implant surface. The BIC percentage was 23% ± 0.2% (mean ± SD), and the BAFO percentage was 20.1% ± 1.2% (mean ± SD) (Table 1).

Nano: 30 Days. A large quantity of newly formed bone and many osteoblasts were present near the implant surface. The trabeculae bone were wide, with a woven immature appearance, and with large osteocyte lacunae. In other areas mature, compact bone with few marrow spaces was present at the interface (Fig. 5A). The BIC percentage was 31.5% ± 2.4% (mean ± SD), and the BAFO percentage was 25.1% ± 1.2% (mean ± SD) (Table 1).

Nano: 60 Days. Mature bone was in direct contact with the implant surface, whereas in other areas a gap or osteoid matrix was interposed between mineralized bone and implant surface (Fig. 5B). Osteoblasts were reduced in numbers in almost all fields. The BIC percentage was 46% ± 4.1% (mean ± SD), and the BAFO percentage was 34.1% ± 1.8% (mean ± SD) (Table 1).

DISCUSSION

The present study aims to evaluate the early bone response of a 300- to 500-nm thickness bioactive ceramic coating deposited by ion–beam-assisted deposition versus a thicker PSCaP-coated surface in a plateau root form implant. A detailed surface physicochemical characterization concerning the two surfaces has been previously addressed.[13] Atomic force microscopy-based texture analysis has shown that the PSCaP presented significantly higher Ra values compared to the nano surface at 1.8 ± 0.25 μm, 0 and 0.66 ± 0.10 μm, respectively. Chemical analysis through time-of-flight secondary-ion mass spectroscopy and x-ray photoelectron spectroscopy showed that the nano group surface presented a 300- to 500-nm thickness, high Ca-P stoichiometry, amorphous coating, whereas the PSCaP surface presented a Ca- and P-based multicrystalline phase 20- to 30-μm thickness coating.[17]

Although nanothickness bioactive ceramic incorporation has been shown to increase the early host-to-implant response,[11,13] the number of studies comparing their early bone response performance to the highly osteoconductive thicker plasma-sprayed coatings is limited.[13] Comparing the same PSCaP and nano surfaces considered in the present study in a dog tibia model, Coelho and Lemons[13] have shown the same degrees of BIC and slightly higher torque-to-interface fracture for the PSCaP-coated surface compared to the nano surface. However, their study[13] used an implant shape that presented early healing sequences comparable to screw root form shape implants, which is substantially different from implant designs that allow the formation of healing chambers after implantation[19], such as the plateau root form implant considered in the present study.

The bone around implant designs where intimate contact between the osteotomy wall and the implant surface results in high degrees of primary stability undergoes localized bone necrosis near the implant surface before bone apposition ensures its biomechanical fixation.[19] Different from this scenario, healing chambers provide little primary stability but have been shown to be rapidly filled with woven bone throughout the volume occupied by the blood clot

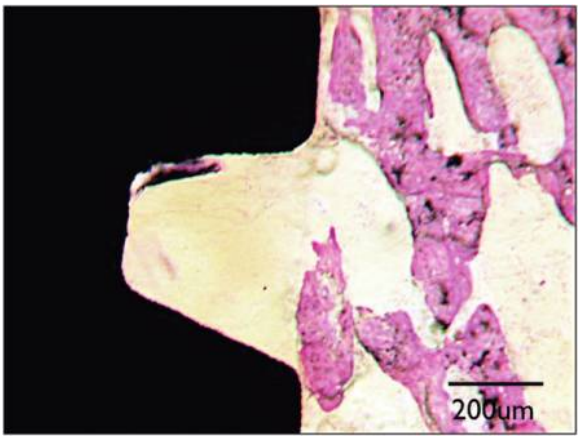

Figure 4.
Optical micrograph of a nano-surface implant at 20 days. Newly formed small bone trabeculae were observed growing toward the coronal implant surface (acid fuchsin and toluidine blue; original magnification ×50).

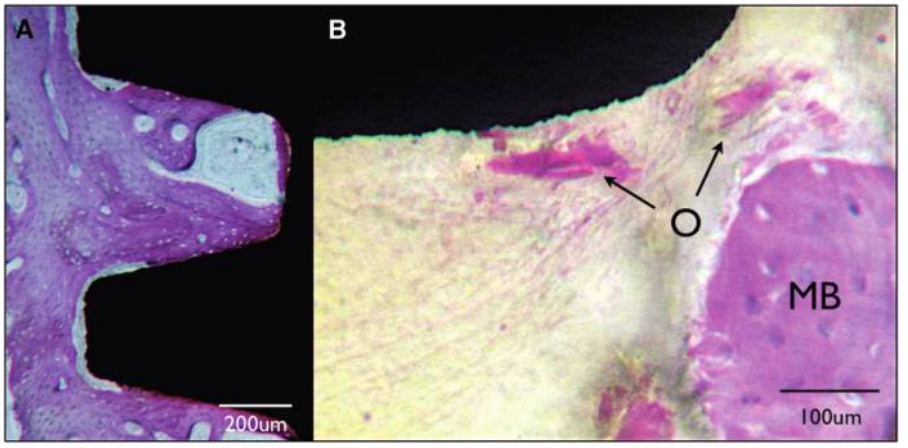

Figure 5.
A) *Intimate contact between bone–nano surface at 30 days (acid fuchsin; original magnification ×50).*
B) *Optical micrograph of a nano-surface implant at 60 days. Mature bone was in direct contact with the implant surface, whereas in other areas a gap or osteoid (O) matrix was interposed between mineralized bone (MB) and implant surface; (arrows) indicate newly formed bone in proximity with the implant surface (acid fuchsin; original magnification ×100).*

immediately after placement for osseointegration achievement,[19] which is in direct agreement with the histomorphologic results obtained in the present investigation.

Although the same general healing pattern was observed for both surfaces throughout the experiment, slight temporal differences were observed where the PSCaP achieved higher degrees of bone organization at earlier times in vivo. The histologic and histomorphometric results of the present study are in agreement with other studies that depicted higher degrees of BIC and in hydroxyapatite-coated implants compared to a smoother control surface.[20,21] We speculate that the significantly higher BIC and BAFO observed at all three times in vivo is likely related to the amount of Ca and P available at early implantation times for the thicker PSCaP coating because of its multiphasic character with crystalline and amorphous phases.[13,14]

Histologically, the plateau root form implant of both surfaces showed osteogenic ingrowth to the surface in non-functional conditions. The same observation was made for plateau root form implants with different surfaces by Granato et al.,[22] who demonstrated significantly higher torque and BIC values for the nano surface versus an uncoated moderately rough surface. According to the present, histomorphologic and histomorphometric results, plateau root form implants may further benefit if bioactive ceramic surfaces are used for rapid integration and consequently biomechanical fixation.

Plasma-sprayed bioceramic coatings have fallen from favor in clinical practice because of inherent potential limitations regarding chemical stability and coating-implant adhesion. However, no conclusive evidence of lower long-term success rates versus smooth or moderately roughened surface has been demonstrated to date. The survival rates reported for hydroxyapatite-coated implants were similar to the survival rates reported for uncoated titanium implants in a recent meta-analytic review. Detailed analysis of these clinical trials did not show that hydroxyapatite-coating compromised the long-term survival of dental implants.[23] On a shorter-term basis, Jeffcoat et al.[24] showed that after 5 years in clinical function, 95.2% of machined titanium-threaded implants and 97.92% of hydroxyapatite-coated threaded implants were successful. This study also demonstrated that 99% of PSHA-coated cylindrical implants experienced less than 2 mm of bone loss.[24] The success rates for the PSHA-coated implants have also been higher compared to titanium plasma-sprayed implants.[25] Thus, controlled prospective and retrospective clinical trials including modern moderately rough uncoated surfaces and bioactive ceramic-coated implants in both nanometer and micrometer range (e.g., PSCaP coatings) are desirable.

CONCLUSIONS

Within the limits of this study, it was possible to state that bioactive ceramic coatings of both thicknesses were biocompatible and osteoconductive. However, the early bone response was favored by the presence of the thicker PSCaP coating.

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