Experimental Evaluation in Rabbits of the Effects of Thread Concavities in Bone Formation with Different Titanium Implant Surfaces

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ABSTRACT

Background: Differences in implant microtexture are important in conditioning the bone response around dental implants.

Purpose: The aim of the present study was an evaluation of the bone response in machined (M), blasted with apatitic calcium phosphate (tricalcium phosphate/hydrossyapatite [HA] blend) particles (resorbable blast texturing [RBT]), and coated with HA implants.

Methods: A total of 48 (16 M, 16 RBT, and 16 HA) threaded screw-shaped implants were inserted into the tibia of 12 rabbits. The specimens were retrieved after 1, 2, 4, and 8 weeks and processed for histology.

Results: All experimental groups showed an increase of the bone-implant contact percentages through the study period. Higher and highly statistically significant differences were found in the percentages of bone observed in the concavities rather than in the convexities of the implants retrieved after 1, 2, and 4 weeks, while no significant differences were found after 8 weeks. In the different time periods, higher percentages of bone-implant contact were found in the RBT and HA-coated implants both in the concavities and in the convexities, but these differences were not statistically significant.

Conclusions: The newly formed bone present in the concavity of the threads was not influenced by the implant surface in the first healing period, while after 4 to 8 weeks, the percentage of bone observed in the concavities and convexities was similar. Additional histological studies are necessary to further evaluate the critical role of the concave geometry in bone differentiation and formation around dental implants.

KEY WORDS: bone growth, concavity, surface blasting, surface roughness, threads shape, titanium implants

INTRODUCTION

Currently, there is a strong interest in the biomaterials surface properties.[1] Different approaches are being investigated to try to obtain an ideal implant surface that is conducive to bone formation in the peri-implant region.[2] The tissue response to biomaterials is influenced by nano, micro, and macrotopography of their surface.[3] Most probably, there may be an optimal microroughness that affects the initial healing processes.[4] The optimal surface roughness (Ra) has not been determined yet, even if Han and colleagues have reported that an Ra of 1.5 μm produced a stronger bone response than smoother or rougher surfaces.[5] The aim is to optimize and possibly shorten the time of osseointegration.[3] A considerable variation exists in surface properties, such as topography, roughness, oxide thickness, oxide composition, and microstructure.[1,5,6] Cells have been shown to relate to different types of surfaces: macrophages, for example, have been shown to affect "rugophilia," while fibroblasts did not adhere to the same surfaces.[4] Stem and differentiating cells feel the substratum, and this fact can mean that migrating and

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DOI 10.1111/cid.12033

attaching cells are able to transform mechanical cues into soluble molecular signals.[7] A large series of implants with different surfaces have been put on the market. The implant surface can be machined (M), or it can be treated with subtractive treatments, such as sandblasting or acid-etching, or with addictive treatments, such as, for example, titanium plasma spray.[8,9] Moreover, grooves can be added to some portions of the implant surface to try to improve the implant initial primary stability.[3] These grooves can also have an influence on cell orientation and migration by the so-called contact guidance.[3] The migration, attachment, and spreading of the osteogenic cells toward the implant surface is of fundamental importance in bone regeneration around the implants; also, the formation of a scaffold composed by fibrin and the activation of the blood cells located at the implant surface most probably plays a role in the healing processes.[4,10,11] The microtexture of the implant surface seems to influence the attachment and growth of bone cells.[12,13] Microroughened surfaces have been shown to present more platelets than M surfaces,[10] and the roughest surfaces showed a higher rate of cell attachment than other types of surfaces.[14] Other researchers have demonstrated that a better bone fixation exists for rougher surfaces.[14] An abrasive blasting of an implant surface seems to increase the Ra, to remove the metal surface contaminants, and to increase the reactivity of the implant surface.[15] In a previous study on immediately loaded implants (Maestro, BioHorizons®, Birmingham, AL, USA) retrieved from man, we did report a striking finding, that is, the presence of newly formed bone inside the thread concavities even at a distance from preexisting bone.[16] We thought that this finding could be explained by the studies of van Eeden and Ripamonti[17] and of Ripamonti and colleagues[18–19] who found that the surface geometry of a material (hydroxyapatite [HA]) was important in the bone formation and that bone formation could initiate in concavities rather than on convexities of the HA substratum. We further hypothesized that the thread concavities of these implants, measuring about 500 µm in diameter and 500 µm in depth, could be of the right size and shape to be conducive to bone formation.[19]

The present study was planned to see if these anedoctal histological results could be confirmed or disproved in an animal study using the same implants with the same thread concavities; we decided also to see if the surface characteristics had also a role in the bone formation in these concavities. Therefore, the aim of the present study was then a comparison of the bone response to threaded implants inserted in rabbit tibia with three different surfaces (M, blasted with HA particles, and HA coated).

MATERIALS AND METHODS

Thread-shaped M implants, implants blasted with apatitic calcium phosphate (tricalcium phosphate/HA blend) particles (resorbable blast texturing [RBT]), and HA-coated implants made of titanium alloy (Specification for Wrought Titanium 6AL-4V ELI Alloy for Surgical Implant Applications [ASTM] F136) (Maestro) were used in this study. The particle size and blast parameters of the RBT process are proprietary. After the blasting procedure, the surface was then cleaned according to internal cleaning procedures and ASTM F86-06 (standard practice for surface preparation and marketing of metallic surgical implants).

Twelve New Zealand white mature male rabbits were used in this study. The protocol of the study was approved by the Ethical Committee of University of Chieti-Pescara, Italy. The implants were inserted into the tibia. Each rabbit received four implants, two implants in the right tibia, and two in the left tibia. A total of 48 implants (16 M, 16 RBT, and 16 HA) were inserted. The rabbits were anesthetized with intramuscular injections of fluanizone (0.7 mg/kg body weight) and diazepam (1.5 mg/kg body weight), and local anesthesia was given using 1 mL of 2% lidocain/adrenalin solution. A skin incision with a periosteal flap was used to expose the bone surface. The preparation of the bone site was done with burs under generous saline irrigation. The implant insertion was performed by hand. The periosteum and fascia were sutured with catgut and the skin with silk. No complications or deaths occurred in the postoperative period. Three animals were killed, with an overdose of intravenous pentobarbital, respectively, after 1, 2, 4, and 8 weeks. A total of 48 implants were retrieved.

Specimen Processing

Implants and surrounding tissues were washed in saline solution and immediately fixed in 4% paraformaldehyde and 0.1% glutaraldehyde in 0.15 M cacodylate buffer at 4°C and pH 7.4, to be processed for histology. The specimens were processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy).[20] The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned, along their longitudinal axis, with a high-precision diamond disc at about 150 μm and ground down to about 30 μm with a specially designed grinding machine. A total of three slides were obtained for each implant. The slides were stained with acid fuchsin and toluidine blue. The slides were observed in normal transmitted light under a Leitz Laborlux microscope (Laborlux S, Leitz, Wetzlar, Germany). Histomorphometry of bone-implant contact percentage in the concavities and convexities of the threads was carried out using a light microscope connected to a high resolution video camera (3CCD, JVC KY-F55B, Q IMAGING, Langley, Surrey (BC), Canada) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX, ASUS, Taipei, Taiwan, China). This optical system was associated with a digitizing pad (Matrix Vision GmbH, Oppenweiler, Germany) and a histometry software package with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc, Milano, Italy).

Three implant for each group were analyzed under a Leo scanning electron microscope (Zeiss, Hallbergmoos, Germany). Roughness measurements were performed for all types of implants, using a Mitutoyo Surftest 211 Profilometer (Mitutoyo Corporation, Tokyo, Japan): an average of three readings was performed for each surface. A total of five implants for each type of surface were analyzed.

Statistical Evaluation

The implant represented the unit of analysis. Analysis of variance was used to test the statistical significance of the differences among the three experimental categories in the bone-implant contact percentages. Bonferroni-corrected *t*-test for unpaired samples was employed as post hoc test. Values of $p < .05$ were accepted as statistically significant.

RESULTS

Scanning Electron Microscopy

M Implants. Typical grooves produced during the manufacturing of the implants were present (Figure 1A). The Ra was 0.81 μ.

RBT Implants. The surface was highly irregular with many small depressions, indentations, and peaks (Figure 1B). The Ra was 2.15 μ.

HA-Coated Implants. The surface was very rough and irregular with the presence of many depressions and peaks (Figure 1C). The Ra was 4.15 μ.

Light Microscopy

One Week (M). Only a few inflammatory cells were present. Newly formed small bone trabeculae could be seen growing toward the implant surface. It was possible to observe a large number of osteoblasts in the process of producing osteoid matrix toward the implant surface. No osteoblasts were detected on the implant surface. The bone-implant contact percentages in the concavities and convexities were 9.2 ± 2 and 0.2 ± 0.8, respectively.

Two Weeks (M). Newly formed, small bone trabeculae could be seen growing toward the implant surface. It was possible to observe a large number of osteoblasts in the process of producing osteoid matrix toward the implant surface (Figure 2). No inflammatory cells were present.

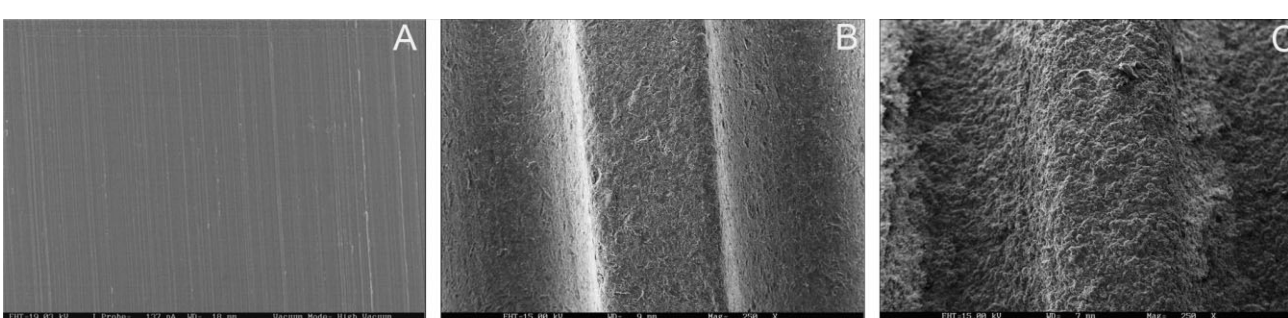

Figure 1 *A*, Machined surface. Grooves produced during the manufacturing of the implants are present, ×1000. *B*, RBT surface. The surface of the implants is highly irregular with many small depressions and indentations and flatter appearing areas, ×250. *C*, HA-coated surface. The surface is highly irregular with the presence of many peaks and valleys, ×250.

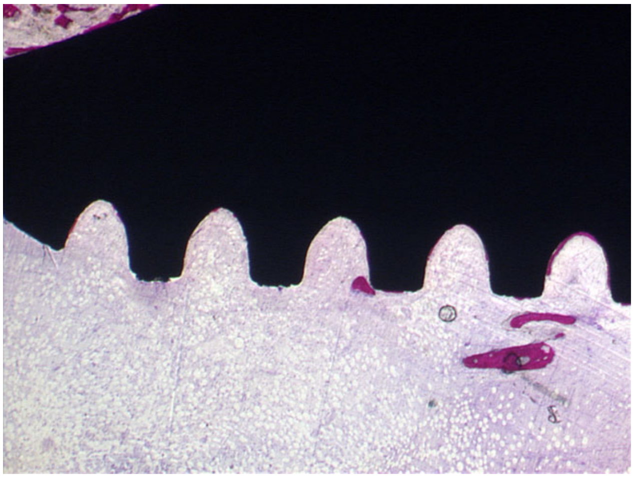

Figure 2 Machined implant after 2 weeks. Newly formed bone is present in the concavities of the implant, while only a few and very small trabeculae are present in the implant convexities. Toluidine blue and acid fuchsin, ×12.

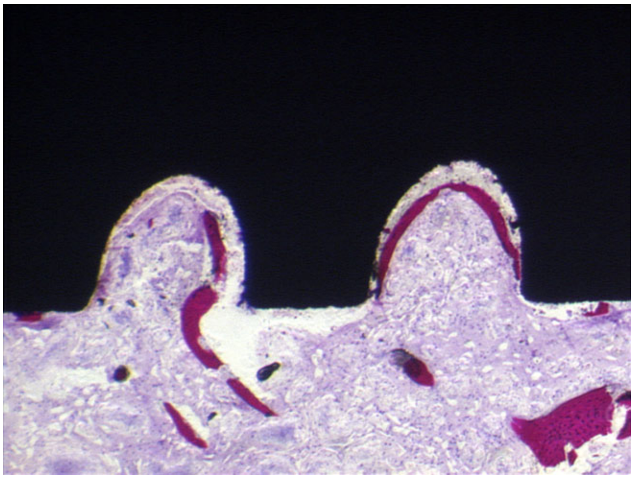

Figure 4 Particular of the previous figure at higher magnification. It is possible to observe newly formed bone in the thread concavities. Toluidine blue and acid fuchsin, ×100.

No osteoblasts were detected on the implant surface. The bone-implant contact percentages in the concavities and convexities were 14 ± 1 and 4 ± 2.2, respectively.

Four Weeks (M). The number of osteoblasts near the implant surface tended to decrease in a significant way. The peri-implant bone was more mature, and the marrow spaces were few in number. A direct contact between implant and bone was observed only in a few areas (Figures 3 and 4). The bone-implant contact percentages in the concavities and convexities were 19.5 ± 2.1 and 12 ± 3.5, respectively.

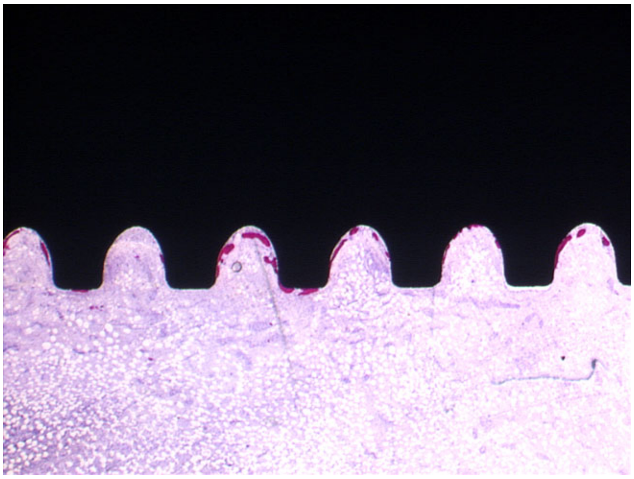

Figure 3 Machined implant after 4 weeks. The quantity of newly formed bone in the concavities of the implants is increased and only a few bone trabeculae are present in the implant convexities. Toluidine blue and acid fuchsin, ×12.

Eight Weeks (M). Osteoblasts were absent in almost all fields. Mature bone was in direct contact with the implant surface, while in other areas, a gap or osteoid matrix was interposed between mineralized bone and implant surface. The bone-implant contact percentages in the concavities and convexities were 26 ± 3.1 and 25 ± 4.2, respectively.

One Week (RBT). A large number of newly formed, intensely stained bone trabeculae was in contact with the implant surface. The osteoblasts produced osteoid matrix directly on the implant surface. Lines of cuboidal-shaped osteoblasts were visible around the implant perimeter. In a few areas, the preexisting bone was being resorbed by osteoclasts that were remodeling the bone that had been prepared during the surgical procedure. The bone-implant contact percentages in the concavities and convexities were 10 ± 2.2 and 1 ± 0.8, respectively.

Two Weeks (RBT). Newly formed, strongly stained bone was found in close contact with the implant surface. The bone trabeculae were wide and contained large osteocyte lacunae. The osteoblasts were actively secreting the osteoid matrix that was undergoing mineralization in some areas (Figure 5). The bone-implant contact percentages in the concavities and convexities were 16 ± 1.4 and 6.1 ± 2.3, respectively.

Four Weeks (RBT). Only in a few portions of the interface actively secreting osteoblasts were observed. Mature

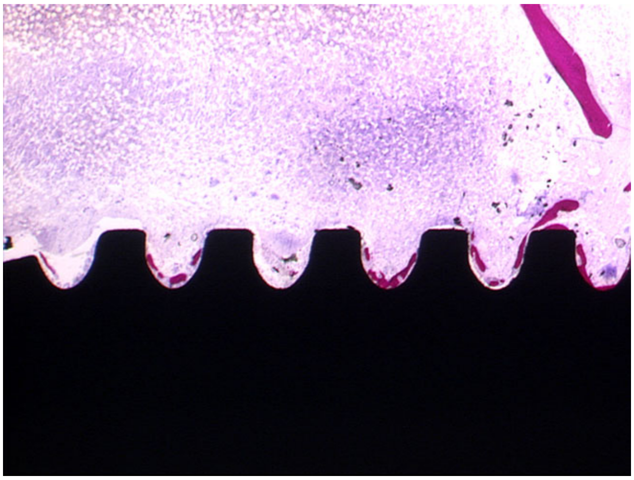

Figure 5 RBT surface after 2 weeks. Newly formed bone is present almost only in the concavities. Only a few bone trabeculae are present in the convexities. Toluidine blue and acid fuchsin, ×12.

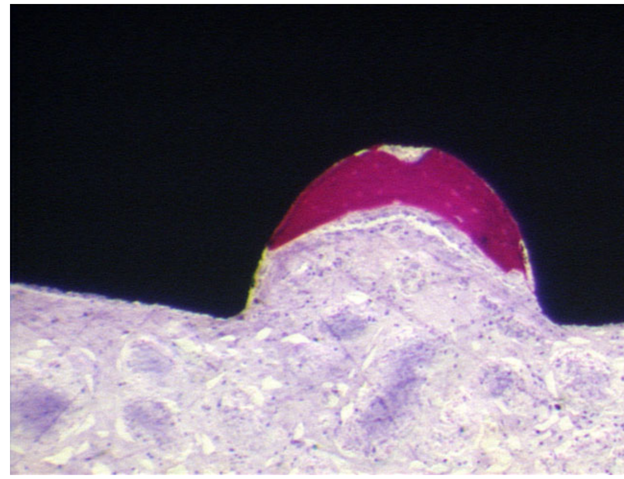

Figure 7 Higher magnification of the previous figure. Newly formed bone and many osteoblasts producing osteoid matrix directly on the implant surface are present. Toluidine blue and acid fuchsin, ×100.

bone and marrow spaces were present in other areas of the interface (Figures 6 and 7). The bone-implant contact percentages in the concavities and convexities were 22.5 ± 2.4 and 13 ± 3.2, respectively.

Eight Weeks (RBT). An increase in the quantity of bone around the implants was observed. Only a few osteoblasts were present. Mature mineralized bone and, only in a few areas, not yet mineralized osteoid matrix were detected at the interface. The bone-implant contact percentages in the concavities and convexities were 28 ± 3 and 24 ± 4.2, respectively.

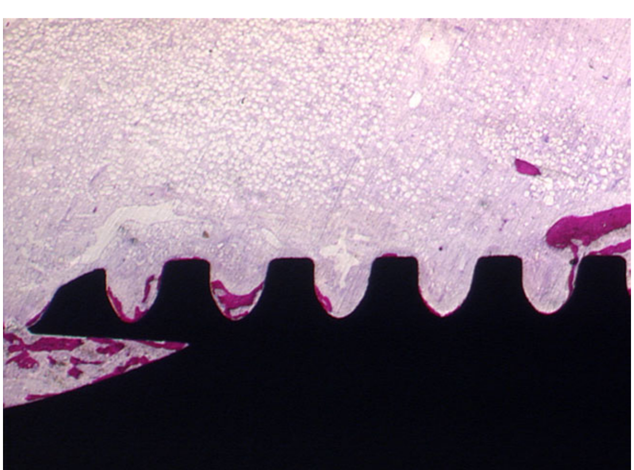

Figure 6 RBT surface after 4 weeks. It is possible to observe an increase of the bone in the concavities. Toluidine blue and acid fuchsin, ×12.

One Week (HA). It was possible to observe a large number of newly formed, intensely stained bone trabeculae that were in contact with the implant surface. The trabeculae were wide, with a woven immature appearance, and with large osteocyte lacunae. The osteoblasts were actively secreting the osteoid matrix that was undergoing mineralization in some areas. The bone-implant contact percentages in the concavities and convexities were 13 ± 2 and 1.3 ± 0.8, respectively.

Two Weeks (HA). The newly formed bone was in close contact with the HA coating. It was possible to observe a large number of newly formed, intensely stained bone trabeculae that were in contact with the HA coating. Osteoid matrix was produced directly on the HA coating. The bonding between the implant metal and the HA coating was very tight (Figure 8). The thickness of the HA coating of the implants appeared to be uniform. The bone-implant contact percentages in the concavities and convexities were 17 ± 1 and 6.1 ± 2.3, respectively.

Four Weeks (HA). It was possible to observe a large number of newly formed, intensely stained bone trabeculae that were in contact with the implant surface. The bonding between the implant metal and the HA layer was very tight and continuous, and in a few areas, the HA was colonized by biologic fluids. A few osteoblasts were actively secreting the osteoid matrix that was

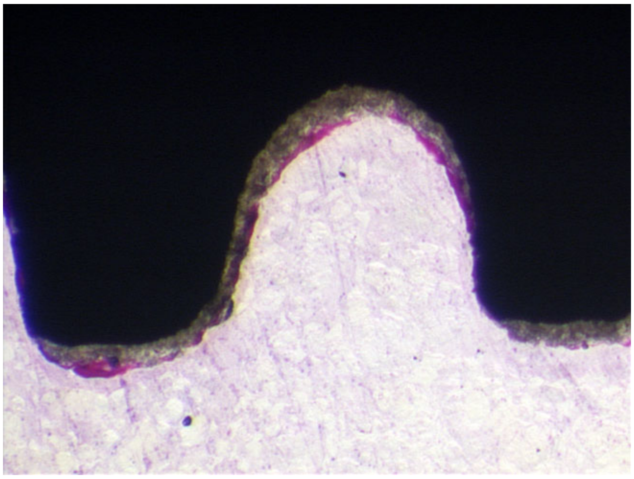

Figure 8 HA-coated surface after 4 weeks. Newly formed bone is in direct contact with the HA coating. Toluidine blue and acid fuchsin, ×50.

undergoing mineralization in some areas. The bone-implant contact percentages in the concavities and convexities were 24.5 ± 2.5 and 15 ± 3.7, respectively.

Eight Weeks (HA). Mature, lamellar bone was present in direct contact with the HA coating. Few osteoblasts were observed. The bone-implant contact percentages in the concavities and convexities were 30 ± 3.1 and 29 ± 1.2, respectively (Figure 9).

Statistical Analysis

All experimental groups showed an increase of the bone-implant contact percentages through the study period. Higher and highly statistically significant differences were found in the percentages of bone that had formed in the concavities rather than the convexities of the implants in the specimens retrieved after 1 ($p = .000$), 2 ($p = .000$), and 4 weeks ($p = .004$). No statistically significant differences were found in the percentages of bone that had formed in the concavities rather than the convexities of the implants in the specimens retrieved after 8 weeks ($p = .703$). In the different time periods, higher percentages of bone-implant contact were found in the RBT and HA-coated implants both in the concavities and in the convexities, but these differences were not statistically significant (Table 1).

TABLE 1 Statistical Analysis

	Concavity	Convexity	*p* Value
1 week (M)	9.2 ± 2	0.2 ± 0.8	.000
1 week (RBT)	10 ± 2.2	1 ± 0.8	.000
1 week (HA)	13 ± 2	1.3 ± 0.8	.000
2 weeks (M)	14 ± 1	4 ± 2.2	.000
2 weeks (RBT)	16 ± 1.4	6.1 ± 2.3	.000
2 weeks (HA)	17 ± 1	6.1 ± 203	.000
4 weeks (M)	19.5 ± 2.1	12 ± 3.5	.003
4 weeks (RBT)	22.5 ± 2.4	13 ± 3.2	.004
4 weeks (HA)	24.5 ± 2.5	15 ± 3.7	.004
8 weeks (M)	26 ± 3.1	25 ± 4.2	.703
8 weeks (RBT)	28 ± 3	24 ± 4.2	.703
8 weeks (HA)	30 ± 3.1	29 ± 1.2	.702

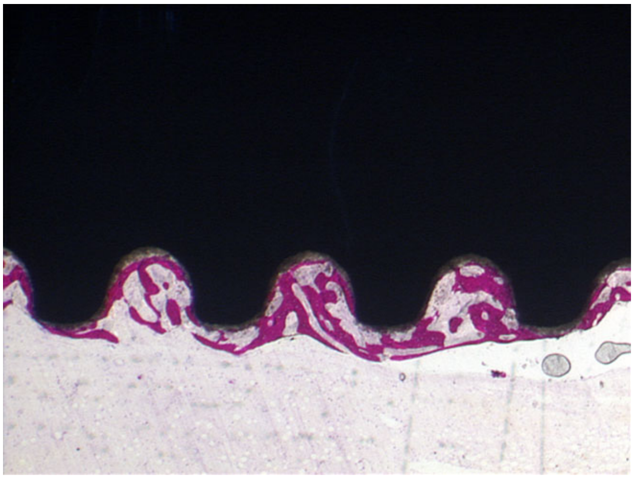

Figure 9 HA-coated surface after 8 weeks. Mature bone is in direct contact with the implant surface. No differences are present between the quantity of bone present inside the concavities and the convexities. Toluidine blue and acid fuchsin, ×12.

DISCUSSION

In the present study, we did find that the bone formation started preferentially in the implant thread concavities during the first healing period. In fact, a higher and highly statistically significant difference was found in the percentages of bone that had formed in the concavities rather than the convexities of the implants in the specimens retrieved after 1, 2, and 4 weeks. The percentages of bone that had formed in the concavities and in the convexities of the implants became similar in the specimens retrieved after 8 weeks. Similar results have been reported by Hall and colleagues and by Burgos and colleagues who found, in a rabbit study, that new bone formation was observed more often in the grooves and in areas with no adjacent host bone, usually as solitary islands with apparently no connection to surrounding and preexisting bone.[3,21,22] In Hall and colleagues'[21] study, about 70% of the grooves were filled by bone.

It has been demonstrated that topography may modulate the osteoblast differentiation,[8] and rougher surfaces produced a higher degree of bone formation around the implants.[23–30] Moreover, the geometric properties of implant surfaces seem to impose also mechanical restrictions on the cell cytoskeletal components that are involved in cell spreading and locomotion.[26–28] It has been reported that the thread geometry may be related to the amount of bone at the implant interface.[31] The Maestro system was designed to increase the surface area of support in relation to bone density, and implants designed for soft bone have more than 30% additional surface area compared with other threaded implant designs with a resulting less stress transferred to the surrounding bone.[28] Square threads, moreover, have been reported to have a higher bone-implant contact percentage and higher reverse-torque values compared with V-shaped thread or reverse buttress thread.[31] An HA coating has been described to produce a faster and greater bone-implant contact percentage and greater reverse-torque resistance when compared with non-coated implants.[32] Threads are used to increase the initial contact area between implant and bone, to improve the initial stability, to enlarge the implant surface area, and to favor dissipation of interfacial stresses.[33] The design of a dental implant should be such that high stress peaks are avoided in the peri-implant bone.[34] One method to decrease the strain in bone is to decrease the stress to the implant and to the prosthesis.[31] As a result, conditions that increase area of support in the bone or methods to decrease force to the prosthesis are appropriate.[31] The smaller the peak stress in the bone, the bigger is the load that can be carried before the onset of bone resorption.[34] A square thread, such as that of the implants used in the present study, provides an optimized surface area for intrusive and compressive load transmission, producing a lower strain profile to bone.[33] Bone has been shown to be the strongest in compression and the weakest in shear loading.[31] A square threaded implant is reported to have a 10-fold reduction in remodeling rate[31] and has demonstrated to show a higher bone to implant contact (BIC) percentage.[33]

We have already seen that the geometry of the substratum affects both cell shape and movement, and, as a result, cell function.[17] Moreover, the geometry of the substratum has been shown to profoundly influence the expression of the chondro-osteogenic phenotype in vivo.[17,35] Biomimetic materials able to induce bone formation through osteoinduction are changing in an important way the techniques of bone regeneration.[35] In previous in vitro studies from our laboratory, we found that microconcavities significantly affected dental pulp stem cells differentiation into osteoblasts.[36,37] The stem cells challenged with concave surfaces differentiated quicker and showed nuclear polarity, an index of secretion, cellular activity, and matrix formation.[36] On the contrary, the worst cell performance was found with the use of convex surfaces.[36] In a series of experiments, most of which on primates, Ripamonti and colleagues have demonstrated that a specific geometry of the substratum of HA determines bone formation by induction within the porous HA, a phenomenon called *geometric induction of bone formation*.[38–46] This geometric induction of bone formation will be helpful in the engineering of newly bone formation in the clinical context.[35] Ripamonti has found that bone formation initiation may be linked to concavities rather than planar or convex surfaces.[35] He also found that the concavity is smart in the sense that it anchors specific endogenous bone morphogenetic proteins at the interface of the HA with the fibrovascular tissue with induction of bone as a secondary response.[46–50] They have also found that in order for these concavities to be active, they must have the following measures: between 800 and 1600 μm in diameter and between 400 and 800 μm in depth.[35]

The concavities of our implants, measuring about 500×500 μm are somewhat in these ranges; moreover, the data of Ripamonti[35] refers to HA specimens, while our data were obtained with titanium implants and there could be differences between these two materials. More recently, Ripamonti and colleagues performed a study with the use of HA-coated titanium implants.[7] They found that a series of repetitive concavities generated an inductive microenvironment for the induction of bone formation. They stated that "the language of the shape is the language of geometry, the language of geometry is the language of a sequence of repetitive concavities that biomimetize the remodeling cycle of the osteonic bone."

What is striking in our implants is that, in every case, in the first healing periods, the bone formation started almost exclusively inside the concavities. Our results showed also an higher percentage of bone-implant contact in the RBT and HA-coated implants both in the concavities and in the convexities, but these differences were, however, not statistically significant. In this experiment, the surface characteristics do not seem to have a relevant role in the percentages of bone formation. Ripamonti[46,51] has shown that the concavities of biomimetic biomaterial matrices were geometric regulators of growth with a shape memory, recapitulating events that occur in the course of normal embryonic development and appearing to act as gates, giving or withholding permission to growth and differentiation. The concavities are thus regulators of bone initiation and deposition during remodeling processes of the skeleton.[46] Furthermore, microconcavity production is a process mimicking physiological conditions within marrow spaces during hematopoiesis, and microconcavities enhance the total area available, in a given volume, for cell membrane interactions.[36] This increase in the contacts of cell membrane serves to augment the exchanges at the level of the cell surface and is a very important step for cell differentiation.[36] The concavities per se are regulators of growth, inducing specific tissue formation and bone induction as in the remodeling processes of the osteonic primate cortico-cancellous bone and act as powerful geometric attractant for bone forming cells.[46,51]

In conclusion, the grooves seem to provide a suitable environment for bone formation, possibly due to mechanical forces, blood clot retention, and presence and gradients of chemotactic and other agents from the healing process.[21,22] The constraining of a cell population into a limited space seems to favor differentiation and bone formation.[22]

Additional studies are necessary to further evaluate the critical role of the concave geometry in bone differentiation and formation around dental implants.[35]

ACKNOWLEDGMENTS

This work was partially supported by the Ministry of Education, University and Research, Rome, Italy.

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