Biomimetic Calcium Phosphate Composite Coating of Dental Implants

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Purpose: *The aim of the present study was to test the hypothesis that calcium phosphate coating of titanium screw-type implants enhances peri-implant bone formation in the jaw.* ***Materials and Methods:*** *Ten adult female foxhounds received experimental titanium screw-type implants in the mandible 3 months after removal of all premolar teeth. Four types of implants were evaluated in each animal: implants with machined titanium surface (the control group), implants coated with collagen I (the collagen-only group), implants with a composite coating of calcium phosphate and mineralized collagen I (the composite group), and implants with calcium phosphate (hydroxyapatite [HA]) coating (the HA-only group). Peri-implant bone regeneration was assessed histomorphometrically after 1 and 3 months in 5 dogs each by measuring bone-implant contact (BIC) and the volume density of the newly formed peri-implant bone (BVD).* ***Results:*** *After 1 month, BIC was significantly enhanced only in the group of implants with composite coating of calcium phosphate and mineralized collagen (*P = .038*). Volume density of the newly formed peri-implant bone was significantly higher in all coated implants after 1 month. No significant difference from baseline was found in BIC for the collagen-only and HA-only groups, but BVD was significantly higher in implants with composite coating (*P = .041*). After 3 months, BIC and BVD were significantly higher in all coated implants than in the controls with machined surfaces.* ***Conclusion:*** *It was concluded that composite coating of dental screw-type implant surfaces using calcium phosphate and collagen can enhance BIC and peri-implant bone formation.* Int J Oral Maxillofac Implants 2006;21:738–746

Key words: *biomimetic coating, calcium phosphate, collagen, dental implants, osseointegration*

Biomimetics involves the employment of microstructures and functional domains of organismal tissue function to design and synthesize new materials for health applications.[1] Biomimetic coating of metal implants for load-bearing intraosseous applications thus would benefit from surface modifications that closely resemble the morphology and chemistry of bone tissue in order to achieve more rapid and/or extensive bone anchorage. This biomimetic approach allows the deposition of calcium phosphate phases on the metal surface under physiologic conditions in simulated body fluids. These coatings are supposed to exhibit structures closer to bone mineral than conventional plasma-sprayed hydroxyapatite (HA) coatings.[2] Modifications in the composition of simulated body fluids have resulted in the formation of different mineral phases.[3–6] Variations in the composition and crystallinity of these coatings affects the in vitro solubility and attachment of bone marrow stromal cells.[7] Comparison of the in vivo behavior of different biomimetic calcium phosphate coatings has shown that dissolution differs according to the organic compounds, structural properties, and thermodynamic stability of the mineral phases used.[8]

Besides mineral phases, the second component of bone tissue is the organic matrix. Collagen, the main component, is combined with noncollagenous proteins. While the latter have distinct functions in controlling mineralization and inducing neovascularia-

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tion and osteogenesis, collagens provide sites for mineralization and also for cellular attachment by exposing RGD- and non-RGD protein sequences that bind to surface receptors such as integrins.[9] A combination of collagen fibers and calcium phosphate mineral phases in a mineralized structure could therefore better mimic the complex extracellular matrix of bone tissue on the surface of a metal implant. The results of a previous study in which mineralized collagen coating on square titanium alloy rods was compared with biomimetic HA coating were ambiguous.[10]

The aim of the present study was to test the hypothesis that the use of a biomimetic composite coating of dental implants using both collagen and calcium phosphates can enhance peri-implant bone formation when used in a clinically relevant model using screw-type implants.

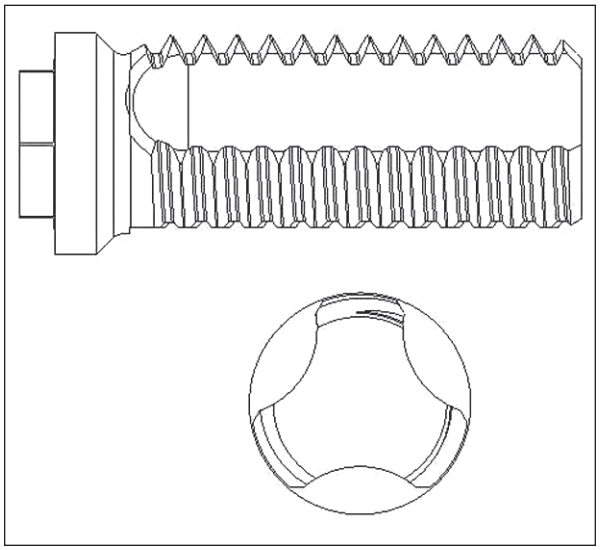


Fig 1 Schematic drawing of the experimental implant.

MATERIALS AND METHODS

The study was performed on 10 adult female foxhounds (average weight approximately 31.0 kg). The dog mandible was chosen because it has been established as a model for peri-implant bone regeneration under clinically relevant conditions.[11–13]

Experimental implants (3i/Implant Innovations, Palm Beach Gardens, FL) were modified from screw-type implants 4 mm in diameter by creating 3 longitudinal grooves to allow for both analysis of bone formation in the peri-implant space and assessment of osteoconductive properties of the surface modifications (Fig 1).

Four types of implants were used:

- **The control group** comprised titanium screw-type implants with a smooth uncoated machined surface (Fig 2a).
- **The collagen-only group** comprised titanium screw-type implants with a collagen I coating. Collagen fibers (Colbar Life Sciences, Herzliya, Israel) were anchored on the implant surface by adsorption on the titanium surface and subsequent anodic polarization (Fig 2b) as described previously.[14,15] Anodization was carried out in potentiostatic mode at 5 V_{SCE} for 60 seconds. Afterwards the collagen layer was thickened by dip coating followed by cross-linking with N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC), leading to a layer density of 44 ± 5 µg/cm^2 on the implant surface.
- **The composite group** comprised titanium screw-type implants with a machined surface covered with a combined HA and mineralized collagen coating. A base layer of HA was produced on the implant surface using an electrochemical-assisted process under cathodic polarization of the sample in a $Ca^{2+}/H_xPO_4^{(3-x)-}$ solution in near-physiologic conditions (pH 6.4, 37°C).[16] To increase the adhesion strength of the layer, an anodization step was included in the calcium phosphate deposition process, leading to partial integration of the HA crystallites into the grown oxide layer.[17] On this base layer, collagen fibrils were adsorbed and cross-linked with EDC. A mineralization step was carried out using the same electrochemical-assisted process as for base layer deposition at a current density of –9 mA/cm^2, for a polarization time of 15 minutes. As a result of this process the surface layer of the coating is formed by a network of completely mineralized collagen fibrils[18] (Fig 2c).
- **The HA-only group** comprised titanium screw-type implants with a machined surface coated with HA. The calcium phosphate coating was prepared in a manner identical to that used for group 3. Taking into account the total charge flow in the deposition process, the amount of calcium phosphate in the coatings of group 3 and 4 differed by less than 13%. The characteristic morphology of the layer is given by HA crystallites with a typical length of 300 nm and a typical diameter of 60 nm (Fig 2d).

Surgical Procedure

Three months after removal of all premolar teeth in the dogs' mandibles, the alveolar crest was re-exposed through a buccal incision after elevation of a lingually based mucoperiosteal flap. Implant sites were prepared in the edentulous area using spiral drills with

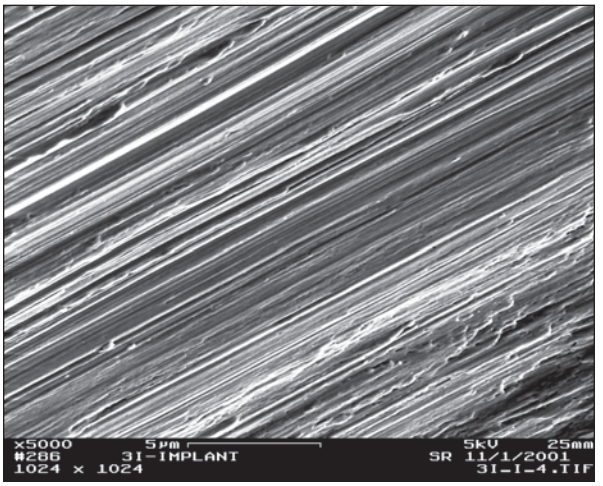


Fig 2a Scanning electron micrographic (SEM) image showing the machined titanium surface. SEM images were obtained with a Gemini electron microscope (Zeiss, Oberkochen, Germany; bar = 5 µm).

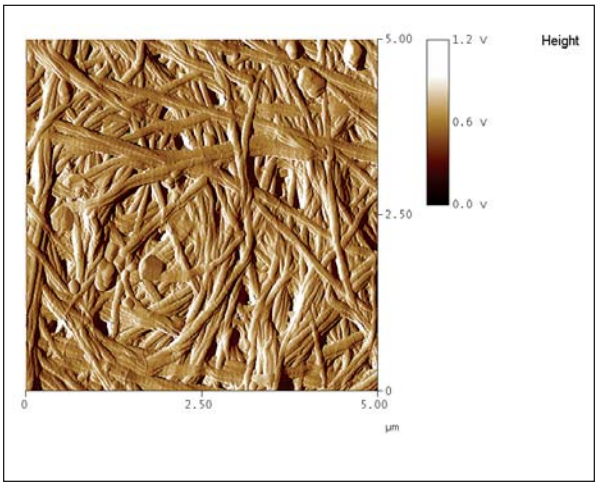


Fig 2b Atomic force microscopic (AFM) image of the collagen-coated titanium surface.

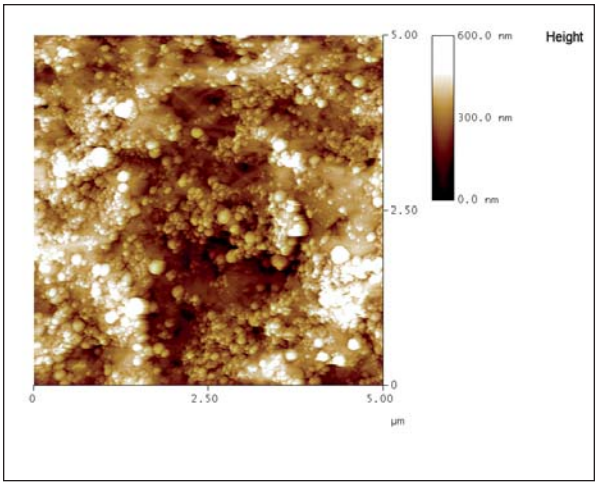


Fig 2c AFM image of the titanium surface coated with mineralized collagen.

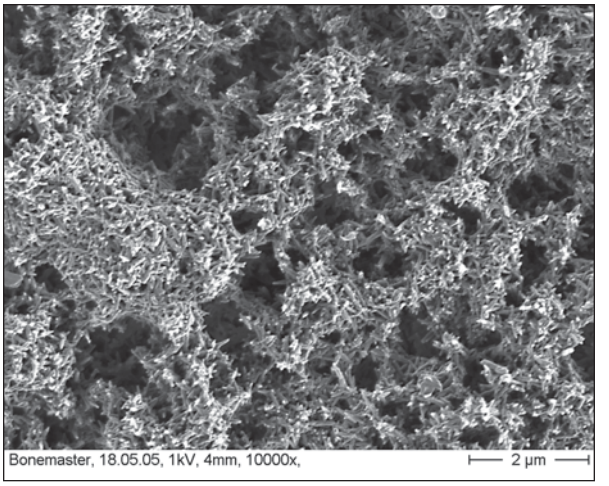


Fig 2d SEM image of the HA titanium surface (bar = 2 µm).

increasing diameters and a screw tap. The implants were placed into a pretapped implant bed to avoid abrasion of the organic coating caused by friction during placement. The positioning of individual implants within the premolar area was varied randomly to neutralize effects of possible variations in bone density. The mean insertion torque during placement was 3.3 Ncm (Osseocare; Nobel Biocare, Göteborg, Sweden). After cover screw placement, the wounds were closed using resorbable polyglactin sutures. Following surgery, the dogs were inspected daily, and oral hygiene procedures were performed once a week.

Five animals were sacrificed at 1 month for retrieval of the implants; the other 3 were sacrificed at 3 months. Mandibular segments were retrieved by bilateral segmental resection from the molar area to the midline and fixated immediately in 4% buffered formalin. The implants were located radiographically, and the mandibular bone was then separated into segments that contained 1 implant each using a diamond-coated saw (Exakt Apparatebau, Norderstedt, Germany). The individual implants with surrounding bone were embedded into methylmethacrylate resin. Undecalcified sections (thickness 30 to 70 µm) of the embedded specimens were fabricated with a diamond-edged blade in a rotating saw (Leitz, Hamburg, Germany) used perpendicular to the long axes of the implants. The resulting specimens were surface stained using toluidine blue and Masson-Goldner staining. Each implant produced between 13 and 15 specimens.

To account for varying degrees of bone density at different heights along the implant body, all speci-

Table 1 Histomorphometric Results (%)

	Control		Collagen only		Composite		HA only	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 month								
BIC	31.5	10.8	41.9	10.5	62.6	17.0	45.2	9.0
BVD	16.1	7.9	22.9	6.7	40.9	11.8	33.0	8.6
3 months								
BIC	41.2	10.9	60.2	7.3	59.0	4.0	61.7	5.3
BVD	40.6	9.9	62.6	14.0	67.3	11.8	58.5	10.3

mens of each implant were evaluated. Active bone formation showing osteoid and osteoblast seams as well as osteoclastic resorption and the nature of the bone-implant contact (BIC) were registered. Histomorphometry was performed on all specimens of all implants using a video camera (Sony, Tokyo, Japan) to record images at 50× magnification. The images were digitized (Axiophot-System; Zeiss), and the implant perimeter was marked on the screen. Areas of the surface with BIC were marked and measured by counting all pixels of the implant perimeter occupied by bone. BIC was expressed as a percentage of the number of pixels of the complete implant perimeter. The volume density of the newly formed peri-implant bone (BVD) was assessed by calculating the percentage of the surface area inside the grooves occupied by bone. This surface area was defined by placing a tangent line on the outer edges of the groove and counting all pixels between this tangent line and the implant perimeter within the groove. Mean values were calculated for each implant and for each group of implants. Differences in group mean values were tested for statistical significance using paired *t* tests within the groups for each observation interval and using unpaired *t* tests for comparisons between the 2 intervals. Differences were considered significant if $P < .05$.

RESULTS

All animals survived the surgical procedures and were available for evaluation. No signs of infection were registered during the healing period or at the time of implant retrieval.

Histologic Results

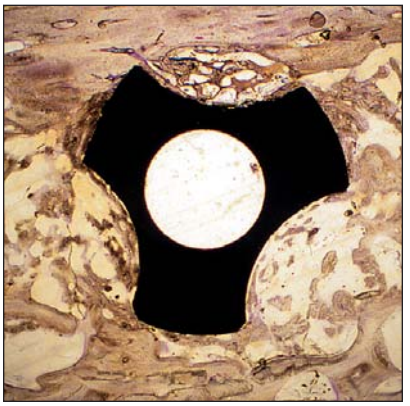
In general, bone density varied across the cross section of the mandible, with an area of lower bone density in the center above the mandibular canal. This pattern was appreciable in all implant locations. Moreover, the thickness of the cortical bone was variable among individual implant locations. Effects of varying bone density in different implant sections and different implant locations on the assessment of BIC and bone density were compensated for by random allocation of implant positions and by evaluation of all specimens of each implant.

1-month Healing Period. After 1 month, young trabecular bone partially filled the grooves around the collagen-coated implants (Fig 3a). Thin trabeculae were in contact with the implant surface in the grooves. New bone was forming along the surface in an osteoconductive pattern (Fig 3b). Bone formation was more extensive in the composite group. The areas of BIC and BVD appeared to be increased in the composite group and exhibited increased vascularity (Figs 3c and 3d). The bone formation observed in the HA-only group was comparable with that observed in the collagen-only group (Figs 3e and 3f). The control implants exhibited very little bone formation within the grooves. Bone contact with the implant surface was located mainly on the outer surface of the implant diameter, which had been in contact with the pre-existing bone (Fig 3g). Only very limited bone contact with small and tiny regenerates was appreciable (Fig 3h).

3-month Healing Period. After 3 months, the structure of peri-implant bone had changed around all implants (Figs 4a to 4h). All implants with a coated surface exhibited very similar features in that the bone around the implants had matured considerably and formed an almost continuous layer of bone on the surface. This resulted in a densification of peri-implant bone (Figs 4a, 4c, and 4e). Higher magnifications revealed a smoother but still active bone surface and signs of remodeling of peri-implant bone in all groups with coated implants (Figs 4b, 4d, and 4f). Control implants exhibited less bone formation, particularly in the groove area, and little BIC beyond the area of the outer surface (Figs 4g and 4h).

Histomorphometric Results

1-month Healing Period. After 1 month, the collagen-only group exhibited a mean BIC of 41.9% ([SD] 10.5), which was not significantly higher than the BIC exhibited by uncoated implants (31.5%; SD 10.8; $P = .098$) (Table 1). The composite group exhibited signif-



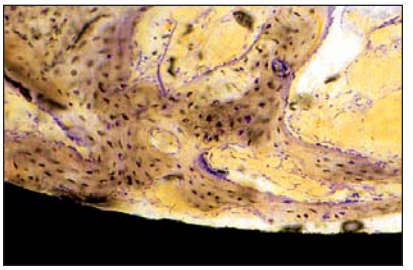
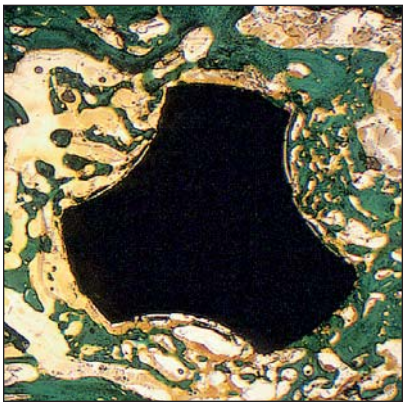


Fig 3a *(left)* Micrograph showing bone regeneration after 1 month in the vicinity of a collagen-coated implant showing immature peri-implant bone formation (toluidine blue; original magnification ×11.5).

Fig 3b *(right)* Micrograph showing a view of bone formed on the surface of a group 2 implant at a higher magnification (toluidine blue; original magnification ×200).



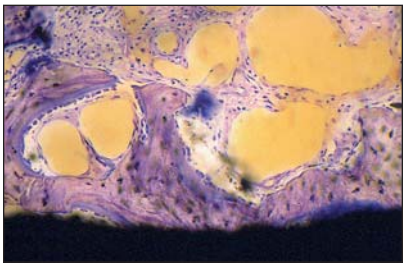
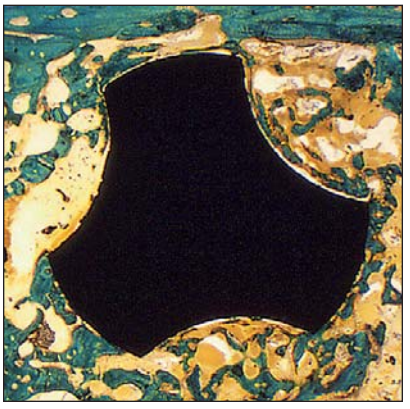


Fig 3c *(left)* Micrograph showing bone regeneration after 1 month in the vicinity of an implant coated with HA and mineralized collagen (group 3) features comparable with those observed in specimens of collagen-coated implants at low magnification (Masson Goldner; original magnification ×11.5).

Fig 3d *(right)* Bone formation with osteoid seams in close contact with the surface of an implant coated with HA and mineralized collagen. Note increased thickness of the bone layer (toluidine blue; original magnification ×200).



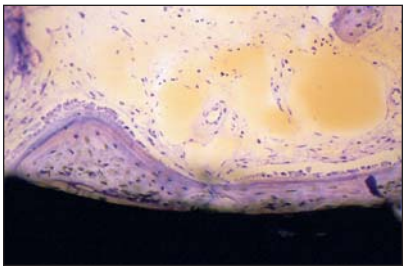
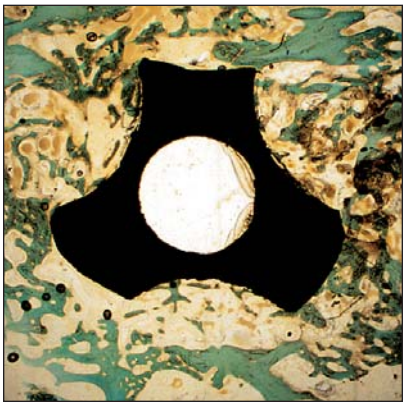


Fig 3e *(left)* Micrograph showing bone regeneration after 1 month in the vicinity of an HA-coated implant showing features comparable with those observed in specimens of collagen-coated implants (Masson Goldner; original magnification ×11.5).

Fig 3f *(right)* Higher magnification showing increased formation of peri-implant bone with osteoid seams and active osteoblasts (toluidine blue; original magnification ×200).



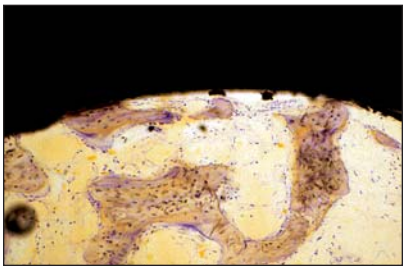


Fig 3g *(left)* Micrograph showing bone regeneration after 1 month in the vicinity of a control implant (group 1). Thin bone formation at a distance from the implant surface is appreciable (Masson Goldner; original magnification ×11.5).

Fig 3h *(right)* A machined implant specimen at a higher magnification. Thin trabeculae and little BIC can be observed (toluidine blue; original magnification ×200).

Fig 4a *(left)* Micrograph showing bone regeneration after 3 months next to a collagen-coated implant, with a continuous layer of bone on the surface (Masson Goldner; original magnification ×11.5).

Fig 4b *(right)* Micrograph showing bone formed on the surface of the implant at a higher magnification (original magnification ×200).

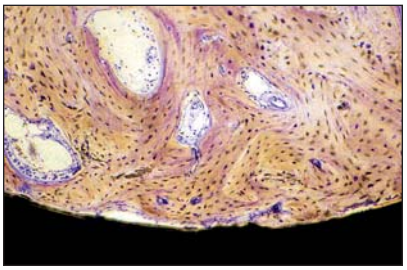
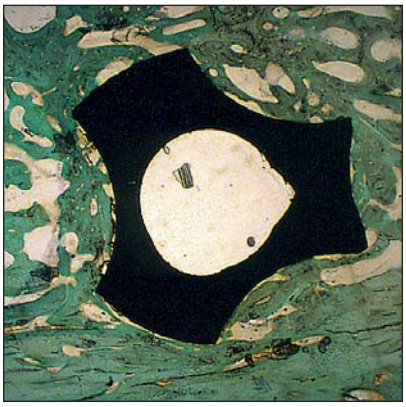


Fig 4c *(left)* Micrograph showing bone regeneration after 3 months in the vicinity of an implant coated with HA and mineralized collagen. The results were comparable to those observed with collagen-coated implants (Masson Goldner; original magnification ×11.5).

Fig 4d *(right)* Micrograph showing bone formed on the surface of an implant coated with HA and mineralized collagen exhibiting signs of remodeling (toluidine blue; original magnification ×200).



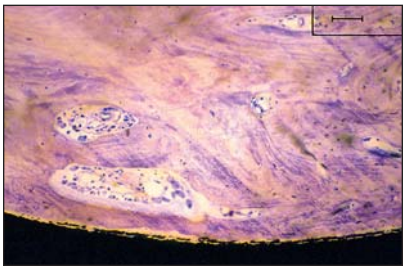
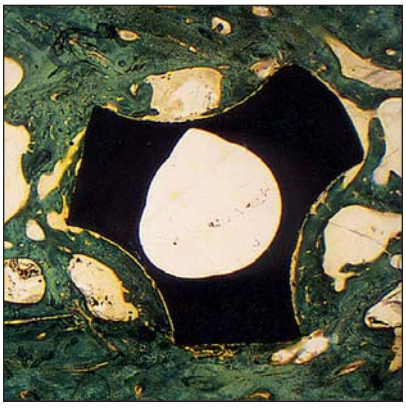


Fig 4e *(left)* Micrograph of an HA-coated implant showing bone regeneration after 3 months (1000 µmol/mL; Masson Goldner; original magnification ×11.5).

Fig 4f *(right)* Micrograph showing bone formed on the surface of an HA-coated implant at a higher magnification. There is a continuous layer of bone on the surface with osteoid seams and osteoblasts (toluidine blue; original magnification ×200).



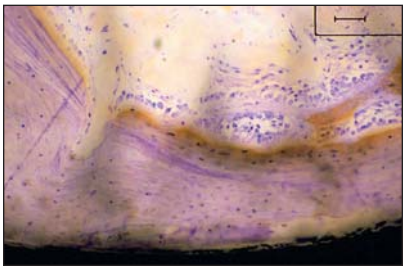
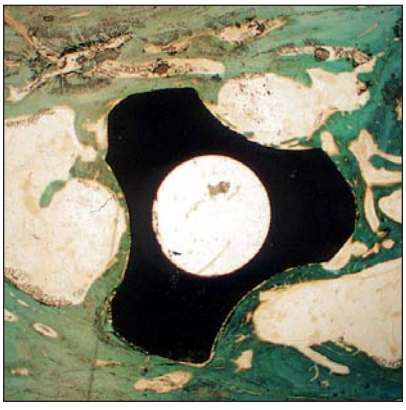
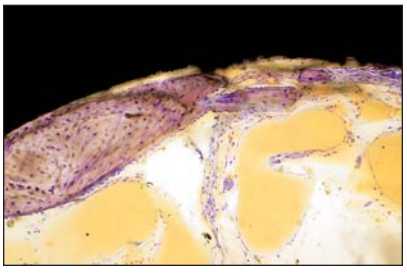


Fig 4g *(left)* Micrograph showing bone formation next to a control implant. Bone density and bone implant contacts appear to be limited mainly to preexisting bone (Masson Goldner; original magnification ×11.5).

Fig 4h *(right)* Sparse and thin bone trabeculae next to a machined implant surface (toluidine blue; original magnification ×200).





icantly greater mean BIC than the machined controls (42.6% versus 31.5%; $P = .038$). Implants coated with HA alone nearly showed a significant increase in average BIC compared with the machined controls, although the mean value was largest in this group (45.2%; SD 9.0; $P = .057$). Mean BIC values of the 3 experimental coatings were not significantly different from each other.

The mean BVD of the newly formed peri-implant bone was 22.9% (SD 6.7) in the grooves of the collagen-only implants. This was significantly higher than the BVD in the control group (16.1%; SD 7.9; $P = .011$). The average BVD values of the other 2 implant groups were also significantly higher: 40.9% (SD 11.8) in the composite group ($P = .001$) and 33.0% (SD 8.6) in the HA-only group ($P = .017$). Differences in mean BVD values between the 3 experimental coatings were significant ($P = .041$).

3-month Healing Period. After 3 months, the mean BIC values had increased significantly only in the collagen-only group (60.2%; SD 7.3; $P = .016$) and the HA-only group (61.7%; SD 5.3; $P = .008$) (Fig 5b). The control group (41.2%; SD 10.9; $P = .132$) and the composite group (59.0%; SD 4.0; $P = .069$) increased in mean BIC; however, the increases were not statistically significant. Nevertheless, mean BIC values of all coatings increased significantly compared to machined surfaces at 3 months ($P = .003$, .004, and .005; for the collagen-only, composite, and HA-only groups, respectively).

In contrast to BIC values, mean peri-implant BVD values increased significantly from 1 to 3 months in all implant groups. Machined surfaces exhibited 40.6% BVD (SD 9.9; $P = .002$); collagen-coated surfaces, 62.6% (SD 14.0; $P < .001$); surfaces with composite coating, 67.3% (SD 11.8; $P = .008$); and HA-coated surfaces, 64.2% (SD 10.3, $P = .003$). Mean BVD values were also significantly higher in all coated implants than in the machined controls ($P = .016$, $<$ .001, and .016 for the collagen-only, composite, and HA-only groups, respectively). Differences in both BIC and BVD values were not significant between the 3 types of coatings after 3 months ($P = .449$ and .165, respectively).

DISCUSSION

Calcium phosphate coating has proved to be beneficial for the anchorage of metal implants in bone tissue[19–25]: Immersion and sinter coating, hot isostatic pressing, sputter coating, and plasma flame spray coating have been employed for coating of dental implants.[19] Most coatings have been associated with increased BIC or an improved ability to bridge peri-implant bone gaps.[20–25] One major disadvantage with plasma flame spray coatings has been the layer thickness of 50 to 100 μm. Mechanical failure and adverse tissue reactions during degradation of separated coating fragments[26] has led to a more biologically oriented approach in recent years. Biomimetic calcium phosphate coating can provide thinner layers that serve the same purposes of cell attraction and attachment but are associated with fewer biologic reactions during degradation.

The fate of biomimetic coatings in vivo is not yet clear. Under neutral pH conditions, in vitro dissolution of biomimetic coatings is mainly dependent on the phase composition, crystallinity, and crystal size,[7] but chemical composition can also alter the behavior of these coatings in vivo. Differences in chemical composition and an acidic environment during early stages of implant healing may promote dissolution of biomimetic calcium phosphate coatings and can affect the longevity of these coatings in vivo. Degradation of biomimetic coating, however, has shown no negative effect on bone behavior in vivo.[26]

An important issue for the use of these coatings on screw-type implants is the mechanical stability of the interface between the coating and implant body, as friction during the placement of self-tapping screws could remove the coating from the implant surface. In previous studies, cracks and delamination of calcium phosphate coatings appeared at forces between 6 and approximately 10 to 12 N, respectively, in electrolytically deposited calcium phosphate layers.[7] In the present study, the insertion torque during the placement of implants into the pretapped implant bed was 3.3 Ncm on average. It is therefore unlikely that substantial delamination of the mineralized surface occurred during implant placement.

In the present study, an experimental coating was created by integrating collagen I, as the main component of the organic bone matrix, into the surface coating so that it would more closely resemble the structure and appearance of natural bone. There are few reports on the use of organic components in biomimetic coating. Simultaneous application of bovine serum albumin (BSA) and calcium phosphate by coprecipitation has shown incorporation of BSA into the mineral crystal lattice work and resulted in the gradual transformation of octacalcium phosphate into carbonate apatite.[5] The sequential use of collagen and calcium phosphate in the present study created a surface that provided a solid calcium phosphate basis on the metal surface of the implant with randomly oriented adsorbed collagen fibers that then became mineralized. The implants with this type of coating showed significantly more BVD and BIC compared to the machined control surfaces after

only 4 weeks. It is remarkable that neither pure collagen nor pure HA coatings were able to achieve significant improvement in BIC values within this timeframe. This suggests that the composite coating has improved osteoconductive properties and supports the hypothesis that a biomimetically mineralized combination of organic bone matrix components and calcium phosphates could improve peri-implant bone reaction in early stages of healing. After 3 months this effect was no longer appreciable, as all types of coatings showed significantly improved BVD and BIC values, and the pure collagen and HA coatings had caught up with the results of the composite coating.

The present results are more encouraging than those of a previous preliminary study that used square implants placed press fit into bur holes.[10] One of the reasons for the positive change in results could be the fact that the distance between the implant surface and the surrounding bone was much smaller in the present study, which would facilitate bone growth onto the surface and may thus have made an improvement in the osteoconductive properties of the coated surface more clear.

CONCLUSION

The present study has shown that coating an implant by biomimetically combining collagen I and calcium phosphates may have a beneficial effect on peri-implant bone regeneration when compared to HA coating alone or collagen coating alone and could improve both peri-implant BVD and BIC in the early stages of healing. However, despite the statistical significance, the quantitative differences were small, and the clinical relevance of these results remains to be shown.

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