Histologic and Histomorphometric Comparison of Immediately Placed Hydroxyapatite-Coated and Titanium Plasma-Sprayed Implants: A Pilot Study in Dogs

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The purpose of this pilot study was to make a histologic and histomorphometric comparison of hydroxyapatite- (HA) coated and titanium plasma-sprayed (TPS) root-form implants that were placed in 2 mongrel dogs immediately after extraction of mandibular premolars. After 8 weeks of healing, the implant-containing segments of the mandible were removed en bloc and bone blocks including implants were sectioned. Histologic and histomorphometric analyses were performed by evaluating bone sections. The mean bone contact percentage of HA-coated implants was 61.84 ± 7.84%, with a range of 52.09% to 75.7%, and the mean bone contact percentage of TPS implants was 51.35 ± 12.1%, with a range of 30.1% to 70.6%. This pilot study suggests that HA-coated implants placed into fresh extraction sockets can achieve better bone contact than TPS implants, but there was evidence that the surface of the HA layer can be resorbed, so long-term stability of HA coatings in immediate implantation must be investigated.
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Key words: experimental study, hydroxyapatite-coated implants, immediate implantation, titanium plasma-sprayed implants

The long-term success of osseointegrated implants, following principles outlined by Brånemark, is well established and well documented.[1–3] According to Brånemark and coworkers, one of the prerequisites for successful osseointegration has been to allow ossification of natural tooth extraction sockets before the placement of implants.[3] Therefore, a healing period of at least 6 months has been recommended between extraction of a tooth and subsequent implant placement.[4] During the healing of extraction sites, alveolar ridge resorption occurs; the degree of alveolar ridge resorption generally depends on the region in which tooth loss is experienced, as well as the amount of time that has passed since extraction.[5] The pressure of removable prosthetic restorations worn during the healing period may also decrease alveolar bone width and height, thereby decreasing the bone volume available for implant placement. To obviate unnecessary bone resorption, under ideal circumstances it might be advantageous to place implants at the time of tooth extraction.[6]

Because of the advantages provided by the elimination of a waiting period for socket ossification; fewer surgical sessions; shortened time of edentulism; preservation of alveolar bone height and width, allowing optimal implant placement in relation to implant length and diameter; and reduced treatment cost, there has been increasing interest in

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the concept of placing implants into fresh extraction sites. A number of clinical and experimental studies have reported successful placement of implants into fresh extraction sites.[7–14] Because of the successful results reported in these studies, immediate implantation has been accepted as a method of implant treatment.

The purpose of this study was to make a histologic and histomorphometric comparison of hydroxyapatite- (HA) coated implants and titanium plasma-sprayed (TPS) implants placed into fresh extraction sites.

Materials and Methods

Two healthy adult mongrel dogs were used in this study. All procedures related to the study were carried out according to the protocol for animal experiments used in the Department of Veterinary Surgery, Faculty of Veterinary, Istanbul University, Turkey.

Two different types of commercially available, as-received, endosteal dental implants were used in this study: *(1)* HA-coated root-form implants (Microvent, Spectra System, Core-Vent Bio-engineering, Dentsply, Milford, DE), and *(2)* TPS root-form implants (Pitt-Easy Bio-Oss, Oraltronics, Bremen, Germany). Prior to surgery, the dogs were presedated; this was followed by laryngeal intubation and halothane inhalation anesthesia. In addition, submucous injections of articain hydrochloride (Ultracain Forte, Turk Hoechst, Istanbul, Turkey) were given as infiltration anesthesia at the operation sites for control of bleeding.

All surgical procedures were performed under aseptic conditions by the same surgical team. Mucoperiosteal flaps were elevated and reflected lingually and buccally so that mandibular third premolars could be extracted after the crowns had been sectioned vertically with a diamond fissure bur in a surgical handpiece under sterile saline irrigation. By using the same atraumatic surgical technique, all mandibular third premolars were extracted from the 2 dogs. To place the implants into the extraction sockets of third premolars, surgical preparation of the implant sites was accomplished according to the manufacturers' written protocols. This included low-speed drilling and irrigation with sterile saline. Two implants were placed in each socket, with the superior edge of the implant flush with the bone. To standardize the surgical procedures, no other surgery, such as flattening of the ridge crest, was performed.

All implants were 3.25 mm in diameter and were slightly larger than the roots of the extracted teeth. By using these implants, intimate bone contact along the full length of implants was obtained. These were selected so that all sockets required similar surgical preparation; this also ensured intimate bone contact with each implant following implant placement. Table 1 indicates the distribution of implants according to size and type in the 2 dogs.

Table 1 Distribution of Implants in Mandibular Third Premolar Sites

	Dog 1		Dog 2	
Implant site	**Left**	**Right**	**Left**	**Right**
Mesial	1	2	1	2
Distal	3	4	3	4

1 = 3.25 × 10 mm HA-coated implant; 2 = 3.25 × 10 mm TPS implant; 3 = 3.25 × 7 mm HA-coated implant; 4 = 3.25 × 8 mm TPS implant.

After the placement of implants, all flaps were closed with interrupted sutures using 3.0 silk sutures, and primary soft tissue closure was achieved without any additional procedure. Following surgery, the dogs were given subcutaneous injection of 1.2 million units of penicillin G (Penadur 6-3-3, Wyeth, Fako Ilaclari AS, Istanbul, Turkey); the injection of penicillin was repeated 4 days after the operation. The sutures were removed 1 week later. The dogs were placed on a soft diet for the duration of the study. None of the implants in this study were loaded. A healing period of 8 weeks was allowed after surgery. After clinical and radiographic data had been recorded, the dogs were sacrificed with an intracardial overdose of sodium pentobarbital. The implants and adjacent tissues were removed en bloc for histologic and histomorphometric examination.

The implants were immersed in 10% neutral buffered formalin for at least 72 hours. The samples were then dehydrated with ascending concentrations of ethanol for 24 hours at each stage. Following transitional acetone immersion, the samples were immersed in 100% polymethylmethacrylate monomer for 24 hours, followed by immersion in a 1:1 ratio of polymethylmethacrylate to methylmethacrylate monomer for 24 hours. The polymethylmethacrylate was made as previously reported.[15] The samples were placed in embedding molds containing polymethylmethacrylate resin for 24 hours. Thereafter, the samples were transferred to fresh methylmethacrylate and bench top–cured at room temperature for 14 to 21 days. Once the plastic was hardened to the touch, the samples were placed into a 37°C oven for final curing for 24 to 72 hours. This protocol produced well-infiltrated samples contained in a clear plastic. The

samples were serial sectioned with a Buehler Isomet saw (Buehler, Lake Bluff, IL) using diamond wafering blades at initial thicknesses of 150 µm. If necessary, the sections were hand-ground with diamond disks to a final thickness of approximately 80 to 100 µm for subsequent analyses. In this manner, 2 to 4 sections were obtained buccolingually for the implants. The sections were stained with a mixture of toluidine blue and basic fuchsin at 50˚C.

Photomicrographs for histologic analyses were taken using a Zeiss Axiophot photomicroscope (Carl Zeiss, Thornwood, NY) at various magnifications for morphometric analysis of the amounts and types of bone and soft tissue present. Photomicrographs were taken with both normal transmitted light as well as with Nomarski illumination. The morphometric analysis was accomplished using a Zenith Z-200 personal computer (Zenith Data Systems, Sacramento, CA), which was interfaced with a Microcomp 39CZ graphic digitizer (SMI, Atlanta, GA). Image analysis was accomplished with the Microcomp PM2 planar morphometry package (SMI).

To perform this analysis, the sections were photographed using Pan-X black and white film, and 8 by 10 inch prints were produced of each section. The photographs were examined, and the areas of interest were outlined with colored markers. When it was difficult to discriminate tissue type, the photographs were microscopically compared to the original sections on the prepared glass slides. The digitizing pad and the Microcomp program were used to measure and record the lengths (in millimeters) of the implant and of the tissues of each particular type in contact with the implant. The tissue length and the total implant length were used to generate the percentage of contact length of each particular tissue type (ie, bone or unmineralized tissues, such as connective tissue or marrow space).

The mean of the bone contact percentages obtained from the individual sections was calculated to provide an overall value for each implant. The bone-implant contact percentage of each section was used for statistical method, rather than the mean bone contact percentage of each implant (n_{tps} = 11, n_{ha} = 14). The statistical analysis, a Mann-Whitney U test, was performed by using SPSS software (release 5.0, SPSS, Chicago, IL).

Results

Clinical Observations. One of the TPS implants was lost during the initial phase of healing (first week), probably because of early tearing of sutures. The remaining 7 implants were clinically osseointegrated at the end of the healing period. No mobility or signs of infection were noted at the implant sites at any time during the study. All 7 implants appeared radiographically to be integrated; that is, no evidence of radiolucency around the implants was noted.

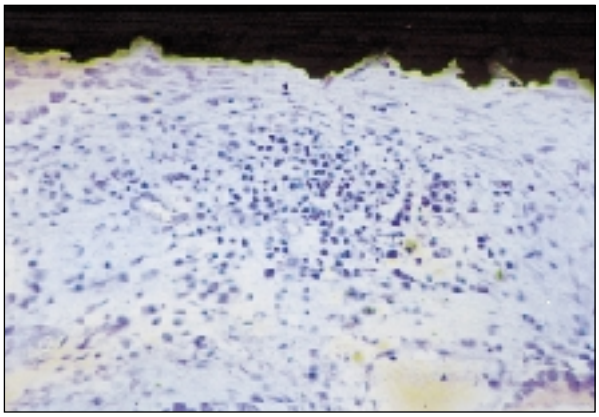


Fig 1 Photomicrograph of chronic inflammatory cell infiltration in the coronal area of TPS implant (routine transmitted light microscopy; toluidine blue; original magnification ×250).

Histologic Observations. Light microscopy assessments demonstrated that the epithelium and mucosal attachment around the cervical area of the TPS implants were normal. Slightly chronic inflammatory cell infiltration was observed in the connective tissue under the epithelium (Fig 1). Although the presence of fibrous tissue was seen in some parts of the bone-implant interface, it was shown that bone closely apposed to the surface of TPS implants (Fig 2). Osseointegration around TPS implants was observed on histologic sections (Fig 3). The presence of hyperemic activity in the Haversian system and osteoblastic activity and the forming osteons could be identified in the apical regions of TPS implants.

The overlying oral mucosa was normal around the HA-coated implants. There was no evidence of acute inflammatory reaction around any of the HA-coated implants. In contrast to the presence of soft tissue in some of the regions of bone-implant interface, newly forming trabecular patterns and osteoblastic activity also were observed in bone supporting HA-coated implants. Bone apposition close to the HA-coated implants was also apparent (Fig 4). In some of the regions around HA-coated implants the presence of osteoclastic activity and gathering of macrophages could be observed. At the surface of HA-coated implants, these foreign-body giant cells occasionally showed incorporated HA particles (Fig 5).

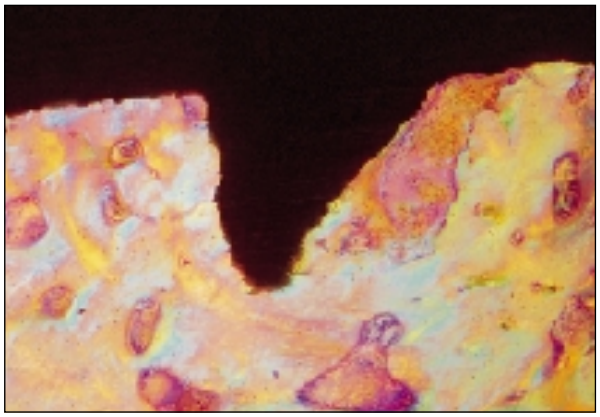


Fig 2 Photomicrograph of TPS implant placed into fresh extraction socket shows bone closely apposed to the surface of the implant. The presence of fibrous tissue can be identified in some regions of the bone-implant interface (Nomarski differential interference microscopy; original magnification ×100).

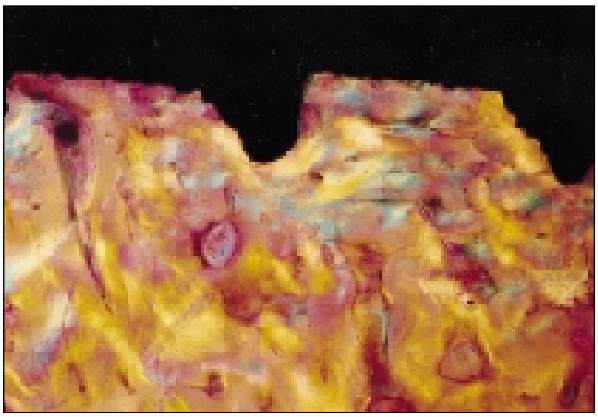
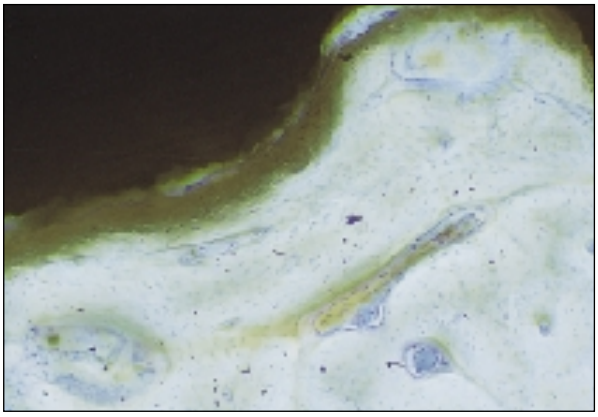
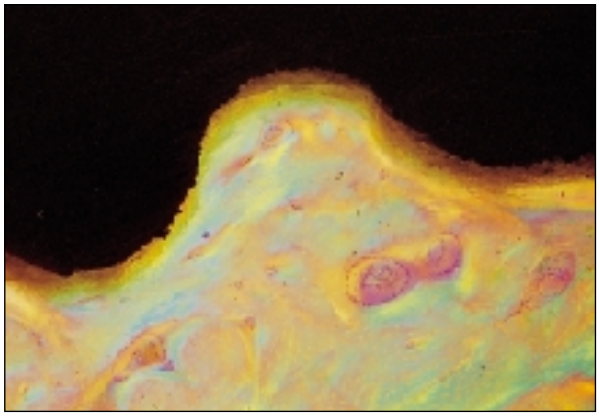
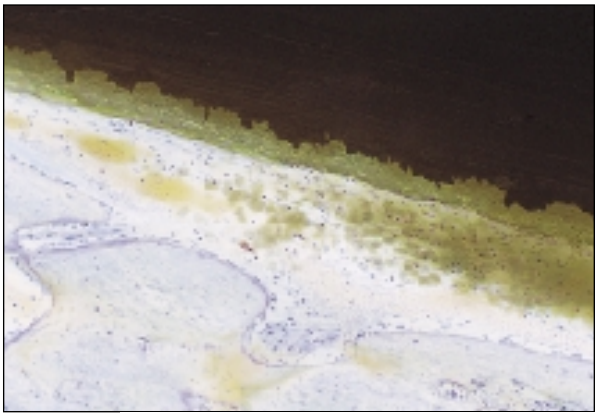


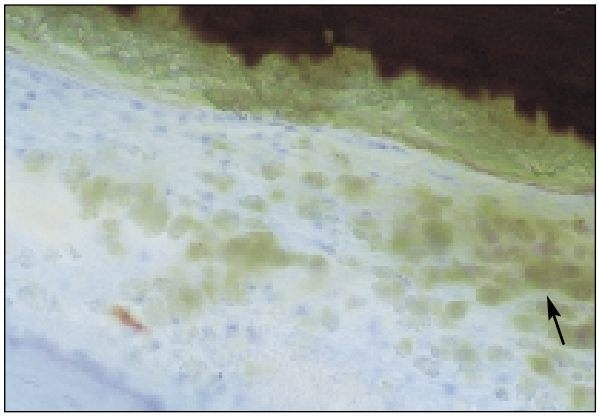
Fig 3 Photomicrograph of close bone contact to TPS implant (Nomarski differential interference microscopy; original magnification ×100).





Figs 4a and 4b Photomicrographs of HA-coated implant show the significant amount of bone apposing the implant, and the thickness of the HA coating is different on the surface of the implant (Fig 4a: Routine transmitted light microscopy; toluidine blue; original magnification ×250; Fig 4b: Nomarski differential interference microscopy; original magnification ×100).





Figs 5a and 5b Photomicrographs of HA-coated implant show the gathering of the macrophages to resorb HA coating (Fig 5a: routine transmitted light microscopy; toluidine blue; original magnification ×100; Fig 5b: routine transmitted light microscopy; toluidine blue; original magnification ×250). Arrow denotes macrophage that gathered.

Table 2 Histomorphometric Results of Average Percentage of Bone and Unmineralized Tissue Contact Length for Each Implant

Implant type	Implant site	Dog no.	Percentage of bone	Percentage of unmineralized tissue
TPS	Distal (right)	1	41.25	58.75
TPS	Distal (right)	2	47.31	52.69
TPS	Mesial (right)	2	65.63	34.37
HA	Distal (left)	1	63.22	37.78
HA	Mesial (left)	1	67.13	32.87
HA	Distal (left)	2	52.17	47.83
HA	Mesial (left)	2	65.24	34.76

TPS = Titanium plasma-sprayed implant; HA = hydroxyapatite-coated implant.

Histomorphometric Results. In the histomorphometric analysis, HA-coated implants showed a higher percentage of bone contact than TPS implants, with an average percentage of bone contact of 61.84% ± 7.84% for HA-coated implants and 51.35% ± 12.1% bone contact for TPS implants. This difference was statistically significant ($P < .05$). Bone contact for the sections of HA-coated implants ranged from 52.09% to 75.7%. Bone contact for the sections of TPS implants ranged from 30.1% to 70.6%. Table 2 provides the computerized histomorphometric results of the average percentage of bone and unmineralized tissue contact length for each implant.

Discussion

In this pilot study, some of the problems associated with the use of immediate implants in the extraction sockets of dogs are similar to those reported previously by Ettinger et al.[16] The most obvious problem was early tearing of sutures, and for that reason, 1 TPS implant was lost in the initial phase of healing. During the healing period, the dehiscence of gingival and mucosal tissues over implants and the exposure of all cover screws was seen. In spite of these problems, all implants were clinically stable and bone was closely apposed to the surfaces of implants at the end of the healing period.

To obtain optimal bone anchorage, most implant systems advocate that the implants be submerged (in a 2-step surgical procedure) during the initial phase of healing.[17] However, it has also been demonstrated that appropriate clinical bone anchorage can be achieved with a nonsubmerged approach (in a 1-step surgical procedure).[18–20] The results of the present study support the concept of a 1-step surgical procedure.

In the data reported by Gottlander and Albrektsson,[21] Weinlander et al,[22] and Sennerby et al,[23] the means of bone-implant interfaces ranged from 21% to 74%. In those studies, healed bone sites were selected for implant placement. The present study provides similar percentages of bone contact, even though the immediate implantation method was used.

In the present study, the HA-coated implants had a significantly higher percentage of bone along their length than did the TPS implants placed in the extraction sockets of dogs. In a recent paper, Weinlander et al,[22] using dogs with HA-coated and TPS implants placed conventionally, reported that HA-coated implants had 71.35% ± 11.79% of their surface in contact with bone, and the average percentage of bone contact was 54.96% ± 10.85% for TPS implants. Gottlander and Albrektsson[21] demonstrated that after 6 weeks in situ, HA-coated implants had higher bone contact than the uncoated titanium implants. Although the implantation method and implant location in the present study were different, the results are similar to those previously reported. In the present study, implants were evaluated at the end of the healing period and the implants were not loaded. Loading of the implants and duration of the time period postplacement can affect the bone-implant contact percentages.[24]

Conclusion

This pilot study suggests that HA-coated implants placed in fresh extraction sockets can achieve better contact with bone than TPS implants. While considering the results of the present study, one must be cognizant of the total sample size of the present study. Also, there is evidence that the surface of the HA layer can be resorbed by macrophages after implantation. Future studies should investigate the long-term stability of HA coatings used in immediate implantation.

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