Enhancing Osseointegration by Capacitively Coupled Electric Field: A Pilot Study on Early Occlusal Loading in the Dog Mandible

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Expeditious postoperative appositional growth of bone to dental implants is desired for clinically successful fixation of oral implants. The present study was performed to evaluate the effect of applying a capacitively coupled electric field (CCEF) followed by functional loading on peri-implant osteogenesis in the dog mandible. Nine adult beagles were used in this study. All premolars on both sides of the mandible were removed from each dog. A physio-odontlam implant (POI, Ti-6Al-4v) with 2 stages (3.7 mm in diameter and 8.0 mm in length), whose surface had been treated with anodic oxidation and sandblasted, was placed into each test site by self-tapping. Daily application of CCEF (8 hours per day) was initiated on the day following surgery and continued for 14 days or 21 days. After CCEF treatment was finished for each period, a prosthetic abutment and a straight post were placed on each implant. Four days after placement of the post, implants were placed under functional loading for 30 days. The dogs were then sacrificed, and histologic and radiographic studies of the mandible were performed. Relatively well calcified, mature bone with a lamellar-like structure was observed by contact microradiography and histologic study (double staining with basic fuchsin-methylene blue) of the peri-implant region on the CCEF-treated samples. In contrast, poorly calcified, immature bone without a lamellar structure was observed in control sites not treated with CCEF. The bone area ratios of the CCEF-treated sides were larger than those of control sides. These results suggest that the application of CCEF after implant placement may enhance peri-implant osteogenesis, even with functional loading. (INT J ORAL MAXILLOFAC IMPLANTS 2001;16:841–850)

Key words: *bone regeneration, dental implant, dog, electric capacitance, occlusal loading*

Osseointegration, which has been defined as a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant, is essential for achieving a successful oral implant.[1] The length of time that is needed before a titanium implant can become functional depends on the state of osseointegration. The restoration process of the bone after oral implantation depends, in part, on the healing capacity of the individual patient. The healing period is generally 6 months following implantation in the maxilla and approximately 3 months following implantation in the mandible, although the healing period varies widely among individuals.[2] If there were a method that could shorten the period of healing or osseointegration, patients could receive a prosthesis sooner. In an attempt to shorten the healing period, capacitively coupled electric fields (CCEF)[3] have been applied in oral implant therapy.[4,5]

Since Brighton and Pollack[3] first reported in 1985 that CCEF treatment stimulates osteogenesis, CCEF has been widely used for the treatment of refractory fractures such as pseudarthrosis and delayed bone repair.[6,7] Ochi and coworkers[4] reported that the application of CCEF promoted bone formation around implants that had been placed in the femora of rabbits. Shigino and associates[5] reported similar results following implant

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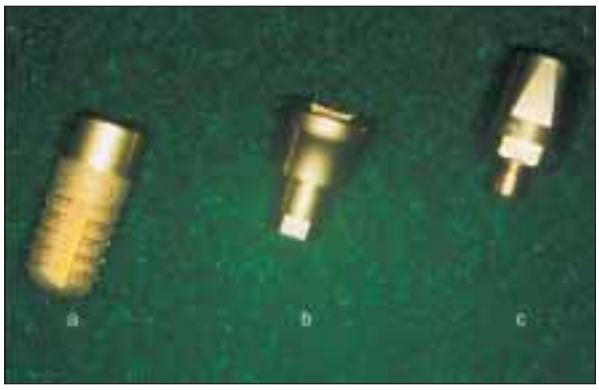


Fig 1a Titanium alloy (Ti-6Al-4V) POI 2-stage implant with a diameter of 3.7 mm and a length of 8 mm. a = implant; b = abutment; c = straight post.

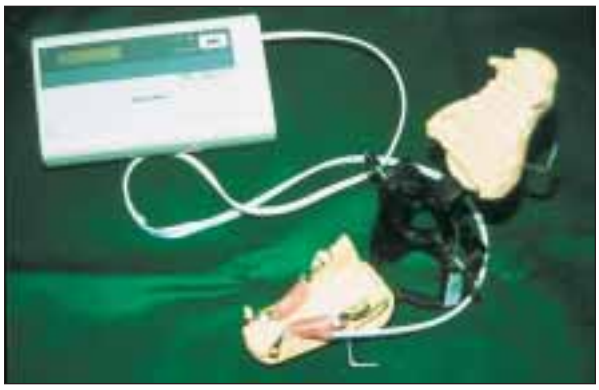


Fig 1b Equipment used to generate CCEF in this study. The attachment of the handmade, detachable oral electrode plate to the left side of the mandible of a dog is shown.

Fig 1c Osteotron II, which was used as the external source that generated CCEF.

placement in the dog mandible. However, only a few studies have examined the bone reactions to early occlusal loading after CCEF treatment. The purpose of this study was to evaluate the effects of CCEF treatment on bone reactions to early occlusal loading after implant placement into the dog mandible via histologic and radiographic examinations.

MATERIALS AND METHODS

Animals

The protocols for animal experimentation described in this paper were approved by the Animal Ethics and Research Committee of the Health Sciences University of Hokkaido, Japan. All of the animal experiments adhered to the guidelines for the care and use of laboratory animals of the university. Nine adult male beagle dogs aged 1.5 to 2 years were used in this study and were randomly assigned into 3 groups of 3 dogs each (groups A, B, and C). The dogs were premedicated with 2 mg/kg diazepam intramuscularly (Horizon, Yamanouchi Pharmaceutical, Tokyo, Japan) and 0.1 mg/kg atropine subcutaneously (Tanabe Pharmaceutical, Osaka, Japan). This was followed by general anesthesia with 2.5 mg/kg sodium thiopental intravenously (Ravonal, Tanabe Pharmaceutical) before the extraction of teeth. The first to fourth premolars were extracted from the left and right sides of the mandible by the procedure described previously.[5]

Implants

A titanium alloy (Ti-6Al-4V) physio-odontlam implant (POI) with 2 stages (FINAFIX, Kyocera, Kyoto, Japan; 3.7 mm in diameter and 8 mm in length) was used in this study. Its surface had been treated with sandblasting and anodic oxidation (Fig 1a).

Implant Placement

Implant placement was performed 3 months after the extractions. After general anesthesia was induced, the oral cavity was cleaned with benzethonium chloride solution (Neostelin Green, Nihon Shika Yakuhin, Shimonoseki, Japan). Infiltration anesthesia with lidocaine hydrochloride (XYLESTESIN-A, ESPE, Seefeld, Germany) was applied to the residual ridge of the mandible. A crestal incision was made from the distal of the canine to the mesial of the first mandibular molar and a mucoperiosteal flap was raised to expose the bone surface. Drill holes were created with a clover drill (#3-37-S, 3.37 mm diameter; Kyocera, Kyoto, Japan), and the area was cooled with running sterile saline using an electric engine (Implanter II, Kyocera) whose speed was set at 800

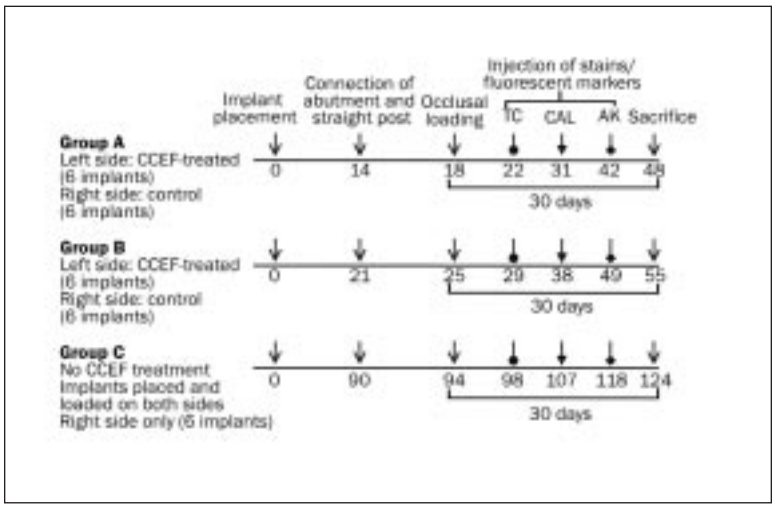


Fig 2 Experimental schedule, with time shown in days. TC = oxytetracycline; CAL = calcein; AK = Alizalin-Komplexon.

rpm/minute. The implant was placed into the osteotomy site by self-tapping, and the mucoperiosteal flap was closed. Two implants were placed on each side of the mandible (n = 6 implants in each group). There were 4 implants per dog (2 on the left mandible and 2 on the right) in a group of 9 dogs, equaling 36 implants overall and thus 12 implants per group.

Capacitively Coupled Electric Field Apparatus

In this study, an electrode made of a gold-platinum alloy (5×15×1 mm) was used for CCEF stimulation. The electrode was held in a removable resin plate with an attachment that permitted the placement or removal of the resin plate onto the left side of the mandible (experimental implant site). An identical resin plate without an electrode was fabricated for the right side of the mandible (control site). The resin plate was held in place by means of the attachment with a band-shaped cast gold-silver-palladium alloy crown that was bonded to each canine and first molar with a resin cement. Each crown/electrode plate complex was without occlusal and functional contacts, so as to prevent looseness of the electrode or excessive pressure on the implant sites. The electrode plate was positioned to touch the mucosa lightly (Fig 1b).

The electric field was applied by a CCEF generator (OSTEOTRON II, Mizuho Ika, Tokyo, Japan) (Fig 1c) 1 day after implant placement. A CCEF of 60-kHz and 10-Vp-p sine wave was applied for 8 hours a day to the left side of the mandible only. The CCEF generator was kept in a closed plastic case, and the case was attached to the back of the dog. An Elizabethan collar was placed around each dog's neck to prevent damage to the electrode and disconnection of wires.

Healing Period

The plates and the CCEF generator were attached only during the period of CCEF stimulation. When a plate was attached or removed, the wound area was cleaned with benzethonium chloride solution. The dogs were medicated for infection control with 250 mg sodium ampicillin intramuscularly (Amipenix for injection, Asahi Kasei Kogyo, Osaka, Japan) in the thigh for 3 days after implant placement. In the experimental groups, CCEF was applied on the experimental side for 14 days (group A) or 21 days (group B); CCEF was not applied on the control side. A conventional osseointegration period group (group C) was maintained for 90 days after implant placement without CCEF treatment (Fig 2). Plates were settled on both sides of the mandible in this group of dogs.

Occlusal Loading

The mucoperiosteum was opened and the implant was exposed. The implant cap was removed and the surrounding bone was trimmed with an abutment reamer (Kyocera, Kyoto, Japan). After washing with saline, a 4-mm-high abutment (Kyocera) was connected to each implant, and the mucoperiosteal flap was sutured. A straight 5-mm post (Kyocera) was attached to the abutment, and an impression was made of each post in the mandible for fabricating a single-tooth crown of gold-silver-palladium alloy. Four days after connection of the abutments, the crowns were then attached to the abutments. Continuous crowns were fixed on the canine to the fourth premolar of the maxilla. The occlusal contacts for each custom-made crown were verified using an articulating ribbon. Occlusal loading was delivered to each dog for 30 days. Axial loading of the implants was provided by the occlusal contacts

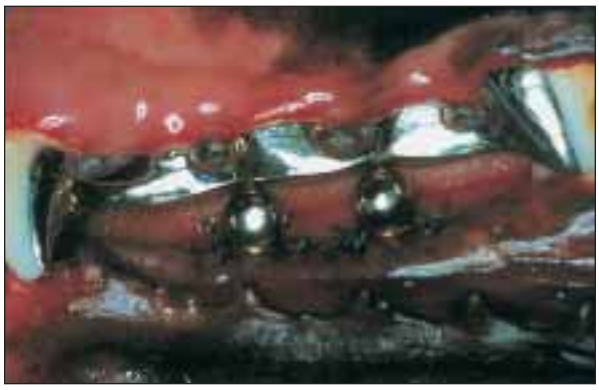


Fig 3 Photograph of implants with abutments and crowns attached for occlusal loading.

of the crowns. The dogs were fed a solid standard diet during the experimental period.

Oxytetracycline (Unacilline for intravenous injection, 60 mg/kg body weight; Showa Yakuhin Kako, Tokyo, Japan) was administered intramuscularly into the thigh as a fluorescent marker for hard tissue 4 days after occlusal loading. Calcein ($C_{30}H_{22}N_{20}O_{13}Na_4$, 8 mg/kg body weight; Kanto Chemical, Tokyo, Japan) was injected on day 13 after occlusal loading, and Alizalin-Komplexon ($C_{19}H_{15}N_8O_{13}2H_2O$, 30 mg/body weight; Merck, Darmstadt, Germany) was injected on day 24 intramuscularly into the thigh to study the amount of osteogenesis over time after occlusal loading (Fig 3).

Histologic Preparation and Examination

Each dog was sacrificed 30 days after occlusal loading. The animals were first anesthetized by general anesthesia. After the head was perfused with a neutralized 10% formalin solution through the carotid artery, the mandible was resected. The specimens were fixed in 10% formalin for an additional 7 days, followed by dehydration in a graded series of ethanols. The specimens were embedded in polyester resin (Rigolac, Ouken Shoji, Tokyo, Japan). Each specimen was cut buccolingually through the center of the implant with a cutting system (BS3000; Exakt, Norderstedt, Germany). The sections were polished with a microgrinding system (MG4000, Exakt) for preparation of nondecalcified specimens.

The sections were polished to a thickness of 100 μm, and contact microradiography (CMR) images were obtained with soft radiographic generation equipment (Sofron Model BSTI 1505CX, Souken, Tokyo, Japan) (focus-sample distance [FSD] 150 mm; voltage of the tube 45 volts; currency in the tube 5 mA; exposure time 5 minutes) on a soft high-resolution radiographic film (PELICULA, Kodak Japan, Tokyo, Japan) to determine the amount of osteogenesis in each section. The film was developed, fixed, water-washed, and dried according to the routine method. The CMR images of the area approximately 2 mm above the apex of the implant (near the screw head) were observed under a transmission optical microscope (BX-50, Olympus, Tokyo, Japan; ocular lens [2.5×] and object lens [10×], referred to as "high magnification" hereafter). For image analysis, low-magnification CMR images were used (ocular lens [2.5×] and object lens [1×], referred to as "low magnification" hereafter).

Eighty-micron sections were examined under a fluorescent microscope (BX-50, Olympus) to examine the time course of osteogenesis. The area approximately 2 mm above the apex of the implant (near the screw head) was studied under high magnification. Oxytetracycline appeared as a yellow-color fluorescence, calcein appeared green, and Alizalin-Komplexon appeared red.

Histologic examination was made on the 60-μm sections. Each section was stained with 2% basic fuchsin solution and 0.1% methylene blue/sodium hydroxide solution. Microscopic examination of the area approximately 2 mm above the apex of the implant (near the screw head) was made under a transmission optical microscope (high magnification).

Image Analysis

Image analysis was accomplished using NIH Image Analysis Software version 1.57 (National Institutes of Health, Bethesda, MD). On each low-magnification CMR image, the area within 0.1 mm of the implant surface was examined to assess the degree of peri-implant osteogenesis using 2 indices: bone contact ratio and bone area ratio. On each image, new bone at the edge and inside the hole was traced. The bone contact ratio was defined as the length of bone surface border that is in direct contact with the implant divided by the perimeter of the implant (× 100%) (Fig 4). The bone area ratio was defined as the area of new bone within 0.1 mm of the implant surface divided by the total area within 0.1 mm of the implant surface (× 100%) (Fig 4), as previously described.[5]

The resultant data were not statistically tested because of the limited number of animals and implants used in this study.

RESULTS

During the experiment, none of the implants exhibited mobility, and inflammatory signs in the surrounding gingiva were not observed in any dog in this study.

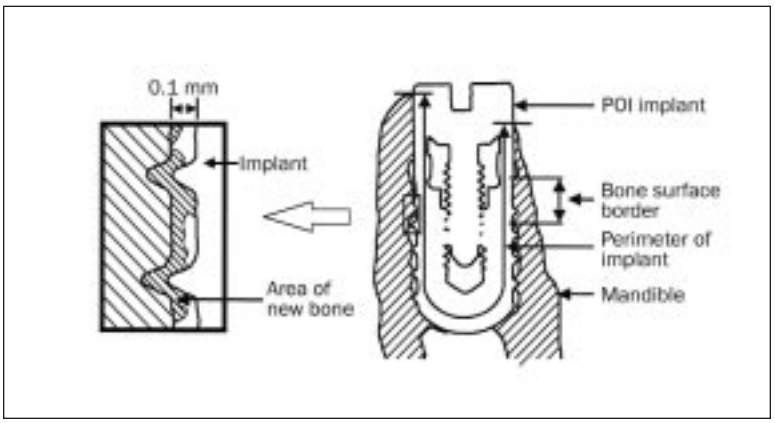


Fig 4 Method of calculation of bone contact ratio and bone area ratio, as taken from a low-magnification CMR image. A dental implant surrounded by new bone *(closely spaced diagonal lines)* and preexisting bone *(widely spaced diagonal lines)* is shown. The bone contact ratio was defined as the length of bone surface border that is in direct contact with the implant, divided by the perimeter of the implant. The bone area ratio was defined as area of new bone within 0.1 mm of the implant surface, divided by the total area within 0.1 mm of the implant surface.

Contact Microradiographic Images

In the CCEF-treated sides of groups A and B and in group C, dense, highly calcified, mature bone tissue had formed near the contact area of the implant (Figs 5b, 5d, and 5e). In contrast, less calcified immature bone tissue was seen in peri-implant areas on the control sides of groups A and B, and bone tissue was sparse (Figs 5a and 5c).

Degree of Osteogenesis as Observed by Fluorescence Labeling

Intense fluorescent areas were seen in the new bone near the implant on the control sides of groups A and B, indicating active osteogenesis (Figs 6a and 6c). In contrast, such active osteogenesis was not observed in the new bone near the implant in the CCEF-treated sides of groups A and B and in group C (Figs 6b, 6d, and 6e); fluorescence labeling was observed only in a narrow area of the new bone near the implant and around the area considered to be the Haversian canal.

Histologic Evaluation

On the CCEF-treated sides of groups A and B and in group C, some areas of new bone near the implant were strongly stained by basic fuchsin/methylene blue (Figs 7b, 7d, and 7e). There were only a few osteocytes in these areas, and mature bone with a lamellar arrangement was seen. By contrast, on the control sides of groups A and B, there were a large number of osteocytes near the implant, along with relatively immature bone without a lamellar structure (Figs 7a and 7c).

Image Analysis

The mean bone contact ratio of the CCEF-treated sides of groups A and B and their respective control sides was similar (Fig 8a). The mean bone area ratio of the CCEF-treated sides of group A was 1.4 times that of the respective control group, and the mean bone area ratio of the CCEF-treated sides of group B was 1.3 times that of the respective control group. The mean bone area ratios of the CCEF-treated sides of groups A and B and of group C were also similar (Fig 8b).

DISCUSSION

Although the exact mechanism by which CCEF application enhances bone repair has not been elucidated, previous in vitro studies showed that CCEF application stimulates human osteoblastic cell proliferation and up-regulates the expression and secretion of insulin-like growth factor-II (IGF-II).[8,9] Application of CCEF increases the level of transforming growth factor-β1 (TGF-β1) mRNA in mouse osteoblastic cells.[10] Other studies have suggested that CCEF treatment not only directly affects the proliferation of osteoblasts but also indirectly affects the rate of blood flow in the microcirculation and thereby the oxygen pressure inside the tissue.[11–13]

Inoue and associates[14] reported that CCEF treatment had no effect on bone growth in patients with old pseudoarthrosis or on delayed bone repair related to the presence of mature cartilage cells and condensation. In these patients, it may be necessary to conduct open osteosynthesis or bone graft to induce hematoma (blood clot). In oral implant surgery, CCEF treatment effectively stimulated osteogenesis near the implant by generating undifferentiated mesenchymal cells.[15] Thus, CCEF may be an appropriate method for promoting osseointegration.

It has been believed that functional loading on an implant restoration in the early period after implant placement prevents osseointegration in the nearby bone[1]; however, the results obtained in the present study suggest that application of CCEF after

Figs 5a to 5e Contact microradiographic images of bone ingrowth into the implants (original magnification ×50).

Fig 5a Representative image from the control side of a group A implant.

Fig 5b Representative image from the CCEF-treated side of a group A implant.

Fig 5c Representative image from the control side of a group B implant.

Fig 5d *(Left)* Representative image from the CCEF-treated side of a group B implant.

Fig 5e *(Right)* Representative image from a group C implant.

Figs 6a to 6e Fluorescent-labeled images (original magnification ×50).

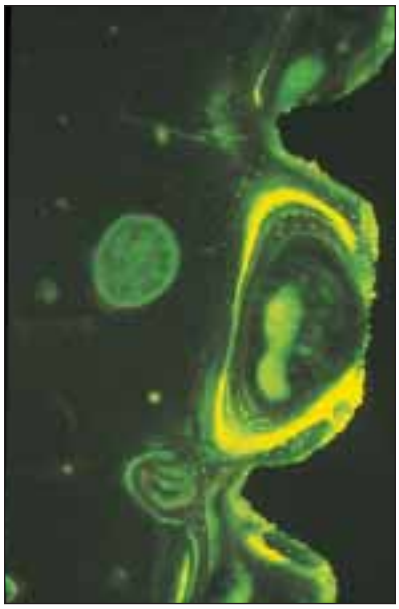


Fig 6a Representative image from the control side of a group A implant.

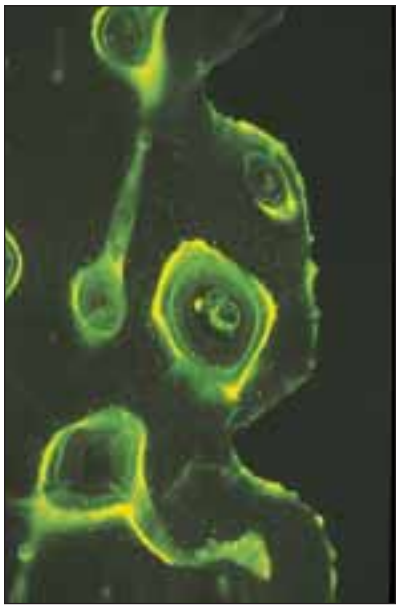


Fig 6b Representative image from the CCEF-treated side of a group A implant.

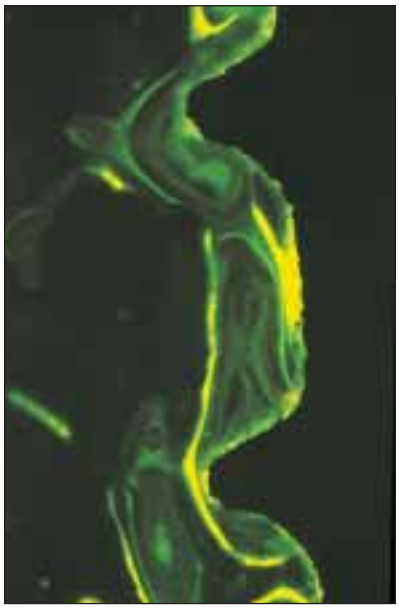


Fig 6c Representative image from the control side of a group B implant.

Fig 6d *(Left)* Representative image from the CCEF-treated side of a group B implant.

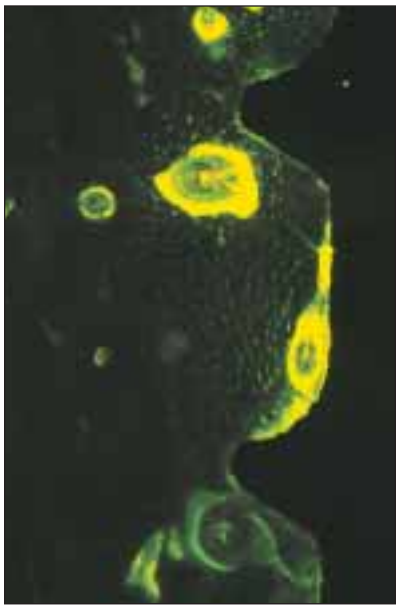
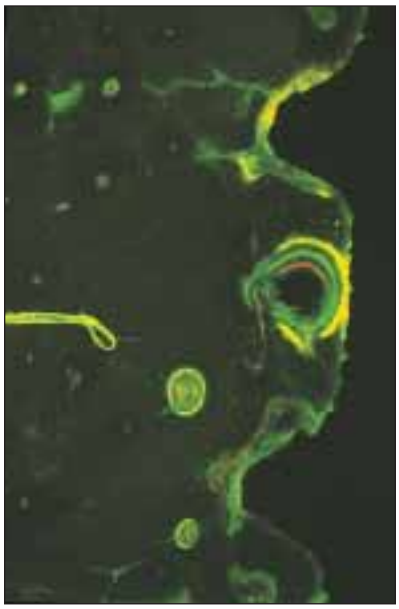


Fig 6e *(Right)* Representative image from a group C implant.



Figs 7a to 7e Sections stained by basic fuchsin/methylene blue (original magnification ×50).

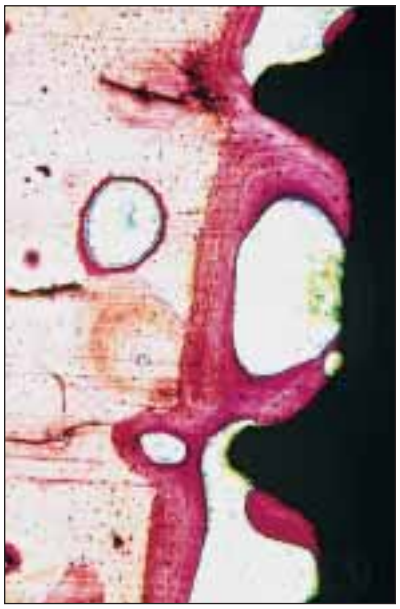


Fig 7a Representative image from the control side of a group A implant.

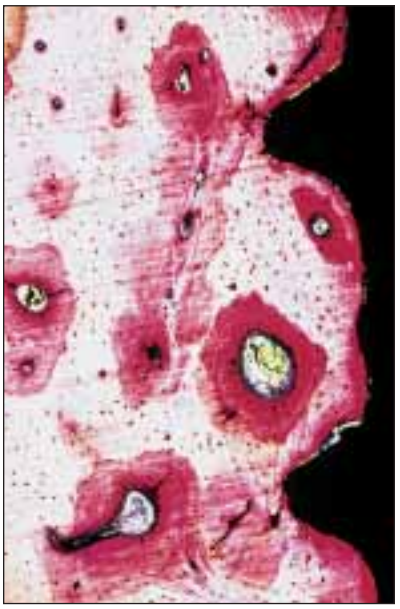


Fig 7b Representative image from the CCEF-treated side of a group A implant.

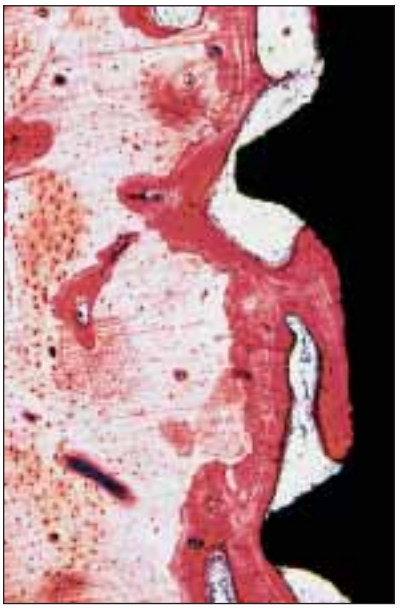


Fig 7c Representative image from athe control side of a group B implant.

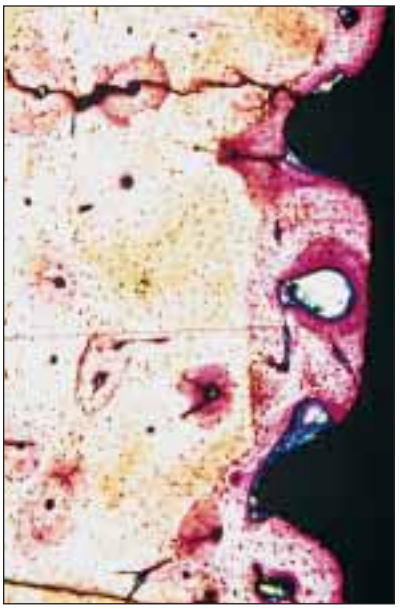


Fig 7d *(Left)* Representative image from the CCEF-treated side of a group B implant.

Fig 7e *(Right)* Representative image from a group C implant.

Fig 8a Effect of CCEF application on the bone contact ratio of the oral implants placed in the mandibles of dogs. Data are shown as mean ± SD (n = 6 for each group).

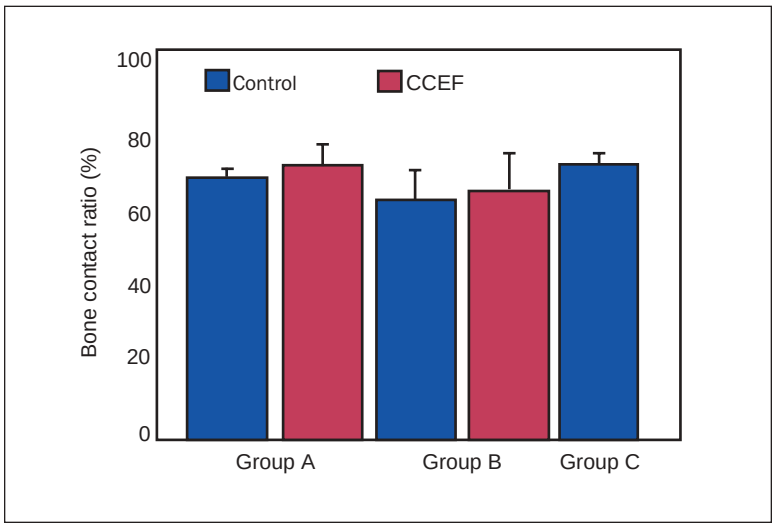
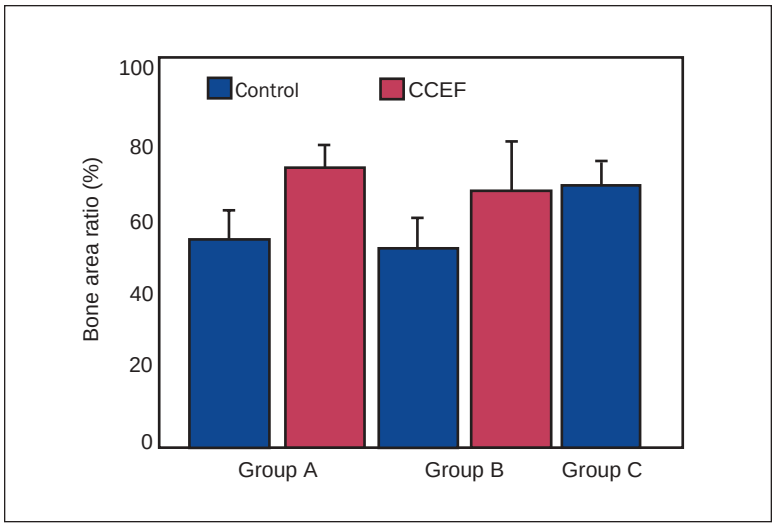


Fig 8b Effect of CCEF application on the bone area ratio of the oral implants placed in the mandibles of dogs. Data are shown as mean ± SD (n = 6 for each group).



implant placement shortens the recovery period of normal occlusal function. Sagara and coworkers[16] compared the degree of bone contact around implants in 2 groups of beagle dogs who did or did not undergo early occlusal loading for 3 months. They found that the bone contact of the group with no occlusal loading after 2-stage (ie, submerged) titanium alloy implant placement was better than that of the group that had undergone occlusal loading 1 week after 1-stage (ie, nonsubmerged) titanium alloy implant placement. In a study by Piattelli and associates,[17] occlusal loading was initiated 15 days after the placement of titanium plasma-sprayed implants in monkeys. Occlusal loading was not implemented on the control implants. Eight months after implant placement, the bone around the loaded implants had a more compact appearance than the bone around the control implants. However, the bone contact ratio of the loaded implants and that of the control implants did not differ significantly.

In the present study, the bone contact ratio and bone area ratio of the groups that did or did not receive CCEF stimulation after the placement of titanium alloy implants into the mandible and that underwent early occlusal loading (as early as day 18) were compared. It was found that the bone area ratio, but not the bone contact ratio, of the implants that had been treated with CCEF was larger than that of the implants that had not been treated with CCEF. These results suggest that the bone density near the implants on the CCEF-treated sides of groups A and B was greater than that on the respective control sides. The bone area ratios of the CCEF-treated sides of groups A and B and of group C did not differ significantly. This result suggests

that CCEF stimulation promoted new bone formation around the implants. The period of 90 days in group C corresponds to the typical healing period in implant therapy. Osseointegration after implant placement in the mandible usually requires approximately 3 months (90 days). Therefore, CCEF stimulation appears to be effective in obtaining quick recovery from occlusal function.

CONCLUSION

The effect of CCEF application for 14 or 21 days (groups A and B) after the placement of implants into the mandibles of beagle dogs, followed by occlusal loading, was studied by histologic and radiographic examination. The following observations can be made:

1. Stimulation via CCEF appears to increase bone area, but not necessarily bone contact, with mandibular endosseous implants in dogs.
2. Application of CCEF appears to promote osteogenesis, which suggests that it shortens the period needed for osseointegration after implant placement.
3. Since CCEF application appears to promote the formation of dense bone near implants, early occlusal loading may be enhanced.

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