Direct Current Electric Stimulation in Implant Osseointegration: An Experimental Animal Study With Sheep

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In an effort to obtain a high-quality bone-implant interface, several methods involving alteration of surface morphological, physicochemical, and biochemical properties are being investigated. The aim of our study was to increase the osseointegration rate and quality and decrease the waiting period of dental implants before loading by using a microelectric implant stimulator device. It imitates microelectrical signals, which occur in bone fractures described in terms of piezoelectric theory. A single dental implant (Zimmer Dental), 3.7 mm in diameter, was inserted into the tibia of sheep bilaterally. Twenty-four dental implants were inserted into 12 sheep. Implant on the tibia of each sheep was stimulated with 7.5 μA direct current (DC), while the other side did not receive any stimulation and served as a control. Animals were sacrificed 1, 2, and 3 months after implantation. Bone segments with implants were processed with unclassified method. The determination of new bone formation and osseointegration around the dental implants was investigated by means of undecalcified method, histomorphologically. No statistically significant difference in bone-to-implant contact (BIC) ratio, osteoblastic activity, and new bone formation was found between the stimulation group and the control group at the late phase of healing (4, 8, and 12 weeks). No evidence was found that electric stimulation with implanted 7.5 μA DC is effective at late phase implant osseointegration on a sheep experimental model.

Key Words: bone formation, electric stimulation, dental implant, osseointegration

INTRODUCTION

Implant survival, healing period, and predictability are important in treatment planning and implant therapy. Improvement of the implant-bone interface still needs additional interest. It is well known that titanium implants can be osseointegrated with surrounding jaw bone after latent periods of several months. The entire healing period of an implant in the mandible or maxilla has been reported to be between 6 weeks and 6 months, although the healing period varies widely among individuals. Implants for early loading and for poor bone quality require additional stimulants for enhanced osteointegration. Regarding this fact, implant laboratory studies have been focusing on higher bone-implant contact rate and quality. Several approaches involving alteration of surface, biochemical, physicochemical, and morphological properties of implants or healing potential of bone are being investigated in an effort to obtain a...
desirable bone-implant interface. Still, new techniques are required that induce postoperative growth of bone into dental implants and shorten the period of healing.

Electric stimulation to promote fracture healing has a long history. Electrically induced bone growth was described by Fukada and Yasuda in 1957. They urged that electrical fields were generated by mechanical stress on bone. Forces that act upon bone compress the tubular structure of bone and induce fluid flow containing ions through the canalicular system, which stimulates bone healing. Wolff’s Law states that a bone becomes adapted during its growth to the functional forces acting upon it. When external forces are applied to bone, electrical signals are generated, and these electrical signals can be described in terms of piezoelectric theory. Scientists have pointed out that there is a relationship between piezoelectricity and callus formation. They have also reported that stress-induced bioelectric potentials are the same signals as in Wolff’s Law.

Functional forces charge bone continuously, and negative and positive electric areas drive bone to remodel. Since the demonstration of the piezoelectric properties of bones, various types of electric stimulations have been widely used to enhance fracture healing and bone formation. Electric stimulation was used to induce osteogenesis, used for treatment nonunion bone fractures, and used to promote bone formation. Also, their effectiveness was studied in vivo and in vitro studies. Generally, 3 major devices have been used for electric stimulation of bone healing: inductive coupling, direct current stimulator, and pulsed electrical electromagnetic field.

Electric stimulation was used successfully for bone healing for treatment of nonunion bone fractures and osteogenesis, a few studies have shown the effect of electric stimulation on bone in growth into titanium implants.

In direct current stimulation, electricity is delivered to biological tissue through electrodes, this is also called the invasive method. The negatively charged electrode, or cathode, is implanted in bone tissue where stimulation is desired, while the positively charged electrode, or anode, is located either subcutaneously or directly to bone. This method allows mimicking of functional force stimulation of bone as in Wolf’s Law but without jeopardizing its stabilization.

The aim of this experimental study was to increase the bone-implant interface rate and contact bone quality with micro direct current (DC) stimulation, which imitates the signals generated in function as in Wolff’s Law.

**Materials and Methods**

**Experimental animals**

The experiment was conducted according to ethical principles of animal investigation in the Department of Veterinary Surgery, Istanbul University, Turkey. All sheep were acclimated (in a controlled environmental isolation facility). Twelve sheep weighing 47 ± 5 kg at the age of 2 years were used in this experiment. Animals were fed, by the associated department, a soft diet meeting their daily calorie requirements. Sheep were divided into 3 groups, and each group had 4 animals that were sacrificed at weeks 4, 8, and 12. Implants of the experimental site (right tibia of sheep) were stimulated with direct electric current from the beginning of the study to the time of sacrifice. Control sites (left tibia of sheep) were not stimulated. This study was performed in compliance with the international and Turkish laws on animal experimentation. The principles and the animal research protocol were approved by the responsible public authorities as requested. Ethical approval number was 2002/38.2002.mar, University of Marmara, Animal Research Ethical Comity.

**Anesthesia and drug administration**

As a preanesthetic, intramuscular 2.0 mg/kg xylazine (50 mg; Rompun, Bayer, Instanbul, Turkey) was administered intravenously. Anesthesia was induced by intravenous 5 mg/kg ketamine (100 mg; Alfasan-Ege, Izmir, Turkey) and maintained by artificial respiration with 1%–2% isoflurane (250 mL; Forane, Abbott, Queensborough, UK).

**Experimental design and electric stimulation**

Constant direct microcurrent stimulator, which possesses the function of feedback control of the applied current intensity and high accuracy, was used in the experiment. The anode electrodes (2 mm in diameter × 5 mm in length) of the stimulator were
made of titanium. The implant was used as a cathode electrode of the system. The electronic chip was protected from body fluids in a 28 × 12 × 4 mm titanium box (Hipokrat, Izmir, Turkey) with biopolymer covers (Hipokrat). Electric wire was covered and insulated with Teflon (Figure 1). The stimulation period was 12 hours per day (6 hours on and 6 hours off); it was powered with 2 CR1220 3V (2 × 35 mAh) batteries (Panasonic, Osaka, Japan).

**Implant surgery and device placement**

After an incision along the (middiaphyseal section) craniomedial part of the tibia, muscles and fascia were dissected and retracted laterally to expose the cortical bone of the tibia. Implants were then placed in the tibia of 12 sheep bilaterally: 1 implant per tibia, one to the left and the other to the right of each sheep. In total, 24 dental implants, 8 mm × 3.7 mm in diameter, (Zimmer Dental) were inserted in the right and left tibia of 12 sheep. The first implant to the left tibia was the study (stimulated), the second implant to the right tibia was the control (not stimulated). Implants were inserted to the cavity prepared with a physiodispenser (electric rotary instrument) at low speed (1200 rpm) under physiologic saline irrigation. The stimulator device was fixed 8 cm distal to the implants (distal-diaphyseal section) medial of the tibia with fixation screws (16 mm × 1.6 mm) (Hipokrat) (Figure 1). The cathode end of the Teflon-coated cable was connected to the dental implant and insulated with a fabricated biopolymer cover screw (Hipokrat) (Figure 2). The anode end of the stimulator cable was connected to the anode titanium pin (Figure 2), which was 2 mm in diameter and 5 mm in length. Flaps were carefully adapted and sutured with interrupted resorbable 3/0 polyglycolic-acid suture (Atramat, Mexico City, Mexico) internally, and the skin was closed with 2/0 Prolene sutures (Atramat). Animals were isolated after surgery. The experimental side (left tibia of each sheep) was stimulated with 7.5 μA DC, while the control side (right tibia) did not receive any stimulation. Animals were fed a standard pellet diet (standard diet 9020, Altromin,
Lage, Germany) and water ad libitum. All sutures were removed after 1 week.

**Postoperative drug administration**

Antibiotics and analgesics (cephalosporin, 1 g/d for 5 days, and ketoprofen, 500 mg/d for 3 days) were administered postoperatively.

**Sacrifice**

Animals were sacrificed at weeks 4, 8, and 12 with an overdose intravenous injection of sodium pentobarbital. During sacrifice, the electrical activities of devices were controlled to check if they had their stimulation function while they were on the animals.

**Spacemen preparation and histologic examination**

Implants with surrounding bone were removed en bloc and immersed in 10% neutral buffered formaldehyde for histomorphometric evaluation. Undecalcified ground sections from the specimens were carried out according to the method described by Donath and Breuner. The specimens were dehydrated in a graded series of ethanol and embedded in methylmethacrylate-based resin (Technovit 7200 VLC, Kulzer & Co, Wehrheim, Germany). The specimens were further cured overnight under blue light (450 nm) below 40°C with water cooling (EXAKT 520, EXAKT, Norderstedt, Germany). A diamond band saw system (EXAKT 300 CL) was used to cut the specimens along a perpendicular plane at the middle of the implant; 150- to 200-μm thick sections were obtained. Afterwards, the sections were reduced to a thickness of 40 μm by using the microparallel grinding system (EXAKT 400 CS). Care was taken to avoid damaging the bone-implant interface.

The final sections were mounted and stained with toluidine blue for histologic and histomorphometric analysis. The digital images of the sections were obtained by a digital camera (Olympus DP 70, Olympus, Tokyo, Japan) attached to a microscope (Olympus BX50) at a magnification of ×4. The obtained images were transferred to a computer, and *Image* analysis software (National Institutes of Health, Bethesda, Md) was used for histomorphometric analysis. Mineralized bone-to-implant contact (BIC) percentage was measured by an experienced blinded investigator to evaluate the bone-implant interface. Nonparametric marking was used to evaluate each specimen (none, 0; mild, 1; moderate, 2; or severe, 3) histologically by degree of osteoblastic activity (oba), necrosis (nec), immature bone (imb), and mature bone formation (mb).

**Statistical analysis**

The BIC data were analyzed using paired Student *t* test. Histologic data were analyzed with nonparametric Wilcoxon signed rank tests. The following hypotheses were checked if they were significant or not. *C* indicates the control group, and *S*, the stimulation group.

1. (C) oba < (S) oba, (C) oba > (S) oba, (C) oba = (S) oba;
2. (C) nec < (S) nec, (C) nec > (S) nec, (C) nec = (S) nec;
3. (C) imb < imb, (C) imb > (S) imb, (C) imb = (S) imb; and
4. (C) mb < (S) mb, (C) mb > (S) mb, (C) mb = (S) mb.

**RESULTS**

Overall, the surgery was well tolerated by the sheep. They had no any complication like exposure of stimulators or implant and infection.

**Histologic and histomorphometric analysis**

Neither group showed necrosis or bone pathology at weeks 4, 8, and 12. BIC values at 4, 8, and 12...
weeks are summarized in Table 1. In the stimulation group, bone contacts were slightly more regular and mostly approximated to the titanium surface compared with the control group at 4 weeks (Figure 3). No statistically significant bone-contact ratio was seen between the stimulation group and the control group at 4 weeks ($P = .164$) (Figure 4; Table 2). Newly formed bone grew close to the implant surface in the stimulation and control group. No significant difference between the control and stimulation group was noted for oba, nec, imb, and mb ($P > .05$) (Table 3).

The bone formation process was well identified by the presence of osteoblasts, and the Haversian system were observed. Figure 8. Histologic sections of control at 12 weeks. Mature implant-bone contact was seen at 12-week histologic section. New bone can be seen as a darker brown layer, which was clearly approximated to the titanium surface. Multiple secondary osteons formed by Haversian remodeling of the surrounding bone (magnification ×4).

### Table 1

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FIGURES 3–8. **Figure 3.** Histologic sections of direct current (DC) stimulation at 4 weeks: bone contact was regular and mostly approximated to the titanium surface. Stimulated implants showed broad-based direct contact at 4 weeks’ observation. Recently remodeled osteons are more dark blue than mature bone. In the center of dark blue circles (white halos) are secondary mineralization radiating from the Haversian canals (magnification ×4). **Figure 4.** Histologic sections of control group at 4 weeks: bone contact was regular, and newly formed bone grew close to the implant surface in the control group. New bone formation was seen at remodeling stage (blue halos) (magnification ×4). **Figure 5.** Histologic sections of DC stimulation at 8 weeks (magnification ×4). Mature bone contact was regular and mostly approximated to the titanium surface. Still, osteoblastic activity focus was seen close to the titanium surface, which underwent remodeling (blue line close to bone-implant contact) (magnification ×4). **Figure 6.** Histologic sections of control at 8 weeks. New bone was matured and identified clearly from old bone with its different color. Bone close to implant mostly approximated to the titanium surface. **Figure 7.** Histologic sections of DC stimulation at 12 weeks. Regular bone-implant contact was seen. Osteoblastic activity due to remodeling was seen at bone-implant contact (blue stained areas near contacts); mature osteoblasts and Haversian system were observed. **Figure 8.** Histologic sections of control at 12 weeks. Mature implant-bone contact was seen at 12-week histologic section. New bone can be seen as a darker brown layer, which was clearly approximated to the titanium surface. Multiple secondary osteons formed by Haversian remodeling of the surrounding bone (magnification ×4).
system was well formed after 4 weeks in both groups (Figure 3). Stimulated implants showed broad-based direct mature bone contact with secondary osteon formation at 4 weeks of observation (Figures 3 and 4). New bone formation following osteoclastic activity was seen in the control group. Newly formed mineralized bone, which showed a darker brown bone pattern, was clearly differentiated from the older bone (Figure 4).

Mature bone contacts of the control group and the electric stimulation group were regular and mostly approximated to the titanium surface at 8-week specimens. Osteoblastic activity focus was seen close to the titanium surface that underwent

| TABLE 2 | Paired Student t test results of bone-to-implant contact (BIC) values of control and study groups at 4, 8, and 12 weeks after implantation |
|---|---|---|---|
| BiC Values | Control (BIC Left Tibia) | Study (BIC Right Tibia) | Control – Study Comparison |
| 4 weeks | Sample size | 4 | 4 | 4 |
| Arithmetic mean (%) | 58.27 | 75.57 | 17.30 |
| Mean difference | 18.87 | 1.83 | 3 |
| Standard deviation | 17.30 | 0.1642 | 19.35 |
| Test statistic t | 3 |
| Degrees of freedom | 1.83 |
| Two-tailed probability | 0.1642 |
| 8 weeks | Sample size | 4 | 4 | 4 |
| Arithmetic mean (%) | 70.12 | 50.77 | 19.35 |
| Mean difference | 17.81 | 1.000 |
| Standard deviation | 2.17 |
| Test statistic t | 3 |
| Degrees of freedom | 3 |
| Two-tailed probability | 0.1182 |
| 12 weeks | Sample size | 4 | 4 | 4 |
| Arithmetic mean (%) | 76.80 | 66.42 | 10.37 |
| Mean difference | 27.44 | 0.5046 |
| Standard deviation | 0.756 |
| Test statistic t | 3 |
| Degrees of freedom | 3 |
| Two-tailed probability | 0.5046 |

| TABLE 3 | Histologic comparison of control group and study groups at 4, 8, and 12 weeks with Wilcoxon signed rank tests* |
|---|---|---|---|---|
| (C) oba – (S) oba | (C) nec – (S) nec | (C) imb – (S) imb | (C) mb – (S) mb |
| 4 Weeks | Z | −1.732† | .000‡ | .000‡ | .000‡ |
| | Asymp sig (2-tailed) | .083 | 1.000 | 1.000 | 1.000 |
| 8 Weeks | Z | −1.414† | .000‡ | −1.000§ | .000‡ |
| | Asymp sig (2-tailed) | .157 | 1.000 | .317 | 1.000 |
| 12 Weeks | Z | −1.000† | .000‡ | .000‡ | .000‡ |
| | Asymp sig (2-tailed) | .317 | 1.000 | 1.000 | 1.000 |

* C indicates control group; S, stimulation group; oba, osteoblastic activity; nec, necrosis; imb, immature bone; mb, mature bone; asymp sig, asymptotic significance.
† Based on positive ranks.
‡ The sum of negative ranks equals the sum of positive ranks.
§ Based on negative ranks.
remodeling in both groups. There was no statistically significant difference in the BIC values (P > .05) between the control and stimulation group at 8 weeks (Table 2). Also, oba, nec, imb, and mb difference between the control and stimulation group was found to be not statistically significant at 8-week histologic sections (P > .05) (Figures 5 and 6; Table 3).

Light-microscopic evaluation at 12 weeks still showed new bone formation and osteoblastic activity in both groups (Figure 7). Osteoblastic activity was decreased in both groups at 8 and 12 weeks gradually and most immature bone was replaced with broad-based direct mature bone contacts around the titanium implants (Figure 8). Differences between the electric stimulation and the control group were not statistically significant for BIC values (P > .05) (Table 2) or oba, nec, imb, and mb (P > .05) (Table 3).

**Discussion**

Several reports encouraged both laboratory and clinical research on electrically induced bone formation and healing with the use of various forms of electric stimulation. Electric stimulation, widely studied with regard to its effect on bone healing, has an established role in the treatment of long bone nonunions. Increased osteogenesis has been reported with use of all major forms of electric stimulation, including direct current, capacitively coupled, and inductively coupled electromagnetic fields. Brighton et al demonstrated in their animal studies that DC stimulation could accelerate fracture healing when the active cathode was placed within the fracture site.

Some other researchers who observed the positive effect of electric stimulation on bone formation point out that electric stimulation affects only less differentiated osteoblasts or preosteoblasts. This effective stage is the differentiation stage, which takes place in the early phases of bone healing. Diniz et al state that the stimulatory effect of electric current gradually decreased when the osteoblasts were stimulated in the differentiation stage, and the stimulation effect was not observed when the osteoblasts were stimulated in the mineralization stage. Jansen et al also reported that electric stimulation in the early stages of cellular maturation was more effective in increasing osteoblast development and mineralization than stimulation in the later stages (calcification and bone formation stages). These study results confirm our study that stimulation of bone at late phases was not effective.

The effect of a 20 μA DC implantable bone growth stimulator on bone production with a “gap healing” model on the horse was evaluated by Collier et al. They report that there was not a significant difference between groups in any of the histomorphometric values measured.

In the literature, relatively few animal studies have evaluated the effect of direct current electric stimulation on implant osseointegration. In previous studies regarding peri-implant bone formation, dental implants were used as cathodes, and negatively charged constant direct currents with a range of 4–100 μA were used to stimulate the surrounding bone tissue. In current levels between 5 and 20 μA, progressively increasing amounts of bone are formed. In a hydrated bone, a minimum of 1.5 V are necessary to create 5 to 20 μA. In our study, we applied 7.5 μA DC and 3V as in the literature.

In some of the studies, bone-implant contact ratio was significantly increased after the capacitive direct current or inductive coupling electrical field applications compared with the control group. Other researchers observed fibrous soft tissue or no difference in the quality and quantity of ingrown bone. Shayesteh et al studied DC stimulation on 4 mongrel dogs. Although statistical analysis becomes slightly weak when the total number of observations in the study is small, their study suggests that local application of an electric current during osseointegration may stimulate bone formation around dental implants and decrease the time for osteointegration.

On the other hand, Buzza et al evaluated the histologic healing process in dental implants under the action of pulsed electromagnetic field (PEMF) at 21 and 42 days. They concluded that there was no quantitative, statistically significant difference in torque between the PEMF and control group. The histologic features did not differ between groups, suggesting that dental implant surfaces do not promote better bone formation when submitted to PEMFs. Shafer et al performed an experimental study on the mandibles of 5 rabbits to evaluate the effect of 7.5 μA DC electric stimulation on the osseointegration of endosseous titanium dental implants. Twenty-eight

A Abbreviations

BIC: bone-to-implant contact
DC: direct current
imb: immature bone
mb: mature bone
ne: necrosis
oba: osteoblastic activity
PEMF: pulsed electromagnetic field

REFERENCES

4. Shigino T, Ochi M, Hirose Y, Hirayama H, Sakaguchi K. Days after placement, the bone surrounding the implants was examined both microscopically and radiographically. Their results show that electric stimulation does not positively affect the healing of bone and needs further investigation.18

In our experimental animal study, no statistically significant difference was found between electric stimulation and control group as with Buzza et al31 and Shafer et al18 at 1, 2, and 3 months, which were at the late phase (mineralization or remodeling) of healing.

The most recent study about accelerating osseointegration of dental implants was an experimental animal study on 10 adult beagle dogs by Song et al. They demonstrated that BIC in 3-week specimens was 1.62 times (P < .05) greater in the experimental group, while there were no statistically significant differences in the 5-week specimens. They suggest that bone formation was accelerated at an early stage by electric stimulation near the implant surface. These study results paralleled our findings at 4, 6, and 12 weeks.

In conclusion, we have found no evidence that electric stimulation with 7.5 μA DC direct current is effective at late-phase implant osseointegration on the sheep experimental model. Further examples of these studies with more animals and specimen may be useful to enlighten this topic.

ABBREVIATIONS

BIC: bone-to-implant contact
DC: direct current
imb: immature bone
mb: mature bone
ne: necrosis
oba: osteoblastic activity
PEMF: pulsed electromagnetic field
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