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Pulsed electromagnetic fields promote bone formation around dental implants inserted into the femur of rabbits

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The present study examined the effect of applying a pulsed electromagnetic field (PEMF) on bone formation around a rough-surfaced dental implant. A dental implant was inserted into the femur of Japanese white rabbits bilaterally. A PEMF with a pulse width of 25 μs and a pulse frequency of 100 Hz was applied. PEMF stimulation was applied for 4 h or 8 h per day, at a magnetic intensity of 0.2 mT, 0.3 mT or 0.8 mT. The animals were sacrificed 1, 2 or 4 weeks after implantation. After staining the resin sections with 2% basic fuchsin and 0.1% methylene blue, newly formed bone around the implant on tissue sections was evaluated by computer image analysis. The bone contact ratios of the PEMF-treated femurs were significantly larger than those of the control groups. Both the bone contact ratio and bone area ratio of the 0.2 mT- and 0.3 mT-treated femurs were significantly larger than the respective value of the 0.8 mT-treated femurs ($P<0.001$). No significant difference in bone contact ratio or bone area ratio was observed whether PEMF was applied for 4 h/day or 8 h/day. Although a significantly greater amount of bone had formed around the implant of the 2-week treated femurs than the 1-week treated femurs, no significant difference was observed between the 2-week and 4-week treated femurs. These results suggest that PEMF stimulation may be useful for promoting bone formation around rough-surfaced dental implants. It is important to select the proper magnetic intensity, duration per day, and length of treatment.

Hiroyuki Matsumoto[1], Morio Ochi[1], Yoshihiro Abiko[2], Yukito Hirose[1], Tohru Kaku[2], Kunihiko Sakaguchi[1]

[1]Department of Fixed Prosthodontics, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061–0293 Japan; [2]Department of Oral Pathology, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061–0293 Japan

Key words: pulsed electromagnetic field – bone formation – dental implant – titanium – rough surface – femur – rabbit

Morio Ochi, Department of Fixed Prosthodontics, School of Dentistry, Health Sciences University of Hokkaido, 1757 Kanazawa, Ishikari-Tobetsu, Hokkaido, Japan, 061–0293
Tel.: +81 1332 3 1211 ext.3355
Fax: +81 1332 3 1427
e-mail: ochident@hoku-iryo-u.ac.jp

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Osseointegrated implants are widely used in dental implantology. The superstructure is seated on the implant after osseointegration occurs. Patients are often inconvenienced by the long period between the time of implantation and placement of the superstructure. Several procedures have been developed to promote osseointegration in orthopedic procedures (Rutherford et al. 1992; Melloring & Nevis 1995; Ijiri et al. 1996; Hanisch et al. 1997). The aim of these procedures is to promote bone formation after the bone is wounded. Chemical and physiologic procedures have been developed to promote bone formation (Martin & Ng 1994; Ryaby 1998; Reddi 1998). Application of a pulsed electromagnetic field (PEMF) is a useful method to promote bone formation, and several studies have demonstrated its clinical usefulness for treatment of orthopedic injuries (Ryaby 1998). A few studies have shown the effect of PEMF on bone ingrowth into porous-coated implants (Martin & Ng 1994; Ijiri et al. 1996; Hanisch et al. 1997; Ryaby 1998; Reddi 1998; Shimizu et al. 1988), which are widely used in cementless implant arthroplasty. Rough-surfaced materials are more widely used than porous surface materials at the bone–implant interface of dental implants. The present study exam-

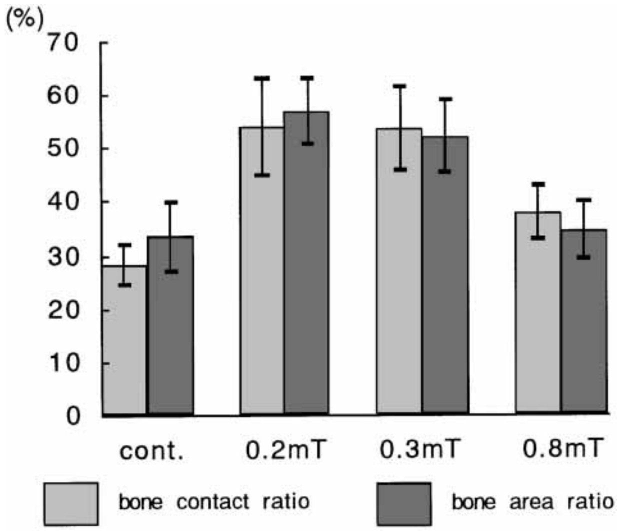


Fig. 1. Effect of magnetic intensity on new bone formation. Stimulation was applied for 8 h per day for 2 weeks.

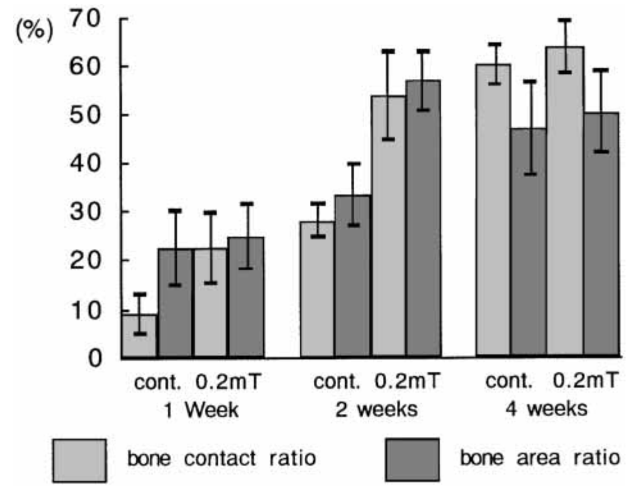


Fig. 3. Effect of the length of treatment on new bone formation. Stimulation was applied at 0.2 mT magnetic intensity for 8 h per day.

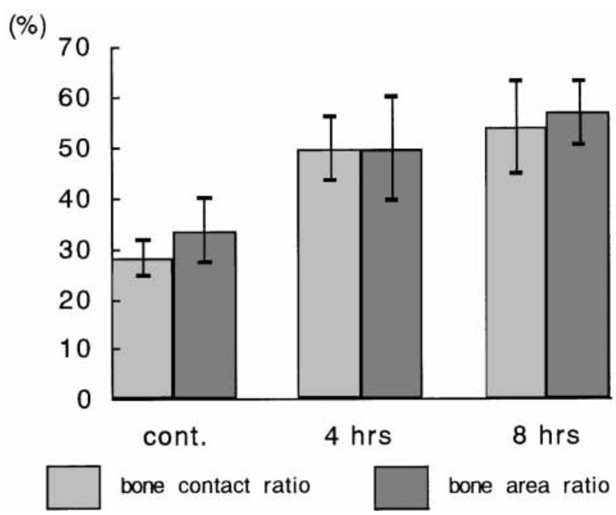


Fig. 2. Effect of duration of stimulation per day on new bone formation. Stimulation was applied at 0.2 mT magnetic intensity for 2 weeks.

ined if PEMF enhances bone formation around a rough-surfaced dental implant, employing an animal model.

Materials and methods

Animals and implant materials

Forty-five adult, male Japanese white rabbits weighing approximately 2.5 kg were purchased from Sankyo Labo Service Co. (Sapporo, Japan). Physio Odontlam Implant (POI) two-piece-type dental implants (FINAFIX; Kyocera Co., Kyoto, Japan), made of titanium alloy (Ti-6Al-4V) with an anodic oxidized surface, were used in this study. The diameter of the implants was 3.2 mm and the length of the endosseous portion was 10 mm. An oxidized layer of 1350 to 1400 Å thickness was deposited on the implant surface by anodic oxidization (Kanematsu et al. 1985), thereby increasing its affinity for the tissue. The portion of the implant surface that is normally in contact with the human gingiva was polished until it shone like a mirror. The portion of the implant surface normally in contact with human bone was blasted.

Implantation and application of PEMF

Implant placement was performed under general anesthesia which was induced by venous adminis-

Table 1. Bone contact ratio and bone area ratio

			Bone contact ratio			Bone area ratio		
			mean		1 SD	mean		1 SD
Fig. 1.	cont	2w 8h	28.1	*	3.6	33.4	*	6.3
	0.2mT	2w 8h	53.8	* *	9.1	56.7	*	6.2
	0.3mT	2w 8h	53.4	* *	7.8	51.9	* *	6.8
	0.8mT	2w 8h	37.7		5.0	34.5		5.4
Fig. 2	cont	2w 8h	28.1	*	3.6	33.4	*	6.3
	0.2mT	2w 4h	49.5	*	6.3	49.6	*	10.1
	0.2mT	2w 8h	53.8		9.1	56.7		6.2
Fig. 3	cont	1w 8h	9.0	*	4.1	22.4		7.8
	0. 2mT	1w 8h	22.5		7.2	24.7		6.9
	cont	2w 8h	28.1	**	3.6	33.4	**	6.3
	0.2mT	2w 8h	53.8		9.1	56.7		6.2
	cont	4w 8h	60.1		4.2	47.1		9.5
	0.2mT	4w 8h	63.7		5.6	50.3		8.3

* $P<0.01$
** $P<0.001$

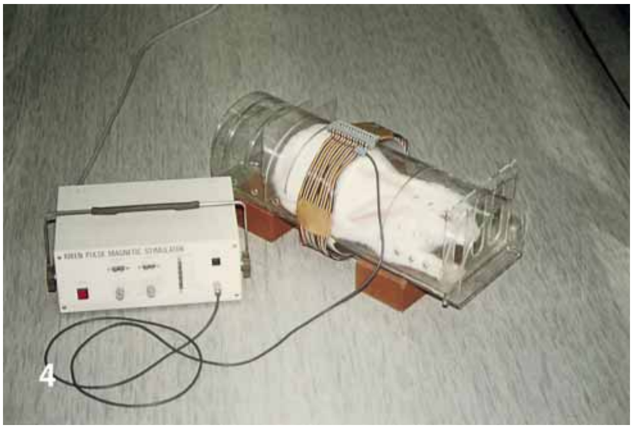
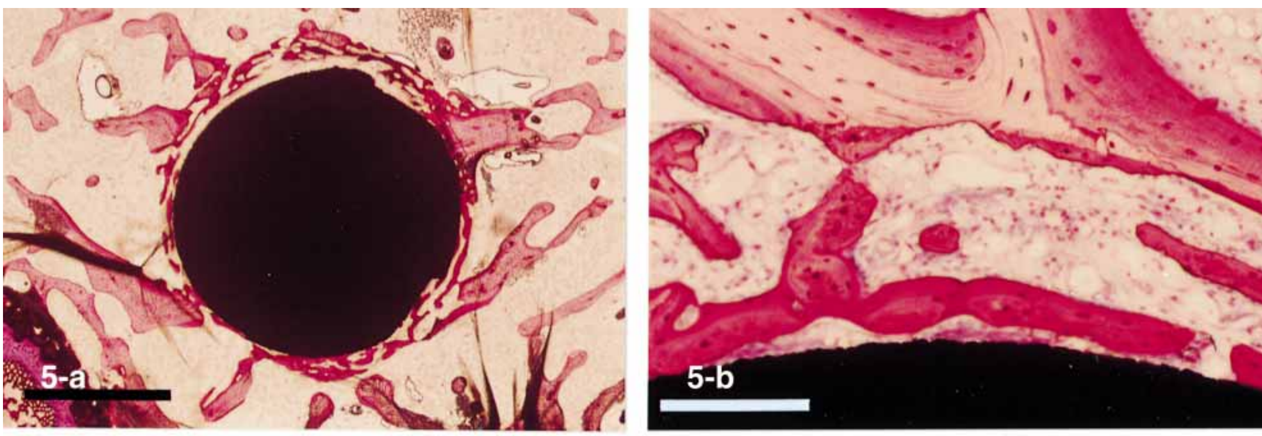
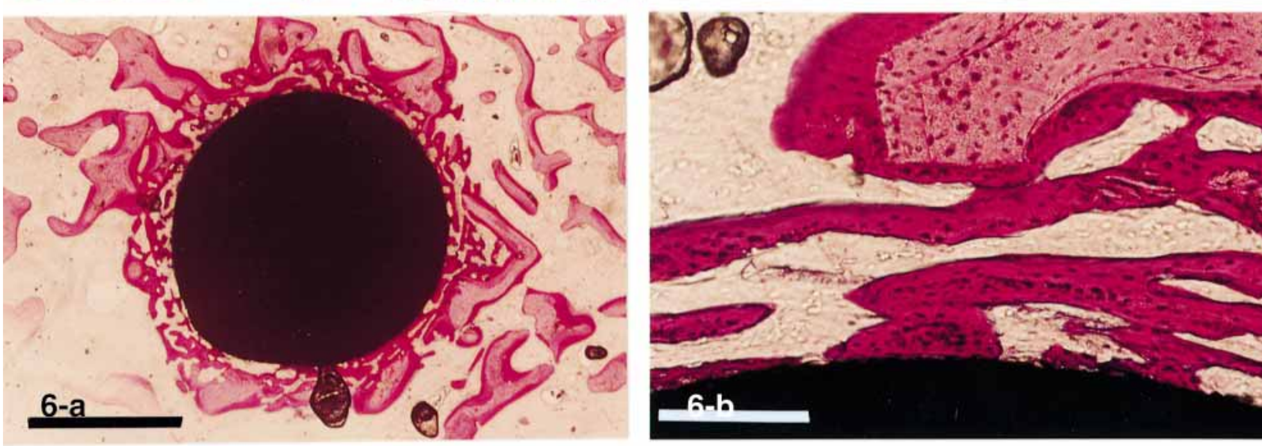


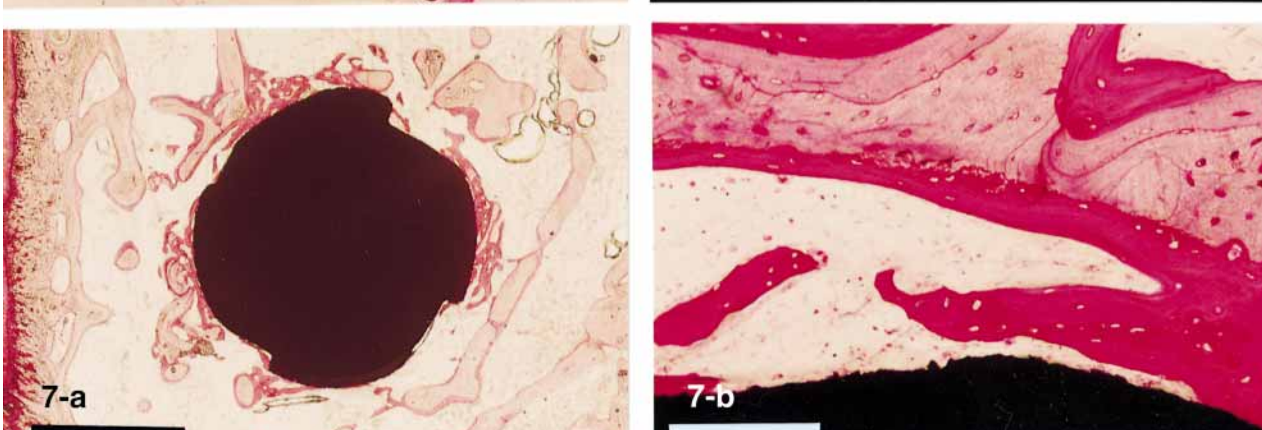
Fig. 4. Diagram of the PEMF generator used for treating rabbit femurs.

Fig. 5. Histological features of a representative section of a femur with a dental implant which was not treated with PEMF, and which was sacrificed 2 weeks after implantation (control group). New bone contained a large number of osteocytes, and had been deposited parallel to the implant surface on the inside of the edge of the drilled hole. The sections were stained with basic fuchsin and methylene blue. The original bone, which stained pink, was surrounded by new bone which stained dark reddish-violet. The junction between original bone and new bone is clearly visible. a: low magnification (×10), bar=2 mm, b: high magnification (×100), bar=0.2 mm.

Fig. 6. Histological features of a representative section of a femur with a dental implant which was treated with PEMF for 8







h per day at a magnetic intensity of 0.3 mT for 2 weeks. The trabecular bone is thick and complex in shape. The trabecular bone in this group was more often in contact with the implant surface than that in the control group sacrificed 2 weeks after implantation. a: low magnification (×10), bar=2 mm, b: high magnification (×100), bar=0.2 mm.

Fig. 7. Histological features of a representative section of a femur with a dental implant which was treated with PEMF for 8 h per day at a magnetic intensity of 0.8 mT for 2 weeks. The trabecular bone is thin, and shows less contact with the implant surface than that in the 0.2 mT- and 0.3 mT-treated groups. a: low magnification (×10), bar=2 mm, b: high magnification (×100), bar=0.2 mm.

tration of 10 mg/kg pentobarbital (Nembutal, Abbott, North Chicago, IL, USA). A hole, 3.6 mm×3.6 mm in size, was drilled at the distal end of each femur, and the implant placed there in the same position and direction being adopted wherever possible. A PEMF generator (Riken Electromagnetic Field Pulse Generator, Institute of Physical and Chemical Research, Saitama, Japan) connected to a band coil (an electric cord coated with soft plastic, coiled 60 times; circumference: 745 mm; resistance: 9.5 Ω) was used. Rabbits which were not treated with PEMF served as the controls. PEMF stimulation was initiated the day after surgery. Both femurs of a rabbit were placed in the circular space of the band coil (Fig. 4), and the femurs were rendered immobile. To observe the effect of the magnetic intensity of PEMF on bone formation, stimulation was applied at 0.2 mT, 0.3 mT or 0.8 mT for 8 h per day for 2 weeks. To observe the effect of the duration of PEMF on bone formation, stimulation was applied daily for 4 h or 8 h at a magnetic intensity of 0.2 mT for 2 weeks. To observe the effect of the length of treatment on bone formation, PEMF stimulation was applied for 1, 2 or 4 weeks at a magnetic intensity of 0.2 mT for 8 h per day in each experimental group, pulse width (25 μs) and pulse frequency (100 Hz) remained constant. Control rats were sacrificed 1, 2 or 4 weeks after implantation. Each of the 6 groups of rabbits treated with PEMF under different conditions and the 3 control groups consisted of 5 rabbits.

Histological examination

The rabbits were sacrificed at the indicated time point by perfusion with 10% neutral formalin via the abdominal aorta. Excised femur samples were fixed for a further 3 days by immersion in 10% neutral formalin. Samples consisting of the implant and surrounding bone tissue were then embedded in polyester resin (Ohken, Tokyo, Japan). They were sliced perpendicular to the major axis of the implant into 30-μm sections, which were stained with 2% basic fuchsin and 0.1% methylene blue solution for light microscopic observation.

Image analysis procedure

The XL 500 computer system (OLYMPUS AVIO, Tokyo, Japan) was used for image analysis. For the purpose, sectioning through samples containing the implant and surrounding bone were chosen at random and then scanned into the computer. Five implant specimens from each group were analyzed. On each image, new bone at the edge and inside the drilled hole was traced in each figure. The bone contact ratio was defined as the length of bone surface border in direct contact with the implant/circumference of the implant [×100 (%)]. The bone area ratio was defined as the area of new bone inside the edge of the drilled hole/area of drilled hole [×100 (%)]. The resultant data were then subjected to variance analysis. Comparisons of group means were performed using the Duncan Multiple Range Test. Differences between group means were considered to be statistically significant at a level of $P<0.01$.

Results

Histological examination

Original bone stained pink and new bone reddish-violet. The junction between original bone and new bone was clearly visible (Figs 5–7). Most new bone contained a large number of osteocytes, and had been laid down parallel to the implant surface on the inside of the edge of the drilled hole. Among the sections obtained 2 weeks after transplantation, the trabecular bone, which was thick and complex in shape, was more often in contact with the implant surface in the femurs treated with PEMF at 0.2 mT and 0.3 mT for 8 h/day than in the non-treated femurs (Figs 5, 6). The trabecular bone in the 0.8 mT-treated femurs was thinner than that in the 0.2 mT- and 0.3 mT-treated ones (Fig. 7). In both the non-treated and 0.2 mT-treated observed 4 weeks after implantation, a thick layer of lamellar trabecular bone had formed on the implant surface, and the bone contact ratio and bone area ratio of these two groups were similar.

Quantitative data

The bone contact ratio and bone area ratio of each experimental group are summarized in Table 1 and the Figs 1–3. The bone contact ratio of the femurs treated with PEMF at 0.2 mT, 0.3 mT and 0.8 mT was significantly larger than that of the control group sacrificed 2 weeks after implantation. The bone area ratio of the femurs treated with PEMF at 0.2 mT and 0.3 mT was significantly larger than that of the control group sacrificed 2 weeks after implantation. No significant difference in either the bone contact ratio or bone area ratio was observed between the 0.2 mT- and 0.3 mT-treated femurs 2 weeks after implantation.

Both the bone contact ratio and bone area ratio of the femurs treated with PEMF for 4 h/day and 8 h/day were significantly larger than the respective value in the control group sacrificed 2 weeks after transplantation. The bone contact ratio and bone area ratio of the femurs treated with PEMF for 4 h/day and those treated for 8 h/day did not differ significantly.

The bone contact ratio and bone area ratio of the 2 week-treated femurs were significantly larger than the respective value of the 1 week-treated ones. No significant difference was observed in either ratio between the femurs treated with PEMF for 2 weeks and those treated for 4 weeks. The bone contact ratio and bone area ratio of the femurs treated with PEMF for 1, 2, and 4 weeks, were significantly larger than in the control groups.

Discussion

The effects of PEMF on bone formation following pseudarthrosis, delayed fracture, fresh fracture and bone graft have been recently reported (Bassett 1984; Bassett & Hess 1984; Buch 1988; Ryaby 1998), but there exists no data relating to PEMF stimulation following the placement of titanium alloy-dental implants with a rough surface at the bone–implant interface. The present study demonstrated that PEMF promotes bone formation around such implants in the rabbit femur.

Two types of surface materials, i.e. titanium and hydroxyapatite-coated titanium, are commonly used in dental implants. The degree to which PEMF promotes bone ingrowth into a porous implant depends upon the nature of the material used. PEMF promotes bone ingrowth into titanium and hydroxyapatite-coated implants, but not into tricalcium phosphate ones (Shimizu et al. 1988; Ijiri et al. 1996). Our results using titanium alloy are consistent with those of Ijiri et al. (1996), who demonstrated that PEMF promotes bone formation on a titanium surface. However, the surface morphology of the dental implant in our study was rough-surfaced, and not porous-surfaced as in the studies of Shimizu et al. (1988) and Ijiri et al. (1996). Groessner-Schreiber & Tuan (1992) reported that among dental implants, the amount of bone which forms on a rough surface is the same as that which forms on a porous one, but greater than that which forms on a smooth surface. PEMF may be just as effective on porous implants as rough-surfaced implants.

Previous investigations have employed electromagnetic powers of 0.1–1.2 mT in PEMF procedures. Low electromagnetic powers such as 0.2 mT and 0.3 mT promoted a greater degree of bone formation than did higher ones (0.8 mT) as in our study, which is consistent with previously published data (Bassett et al. 1975; Matsunaga et al. 1991; Ochi 1993). It may be important to maintain a certain electromagnetic power during the PEMF procedure.

The duration of stimulation may also be an important factor. Ijiri et al. (1993), showed that stimulation for 10 h/day promoted a greater degree of bone formation than did stimulation for 5 h/day. Our results, however, did not show a significant difference between the femurs treated with PEMF for 4 h/day and those treated for 8 h/day. The amount of bone which had formed in the femurs treated with PEMF for 2 weeks and those treated for 4 weeks did not differ significantly; however, there was a significant difference in the amount of bone which had formed over 2 weeks and over 4 weeks in the non-treated groups. It is important to determine the optimal stimulation time per day and the optimal duration of treatment with PEMF in promoting osseointegration in patients with dental implants.

Possible mechanisms underlying PEMF stimulation of osteogenesis include promotion of vascularization, collagen production, and/or osteogenic cell proliferation and differentiation (Matsunaga 1986; Yen-Patton et al. 1988; Bodamyali et al. 1988; Aaron et al. 1989), but this aspect is beyond the scope of the present study.

In conclusion, PEMF stimulation may be useful for promoting bone formation on rough-surfaced dental implants. It is important to determine the minimal essential magnetic intensity, as well as the requisite duration of stimulation per day and the period of time over which this should be continued.

Acknowledgement

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Résumé

L'étude présente a examiné les effets de rayonnements électromagnétiques pulsés (PEMF) sur la formation osseuse autour d'implants dentaire à surface rugueuse. Un implant a été placé dans des fémurs de lapins blancs japonais. Un PEMF à variation de pulsation de 25 μs et de fréquence de pulsation de 100 Hz ont été appliqués. La stimulation PEMF a été appliquée pendant quatre heures ou huit heures par jour avec une intensité magnétique de 0.2 mT, 0.3 mT ou 0.8 mT. Les animaux ont été tués une, deux ou quatre semaines après l'implantation. Après coloration des coupes en résine avec de la fuschine basique 2% et du bleu de méthylène 0.1%, la néoformation osseuse autour des implants a été étudiée par analyse d'image sur ordinateur. Les proportions de contact osseux des fémurs étaient significativement plus importantes après traitement PEMF que dans le groupe contrôle. Tant la proportion de contact osseux que la proportion d'aires osseuses des fémurs traités avec 0.2 mT et 0.3 mT étaient significativement plus importantes que les valeurs respectives des fémurs traités avec 0.8 mT ($P>0.001$). Aucune différence significative dans la proportion de contact osseux ou dans la proportion des aires osseuses n'a été observée lorsque le PEMF était appliqué durant quatre ou huit heures par jour. Bien qu'une quantité plus importante d'os ait été formée autour des implants sur fémurs traités après deux semaines par rapport à ceux traités après une semaine, aucune différence statistiquement significative n'a été observée entre les fémurs traités pendant deux et quatres semaines. Ces résultats suggèrent que la stimulation PEMF peut être utile pour promouvoir la formation osseuse autour des implants dentaires avec surface rugueuse. Il est important de sélectionner l'intensité magnétique idéale du rayonnement, sa durée (par jour) et la longueur du traitement.

Zusammenfassung

Die vorliegende Studie untersuchte den Effekt der Anwendung eines pulsierenden elektromagnetischen Feldes (PEMF) auf die Knochenbildung um ein dentales Implantat mit einer rauhen Oberfläche. Ein dentales Implantat wurde beidseits in den Femur von weissen japanischen Kaninchen eingesetzt. Es wurde ein PEMF mit einer Pulsweite von 25 µs und einer Pulsfrequenz 100 Hz angewendet. Die PEMF-Stimulation wurde für 4 oder 8 Stunden pro Tag mit einer magnetischen Intensität von 0.2 mT, 0.3 mT oder 0.8 mT angewendet. Die Tiere wurden 1, 2 oder 4 Wochen nach der Implantation geopfert. Nach der Färbung der Kunststoffschnitte mit 2% basischem Fuchsin und 0.1% Methylenblau wurde der neugebildete Knochen um die Implantate auf den Schnittpräparaten mittels computergesteuerter Bildanalyse ausgewertet. Der Anteil an Knochenkontakt war bei den PEMF-behandelten Femuren signifikant grösser als bei den Kontrollstellen. Sowohl die Anteile an Knochenkontakt als auch die Anteile an Knochenfläche der mit 0.2 mT und 0.3 mT behandelten Femuren waren signifikant grösser als die entsprechenden Werte der mit 0.8 mT behandelten Femuren ($P<0.001$). Es bestanden keine signifikanten Unterschiede im Anteil an Knochenkontakt oder Knochenfläche wenn PEMF entweder 4 Stunden oder 8 Stunden pro Tag angewendet wurde. Bei den 2 Wochen behandelten Femuren hatte sich signifikant mehr Knochen gebildet als bei den 1 Woche behandelten Stellen. Jedoch konnten zwischen den 2 Wochen behandelten Femuren und den 4 Wochen behandelten Stellen keine signifikanten Unterschiede gefunden werden. Diese Resultate lassen vermuten, dass die PEMF-Stimulation bei der Förderung der Knochenbildung um dentale Implantate mit rauher Oberfläche hilfreich sein kann. Es ist wichtig, die geeignete magnetische Intensität, die richtige Dauer der Behandlung pro Tag und die korrekte Länge der Behandlung auszuwählen.

Resumen

El presente estudio examinó el efecto de aplicar un campo electromagnético pulsatil (PEMF) en la formación de hueso alrededor de implantes dentales de superficie rugosa. Se insertó un implante en el fémur de conejos blancos japoneses bilateralmente. Se aplicó un PEMF con una anchura de pulso de 25 µs y una frecuencia de pulso de 100 Hz. Se aplicó una estimulación de PEMF durante 4 u 8 horas por día, con una intensidad magnética de 0.2 mT, 0.3 mT o 0.8 mT. Los animales se sacrificaron 1, 2 o 4 semanas tras la implantación. Tras teñir las secciones de resina con fucsina básica al 2% y azul de metileno al 0.1%, se evaluó el hueso neoformado alrededor del implante en las secciones tisulares por medio de análisis de imagen computarizado. Los índices de contacto óseo de los fémures tratados con PEMF fueron significativamente mayores que los del grupo de control. Tanto el índice de contacto óseo como el índice de área ósea de los fémures tratados con 0.2 mT y 0.3 mT fueron significativamente mayores que los valores respectivos de los fémures tratados con 0.8 mT ($P<0.001$). No se observó diferencia significativa entre el índice de contacto óseo o índice de área ósea tanto si el PEMF se aplicó 4 hr/día o 8 hr/día. Aunque se formó una cantidad significativamente mayor de hueso alrededor del implante de los fémures tratados durante 2 semanas que de los fémures tratados durante 1 semana, no se observó diferencia significativa entre los fémures tratados durante 2 semanas y los de 4 semanas. Estos resultados sugieren que el PEMF puede ser útil para promover la formación ósea alrededor de implantes dentales de superficie rugosa. Es importante seleccionar la intensidad magnética adecuada, duración diaria y longitud de tratamiento.

要旨

本研究は、パルス電磁場（PEMF）の適用が粗面の歯科インプラント周囲の骨形成に及ぼす影響を調べた。日本白色家兎の両側大腿骨に歯科インプラントを埋入した。PEMF をパルス幅２５μ秒及びパルス周波数１００Hz で適用した。PEMF の刺激は、０．２mT、０．３mT または０．８mT の磁場強度で、１日４時間または８時間適用した。動物は埋入１週間、２週間または４週間後に屠殺した。樹脂切片を２％の塩基性フクシンと０．１％のメチレン・ブルーで染色した後、組織切片上でインプラント周囲の新生骨を、コンピュータ画像解析によって評価した。PEMF で処理した大腿骨の骨接触率は、対照群のそれより有意に高かった。０．２mT 及び０．３mT で処理した大腿骨の骨接触率及び骨面積率は０．８mT で処理した大腿骨の値よりも有意に高かった（p＜０．００１）。PEMF の適用時間が１日４時間か８時間かでは、骨接触率あるいは骨面積率に有意差は認められなかった。２週間処理した大腿骨においてインプラント周囲の骨形成量は１週間処理した大腿骨より有意に多かったが、２週間と４週間の間には有意差は見とめられなかった。これらの結果は、PEMF の刺激は粗面の歯科インプラント周囲の骨形成促進に有効であり得ることを示唆している。磁場強度、１日あたりの適用時間及び治療期間を適切に選択することが重要である。

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