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Electrical Field Effect on Peri-Implant Osteogenesis: A Histologic and Histomorphometric Study

Graciela Ana Giannunzio, DDS,* Rodolfo Carlos Speerli, EEE,† and María Beatriz Guglielmotti, DDS, PhD‡

Purpose: *Tissue response to injury, as occurs during wound healing, is a well-organized biologic event. Both clinical and experimental studies have shown external electrical stimulation to enhance tissue repair. The effect of* in situ *electrical stimulation has been studied in experimental models of fracture healing, ostectomy, osteogenic distraction, and implants. The aim of the present study was to evaluate the effect of an electrical field on peri-implant wound healing, using an experimental model that involved placing a metallic laminar implant in rat tibia.*

Materials: *Forty male Wistar rats weighing approximately 100 g were used. A titanium laminar implant ($6 \times 1 \times 0.1$ mm^3) (Implant Vel, Buenos Aires, Argentina) was inserted through the hole and placed in the medullary compartment. The tissues were then repositioned and sutured carefully. An electric field generator (ECCEL, DAM, Argentina) was used to deliver the electric stimulus. The electric field plate was placed on the skin of both hind limbs. In sham group, the animals were subjected to the same procedure without connecting the plate to the electric field generator. All the animals were killed by ether overdose at 15 days of postimplantation. The tibiae were resected, fixed in 20% formalin, radiographed, and processed for embedding in methyl methacrylate. The ground sections were stained with 1% toluidine blue. The following parameters were evaluated: peri-implant bone volume and percentage of osseointegration. Statistical analysis of the results was performed using ANOVA ($P < 0.05$).*

Results: *Application of external positive or negative electrical fields using the experimental model (post-titanium implant bone healing in rat tibia), under the conditions stated herein, was found to enhance peri-implant lamellar bone volume compared with sham-treated animals.*

Conclusion: *The use of a device generating a positive/negative electrical field resulted in the presence of woven bone. (Implant Dent 2008;17:1–●●●)*

Key Words: osseointegration, electrical field, osteogenesis, metal implant

Tissue response to injury, as occurs during wound healing, is a well-organized biologic event. Both clinical and experimental studies have shown that external electrical stimulation enhances tissue repair.

In 1940, Burr *et al*[1] found that electronegative polarity exerted a positive effect on the proliferation stage of soft tissue wound healing. In 1957, Fukada and Yasuda[2] reported the first findings on the endogenous electrical properties of bone tissue. The authors found that mechanical stress generated an electric potential, possibly because of a piezoelectric effect, and that areas of active growth and bone repair were electronegative when compared with less active areas.

Studies have demonstrated that exogenous electrical stimulation induces osteogenesis in the tissue surrounding a negative electrode.[3,4] In 1974, Bassett *et al*[5] found that electromagnetic fields increase cellular electric potential, accelerating the rate of tissue repair and reducing edema and pain. However, the authors failed to clarify which of the 2 fields, electrical or magnetic, were actually responsible for these effects. In a study on mitotic activity in lymphocytes of rat thymus performed in 1992, Liburdy[6] observed that the electrical field, whether direct or induced by a magnetic field, interacted with calcium transport through the cell membrane.

Constant advances in technology lead to continuous development of devices used in physiotherapy employing electromagnetic waves such as short wave, microwave, and magnetotherapy. The former 2 deliver high frequency waves, thus generating heat in the tissues and in the implants as a side effect. Conversely, magnetotherapy involves low frequency variable magnetic fields that exert an effect on the electric charge of the cells and tissues, without generating heat.

Magnetic fields were used for therapeutic purposes at a time when the application of electrical fields was difficult because of the lack of adequate technology. Current availability of semiconductors and proper insulat-

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Table 1. Characteristics of Electrical Fields Applied to Each Experimental Group and Sham Group

	Sham	Experimental		
	Group I (n = 10)	Group II (n = 10)	Group III (n = 10)	Group IV (n = 10)
Device	—	ECCEL 122	ECCEL 222	ECCEL 222
Polarity	—	Positive–Negative	Negative	Positive
Intensity (μA/cm^2)	—	1.68	2.64	2.64
Frequency (Hz)	—	115	650	650
Applications	6	6	6	6
Duration of each application (min)	20	20	20	20

ing materials have allowed for the development of electrical field generating devices for therapeutic use with virtually no magnetic component.

The effect of *in situ* electrical stimulation has been studied in experimental models of fracture healing, ostectomy, osteogenic distraction, and implants.[7–9] Clinical studies have evaluated its effect on nonunion fracture healing, bone graft attachment to bony defects, and osteoradionecrosis among others. All the above showed that the effect of *in situ* electrical stimulation varies substantially depending on the intensity of the stimulus, the polarity of the applied current, its duration, whether it is continuous or pulsed, and on the metal used as an electrode.[4]

The aim of this study was to evaluate the effect of an electrical field on peri-implant wound healing, using an experimental model that involved placing a metallic laminar implant in rat tibia.[10]

Materials and Methods

Animals

Forty male Wistar rats weighing approximately 100 g were used. The animals were housed in plastic cages and maintained on a 12:12-h light:dark cycle. They were fed rat chow and water *ad libitum*. The National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH publication No. 85-23, revised 1985) were observed.

Surgical Procedure

The animals were anesthetized by intraperitoneal injection of 8 mg/100 g body weight of ketamine hydrochloride (Ketalar, Parke-Davis, Morris, Plains, NJ) and 1.28 mg/100 g body weight of xylazine (Rompum, Bayer Argentina, SA).

Both tibiae were shaved and a 1.5-cm long incision was made at the level of the tibial crest. The subcutaneous tissue, muscle, and ligaments were dissected to expose the external surface of the tibia at the level of the diaphysis. Using a round burr, a 1.5-mm diameter hole was drilled manually so as to avoid overheating and damage to the bone tissue. A titanium laminar implant (6 × 1 × 0.1 mm^3) (Implant Vel, Buenos Aires, Argentina) was inserted through the hole and placed in the medullary compartment. The tissues were then repositioned and carefully sutured.

Electrical Field Application

The animals were assigned to 1 of 4 groups (n = 10), 3 experimental groups and 1 sham group (Table 1).

In experimental groups, an electric field generator (ECCEL, DAM, Argentina) was used to deliver the electric stimulus. The animals were sedated during each procedure by intraperitoneal administration of 25 mg/kg body weight of diazepan (Roche, USA). All the animals were exposed to a total of six 20-minute applications, which were administered every other day. The electric field plate was placed on the skin of both hind limbs (Fig. 1).

In sham group, the animals were subjected to the same procedure without connecting the plate to the electric field generator. All the animals were killed by ether overdose at 15 days of postimplantation.

Histologic Processing

The tibiae were resected, fixed in 20% formalin, radiographed, and processed for embedding in methyl methacrylate. Three cross-sections perpendicular to the longest axis of the implant were obtained from each tibia,

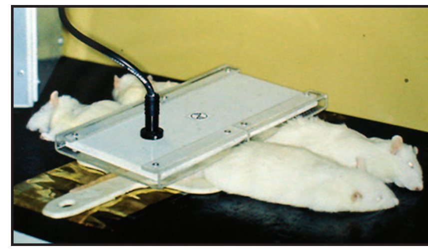

Fig. 1. Notice the rat receiving the electrical field, under sedation.

and ground to a thickness of 50 μm. The ground sections were stained with 1% toluidine blue.

Histomorphometric Analysis AQ: 2

Histomorphometric determinations were performed on the sections using a light microscope (Zeiss Axioscop 2 MOT; Carl Zeiss, Jena, Germany) online with an image-analysis system (Kontron KS300 version 2; Kontron Elektronik, Munich, Germany). T1

The following parameters were evaluated: peri-implant bone volume and percentage of osseointegration. Statistical analysis of the results was performed using ANOVA ($P < 0.05$).

Results

None of the animals showed alterations in body weight, behavior, or general health. F1

All the implants were inside the diaphyseal area of the tibia 15 days postimplantation, as shown by the radiographic study. Macroscopic examination confirmed satisfactory healing of soft tissues in all the animals.

Microscopic observation evidenced lamellar bone tissue in contact with the implant surface in sham-treated animals (group I) (Fig. 2, *a–c*). F2

Animals of group II (positive–negative polarity) treated with ECCEL

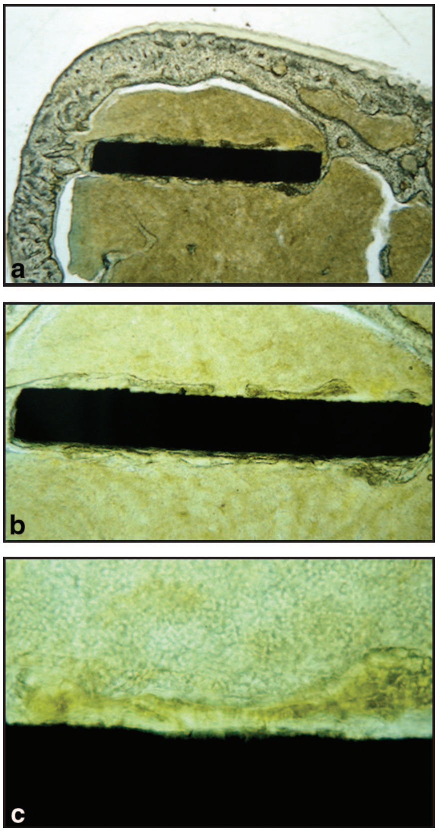

Fig. 2. Sham group. (a) Grinding section showing close bone apposition to titanium laminar implant (original magnification ×50). (b) At higher magnification, the intimate contact and the full congruency at the level of the bone–implant interface are evident (original magnification ×100). (c) Notice the bone close to the implant surface (original magnification ×400).

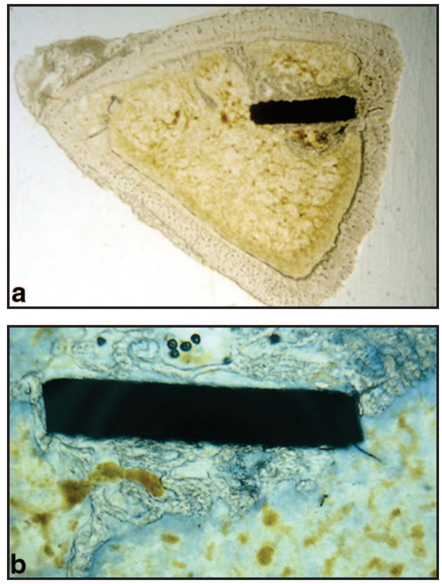

Fig. 3. Experimental group. Positive–negative polarity. (a) Notice the presence of woven bone formation in the peri-implant microenvironment (original magnification ×25). (b) Scarce osseointegration is evident at the higher magnification (original magnification ×100).

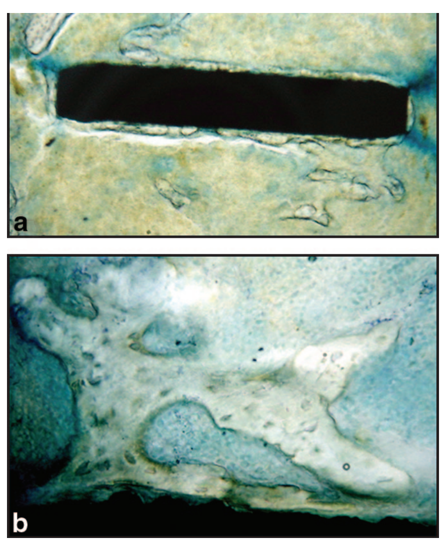

Fig. 4. Experimental group. Negative polarity. (a) Grinding section showing bone apposition to the implant (original magnification ×100). (b) At higher magnification note the close contact of the bone–implant interface (original magnification ×400).

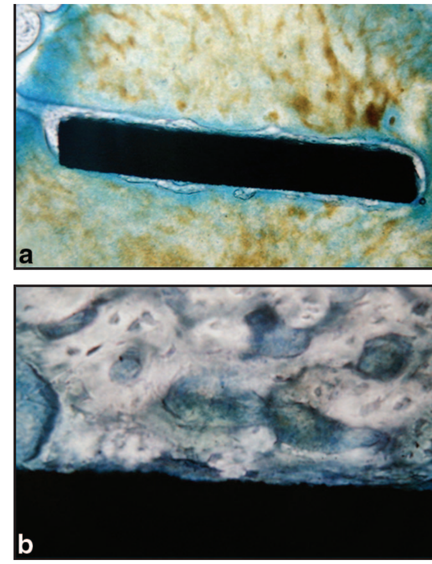

Fig. 5. Experimental group. Positive polarity. (a) Grinding section showing bone apposition to the implant (original magnification ×100). (b) At higher magnification note the close contact between bone and implant (original magnification ×400).

122 presented woven bone tissue surrounding the implant (Fig. 3, *a* and *b*). F3

Both groups III (negative polarity) and IV (positive polarity) treated with ECCEL 222 presented lamellar bone tissue partly in contact with the implant (osseointegration) (Figs. 4, *a* and b and 5, *a* and *b*); the bone was thicker than that observed in the sham group. F4 F5

Statistical analysis of the histomorphometric results showed a significant difference in the percentage of osseointegration when comparing group I (mean, 66 ± 7) and group II (mean, 11 ± 3) $P < 0.05$ and groups I and III (mean, 52 ± 6) $P < 0.05$; whereas no difference was observed between group I and IV (mean, 58 ± 12). In addition, comparison between groups II and III ($P < 0.05$) and groups II and IV ($P < 0.05$) showed a significant difference, unlike comparison between groups III and IV.

Significant differences in peri-implant bone volume were observed between groups I (mean, 5 ± 1) and II (mean, 13 ± 3) $P < 0.05$, groups I and III (mean, 11 ± 3) $P < 0.05$, and between groups I (mean, 5 ± 1) and IV (mean, 16 ± 5) $P < 0.05$. In addition, comparison between groups III and IV $P < 0.05$ showed a significant difference. No significant differences in peri-implant bone volume were observed between groups II and III or between groups II and IV (Table 2). T2,AQ:3

Discussion

Geddes[11] described that the concept of electrical stimulation to elicit fracture-healing dates from A. Boyer (1816) specifically for the healing of nonunion fractures. Several reports encouraged both laboratory and clinical research on electrically induced bone formation and healing with use of various forms of electrical stimulation.[12] Brighton *et al*[13] stated that "the clinical effectiveness of bone-growth stimulation proved to be easier to demonstrate the mechanism or mechanisms of action of electrically induced osteogenesis."

Peri-implant bone healing provides a suitable model for the study of bone formation and can be considered to be a sensitive indicator of bone damage under different experimental conditions. The experimental model used in this study allows studying *de novo* bone formation in the peri-implant bone marrow of rat tibia under different local or systemic conditions.

Our results clearly show that the use of electrical fields with positive or

Table 2. ANOVA Statistical Analysis of the Peri-Implant Bone Volume and Percentage of Osseointegration

	Sham	Experimental		
	Group I	Group II 122 (+, −)	Group III 222 (−)	Group IV 222 (+)
% Osseointegration (mean ± SD)*	66 ± 7	11 ± 3	52 ± 6	58 ± 12
Peri-implant bone volume, mm^2 (mean ± SD)†‡	5 ± 1	13 ± 3	11 ± 3	16 ± 5

* A significant difference ($P < 0.05$) was found when comparing groups I and II, I and III, II and III, and II and IV, whereas no significant difference was observed when comparing groups I and IV or III and IV.

† A significant difference ($P < 0.05$) was found when comparing groups I and II, I and III, I and IV, and III and IV, whereas no significant difference was observed when comparing groups II and IV or II and III.

‡ mm^2 projection of the histological sections.

negative polarity (1.68 $\mu A/cm^2$, 115 Hz) delays bone healing, as evidenced by the presence of woven bone. Application of electrical fields with either negative or positive polarity (2.64 $\mu A/cm^2$, 650 Hz) was found to increase peri-implant bone volume when compared with sham-treated animals.

Although most studies using electrical fields have used negative polarity, the favorable effect of electropositivity on wound healing has also been described. Weiss *et al*[3] stated that many investigators have found enhanced wound healing when the polarity was changed during the course of treatment. It is possible that direct electronegative stimuli accelerate the rate of healing increasing both cellular proliferation and collagen synthesis. However, the mechanism by which this cellular activation occurs is yet to be clarified.

Recent findings reported by Brighton *et al*[13] lend further support to the hypothesis that differences in the dose–response (bone-cell proliferation) of the various forms of electrical stimulation are due to differences in signal transduction.

The findings reported in the literature and the results obtained using this experimental model evidence the need for further research on this noninvasive treatment involving application of external electrical fields, so as to optimize its clinical use for soft and hard tissue repair.

Conclusion

Application of external positive or negative electrical fields using the experimental model (post-titanium implant bone healing in rat tibia), and under the conditions stated herein, was found to enhance peri-implant lamellar bone volume compared with sham-treated animals.

The use of a device generating a positive or negative electrical field resulted in the presence of woven bone.

Disclosure

The authors claim to have no financial interest in any company or any of the products mentioned in this article.

Acknowledgments

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References

1. Burr HS, Taffel M, Harvey SC. An electromagnetic study of the healing wound in man. *Yale J Biol Med*. 1940;12:483-485.
2. Fukada E, Yasuda I. On the piezoelectric effect of bone. *J Physical Soc Japan*. 1957;12:1158-1162.
3. Weiss DS, Kirsner R, Eaglstein WH. Electrical stimulation and wound healing. *Arch Dermatol*. 1990;126:220-225.
4. Bassett CA, Pawluk RJ, Becker RO. Effects of electric currents on bone in vivo. *Nature*. 1964;204:652-655.
5. Bassett CA, Pawluk RJ, Pilla AA. Augmentation of bone repair by inductively couple electromagnetic field. *Science*. 1974;184:575-577.
6. Liburdy RP. Calcium signaling in lymphocytes and ELF fields. Evidence for an electric field metric and a site of interaction involving the calcium ion channel. *FEBS Lett*. 1992;301:53-59.
7. Colella SM, Miller AG, Stang RG, et al. Fixation of porous titanium implants in cortical bone enhanced by electrical stimulation. *J Biomed Mater Res*. 1981;15:37-46.
8. Pankaj RN. A histologic evaluation of the effect of electrical stimulation on osteogenic changes following placement of blade-vent implants in the mandible of rabbits. *J Oral Implantol*. 1998;24:185-195.
9. Shandler H, Weinstein S, Nathan L. Facilitated healing of osseous lesions in the canine mandible after electrical stimulation. *J Oral Surg*. 1979;37:787-792.
10. Cabrini RL, Guglielmotti MB, Almagro JC. Histomorphometry of initial bone healing around zirconium implant in rats. *Implant Dent*. 1993;2:264-267.
11. Geddes LA. A short history of the electrical stimulation of excitable tissue. Including electrotherapeutic applications. *Physiologist*. 1984;27(1 suppl):S1-S47.
12. Brighton CT, Hozack WJ, Brager MD, et al. Fracture healing in the rabbits fibula when subjected to various capacitively coupled electrical field. *J Orthop Res*. 1985;3:331-340.
13. Brighton CT, Wang W, Seldes R, et al. Signal transduction in electrically stimulated bone cells. *J Bone Joint Surg*. 2001;83-A:1514-1521.

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Abstract Translations

GERMAN / DEUTSCH

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Auswirkungen elektrischer Felder auf die Osteogenese im Implantatumlagernden Bereich: eine histologische sowie histomorphometrische Studie

ZUSAMMENFASSUNG: Zielsetzung: Die Reaktion des Gewebes auf Verletzungen, wie dies auch bei der Wundheilung auftritt, kann als gut organisiertes biologisches Ereignis bezeichnet werden. Sowohl klinische als auch experimentelle Studien haben gezeigt, dass eine elektrische Stimulation von außen zur Wiederherstellung des betroffenen Gewebes beitragen kann. Die Auswirkungen einer elektrischen Stimulation *vor Ort* wurden in experimentellen Modellen bei der Heilung von Brüchen, nach Ostektomie, osteogener Distraktion sowie bei Einsatz von Implantaten untersucht. Die vorliegende Studie zielte darauf ab, die Auswirkungen bei Anlage eines elektrischen Feldes auf die das Implantat umlagernden Gewebeteile bei Wundheilung zu ermitteln und bewerten. Dazu fand ein experimentelles Modell Anwendung, das die Einpflanzung eines metallischen Laminarimplantats in das Schienbein von Ratten vorsah. ***Materialien und Methoden:*** Als Versuchstiere wurden 40 männliche Wistar-Ratten mit einem Gewicht von jeweils ca. 100g eingesetzt. Ein laminäres Titanimplantat (6 × 1 × 0,1 mm) (Implant Vel, Buenos Aires, Argentinien) wurde durch eine Öffnung eingeführt und in den medullären Bereich eingesetzt. Die betroffenen Gewebeteile wurden neu ausgerichtet und sorgfältig genäht. Mittels elektronischem Gerät (ECCEL, DAM, Argentinien) wurde ein elektrischer Impuls ausgesendet. Die elektrische Feldplatte wurde auf die beiden hinteren Gliedmaßen aufgebracht. Blindgruppe: Die Tiere wurden der gleichen Prozedur unterzogen, allerdings lag bei diesen kein Strom vom Generator an der Platte an. Alle Versuchstiere wurden mit einer Überdosis an Áther 15 Tage nach der Implantierung getötet. Die Schienbeine der Tiere wurden entnommen, in 20-prozentigem Formalin fixiert, geröntgt und zur Einbettung in Methyl-Methacrylat vorbereitet. Die Bodenabschnitte wurden mit 1-prozentigem Toluidinblau eingefärbt. Die nachfolgenden Parameter wurden bewertet: das Knochengewebsvolumen im das Implantat umlagernden Gewebe sowie der Prozentsatz der Knochengewebsintegration. Über ANOVA ($p < 0.05$) wurde eine statistische Analyse der Ergebnisse durchgeführt. ***Ergebnisse:*** Die Anwendung externer positiver und/oder negativer elektrischer Felder unter Anwendung des experimentellen Modells (Post-Titan-Implantat-Knochenheilung in Schienbeinen bei Ratten) sowie unter den in diesem Bericht geschilderten Bedingungen stellte sich bei einem Vergleich zu der Blindversuchsgruppe als vorteilhaft und fördernd für die Bildung von Lamellenknochen im das Implantat umlagernden Bereich heraus. ***Schlussfolgerung:*** Durch die Anlage eines Geräts zum Aufbau positiver/negativer elektrischer Felder wurde die Bildung von Geflechtknochen hervorgerufen.

SCHLÜSSELWÖRTER: Knochengewebsintegration, elektrisches Feld, Osteogenese, Metallimplantat

SPANISH / ESPAÑOL

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Efecto del campo eléctrico en la osteogénesis periimplante: Un estudio histológico e histomorfométrico

ABSTRACTO: Propósito: La respuesta del tejido a las lesiones, como ocurre durante la curación de una herida, es un evento biológico bien organizado. Estudios clínicos y experimentales han demostrado que la estimulación eléctrica externa mejora la reparación del tejido. El efecto de la estimulación eléctrica *in situ* se ha estudiado en modelos experimentales de curación de fracturas, osteotomías, distracción e implantes osteogénicos. El objetivo de este estudio fue evaluar el efecto de un campo eléctrico en la curación de una herida periimplante, usando un modelo experimental que involucró la colocación de un implante laminar metálico en la tibia de un ratón. ***Materiales y Métodos:*** Se usaron cuatro ratones machos Wistar que pesaban aproximadamente 100g de peso corporal. Un implante laminar de titanio (6 × 1 × 0,1 mm) (Implant Vel, Buenos Aires, Argentina) se colocó a través del agujero en el compartimiento medular. Los tejidos se reposicionaron y suturaron cuidadosamente. Se usó un generador de campo eléctrico (ECCEL, DAM, Argentina) para crear el estímulo eléctrico. La placa del campo eléctrico se colocó en la piel de las dos extremidades traseras. Grupo de control: Los animales fueron sometidos al mismo procedimiento sin conectar la placa al generador de campo eléctrico. Todos los animales fueron sacrificados a través de una sobredosis de éter de 15 días después de la colocación del implante. Las tibias fueron resecadas, fijadas en formalina al 20%, radiografiadas y procesadas en metacrilato de metilo. Las secciones trituradas fueron manchadas con azul de toluidina al 1%. Se evaluaron los siguientes parámetros: volumen de hueso periimplante y porcentaje de oseointegración. Los análisis estadísticos de los resultados se realizaron usando

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ANOVA ($p < 0.05$). ***Resultados:*** La aplicación de campos energéticos negativos y/o positivos usando el modelo experimental (curación del hueso del implante postitanio en la tibia de ratones) y bajo las condiciones indicadas aquí, demostró mejorar el volumen de hueso lamelar perimplante, comparado con los animales del grupo de control. ***Conclusión:*** El uso de un dispositivo que genera un campo eléctrico positivo o negativo resultó en la presencia de un hueso integrado.

PALABRAS CLAVES: Oseointegración, campo eléctrico, osteogénesis, implante de metal

PORTUGUESE / PORTUGUÊS

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Efeito do campo elétrico sobre a osteogênese do periimplante: Estudo histológico e histomorfométrico

RESUMO: Objetivo: A resposta do tecido à lesão, conforme ocorre durante a cura da ferida, é um evento biológico bem-organizado. Estudos clínicos e experimentais mostraram que o estímulo elétrico externo intensifica a reparação do tecido. O efeito de estímulo elétrico *in situ* foi estudado em modelos experimentais de cura de fratura, ostectomia, distração osteogênica e implantes. O objetivo do presente estudo era avaliar o efeito de um campo elétrico sobre a cura da ferida do periimplante, usando-se um modelo experimental que envolva um implante laminar metálico em tíbias de ratos. ***Materiais e Métodos:*** Quarenta ratos Wistar machos pesando aproximadamente 100g de peso corporal foram usados. Um implante laminar de titânio ($6 \times 1 \times 0.1$ mm) (Implant Vel, Buenos Aires, Argentina) foi inserido no orifício e colocado no compartimento medular. Os tecidos foram então reposicionados e suturados cuidadosamente. Um gerador de campo elétrico (ECCEL, DAM, Argentina) foi usado para provocar o estímulo elétrico. A placa de campo elétrico foi colocada sobre a pele de ambos os membros posteriores. Suposto grupo: Os animais foram sujeitos ao mesmo procedimento sem conectar a placa ao gerador de campo elétrico. Todos os animais foram mortos por overdose de éter 15 dias após a implantação. As tíbias foram ressecadas, fixadas em formalina a 20%, radiografadas e processadas para incrustar no metil metacrilato. As seções de base foram manchadas com azul de toluidina a 1%. Os seguintes parâmetyros foram avaliados: volume do osso de periimplante e porcentagem de osseointegração. A análise estatística dos resultados foi realizada usando-se ANOVA ($p < 0.05$). ***Resultados:*** Descobriu-se que a aplicação de campos elétricos externos positivos e/ou negativos usando-se o modelo experimental (cura de osso de implante pós-titânio em tíbias de ratos) e sob as condições declaradas neste intensificou o volume do osso lamelar de periimplante, em comparação com animais supostamente tratados. ***Conclusão:*** O uso de um dispositivo gerador de campo elétrico positivo/negativo resultou na presença de osso entrelaçado.

PALAVRAS-CHAVE: Osseointegração, campo elétrico, implante de metal

RUSSIAN / РУССКИЙ

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Влияние электрического поля на периимплантатный остеогенез: гистологическое и гистоморфометрическое исследование

РЕЗЮМЕ: **Цель.** Ответная реакция ткани на травму, т.е. заживление раны, – это хорошо организованный биологический процесс. И клинические, и экспериментальные исследования продемонстрировали, что внешняя электростимуляция улучшает восстановление ткани. Влияние местной электростимуляции исследовалось на экспериментальных моделях срастания перелома, иссечения кости, вытяжения кости и имплантатов. Цель данного исследования – оценить влияние электрического поля на заживление периимплантатной раны с помощью экспериментальной модели, созданной путем помещения металлического пластинчатого имплантата в большеберцовую кость крысы. **Материалы и методы.** Использовалось сорок мужских особей крыс Уистара весом приблизительно 100 г. Титановый пластинчатый имплантат (6х1х0,1мм) (Имплантат Vel, Буэнос-Айрес, Аргентина) вставили через разрез и поместили в костно-мозговой канал. Затем ткани вернули на прежнее место и тщательно наложили швы. Для создания электростимулирующего эффекта использовали генератор электрического поля (ECCEL, DAM, Аргентина). На кожу обеих задних конечностей поместили пластинку с электрическим полем. Группа ложной стимуляции: животных подвергли такой же процедуре, но без подключения пластинки к генератору электрического поля. Всех животных умертвили передозировкой эфира через 15 дней после имплантации. Большеберцовые кости резецировали, обработали 20-процентным раствором формалина, сделали рентгеновские снимки и погрузили в метилметакрилат. Основные участки окрасили 1% раствором толуидинового синего. Оценивались следующие параметры: объем периимплантатной кости и процент остеоинтеграции. Статистический анализ результатов выполнен с помощью ANOVA ($p<0.05$). **Результаты.** Был сделан вывод о том, что применение внешних положительных и (или) отрицательных полей в экспериментальной модели (заживление кости после установки титанового имплантата в большеберцовую кость крысы) при описанных выше условиях способствует увеличению объема периимплантатной пластинчатой кости по сравнению с животными, которым симулировали лечение.**Вывод.** Использование устройства, генерирующего положительное/отрицательное электрическое поле, привело к формированию пластинчатой ретикулофиброзной костной ткани.

КЛЮЧЕВЫЕ СЛОВА: оссеоинтеграция, электрическое поле, остеогенез, металлический имплантат.

TURKISH / TÜRKÇE

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Peri-implant osteogenezinde elektriksel alanýn etkisi: Histolojik ve histomorfometrik bir çalýþma

ÖZET: **Amaç:** Bir yaranın iyileşmesinde görülen yaralanmaya karşı dokusal yanıt, iyi bir organizasyon gösteren biyolojik bir olaydır. Klinik ve deneysel çalışmalar, dışarıdan (eksternal) elektriksel stimülasyonun doku onarımını arttırdığını göstermiçstir. *Ýn situ* elektriksel stimülasyonun etkisi, kırık iyileçsmesi, ostektomi, osteojenik distraksiyon ve implantların deneysel modellerinde araçstırılmıştır. Bu çalışmanın amacı, peri-implant yara iyileçsmesinde bir elektriksel alanın etkisini, metal bir laminar implantın sıçan tibia kemiğine yerleçstirildiği deneysel bir modelde değerlendirmekti. **Gereç ve Yöntem:** Bu çalışmada yaklaşık 100 g vücut ağırlığına sahip, 40 adet erkek Wistar sıçan kullanıldı. Bir titanyum laminar implant (6x1x0.1mm) (Implant Vel, Buenos Aires, Arjantin) insizyondan sokularak medullar kompartmana yerleştirildi. Sonra dokular yeniden konumlanıp, dikkatlice sutür atıldı. Elektriksel stimülasyon için bir elektrikli alan jeneratörü (ECCEL, DAM, Arjantin) kullanıldı. Elektriksel alan plakası, sıçanlarda iki arka bacağın derisine yerleştirildi. Plasebo grubu: Plaka, elektriksel saha jeneratörüne bağlanmadan hayvanlara aynı prosedür uygulandı. İmplantasyondan 15 gün sonra hayvanların hepsi yüksek bir eter dozu ile öldürüldü. Tibia kemiklerine reseksiyon yapılarak, bunlara %20 formalin uygulandı. Daha sonra radyografi uygulanan tibia, metil metakrilata gömülmek üzere işlemden geçirildi. Öğütülmüş kısımlar, %1 toluidin mavi ile boyandı. Değerlendirilen parametreler, peri-implant kemik hacmini ve osseointegrasyon yüzdesini içerdi. Sonuçların istatistiksel analizinde ANOVA ($p<0.05$) kullanıldı. **Bulgular:** Burada belirtilen koşullar altında, deneysel model (sıçan tibia'sında titanyum implant sonrası kemik iyileşmesi) kullanılarak dışarıdan (eksternal) pozitif ve/veya negatif elektriksel alan uygulamasının plasebo uygulanan hayvanlara göre peri-implant lamelar kemik hacmini arttırdığı görüldü. **Sonuç:** Pozitif/negatif elektriksel alan yaratan bir cihazın kullanılması kemik örülmesine neden olmuştur.

ANAHTAR KELÝMELER: Osseointegrasyon, elektriksel alan, osteogenez, metal implant. F6–8

JAPANESE / 日本語

インプラント周辺骨形成におけるElectrical Field効果：組織学ならびに組織形態測定学研究

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研究概要： **目的：** 損傷に対して起る組織反応は、外傷治癒で発生するように、合理的に組織化された生物学的事象である。臨床と実験両分野の研究で、外部から与える電気刺激が組織修正増進を促すことが既に明らかになっている。骨折治癒をはじめ骨切手術、仮骨延長手術やインプラントの実験モデルで in situ（本来の場所における）電気刺激効果は研究されてる。当研究はラットの頸骨にメタル薄板インプラントを挿入した実験モデルを使い、インプラント周辺の外傷治癒に対するElectrical Field効果の評価を目的とした。

研究素材と方法： 約100gの体重で雄のWistar種ラット40匹を使用し、チタン製薄板インプラント（6×1×0.1 mm）（Implant Vel, アルゼンチン、ブエノスアイレス）を孔を通して挿入し、medullary compartment(骨髄隔室部)へ埋入した。埋入後は慎重に組織を適切な位置に戻し縫合し、Electrical Field発生装置（ECCEL, DAM, アルゼンチン）を使い電気刺激を通電した。 ラット両後足の皮膚にはElectrical Fieldプレートを装置した。シャムグループでは実験動物をElectrical Field発生装置のプレートに接続せずに同じ処置を受けさせた。すべての実験動物はインプラント術後15日目にエチルエーテル過量投与で死亡させた。頸骨を切除し20%のホルマリンに定着し、レントゲン撮影しメタクリル酸メチルに封埋処理をした。粉砕したセクションは1%の青色トルイジンで染色し、次のパラメーターを評価した：インプラント周辺骨量とオッセオインテグレーションの割合。ANOVA ($p<0.05$)を使用した統計的分析結果。

結果： 使用した実験モデル（ラット頸部チタンインプラント術後骨組織治癒）を本文既述のコンディションのもとで対象のシャムグループ実験動物と比較すると、外部からポジティブおよび、あるいはネガティブのElectrical Fieldを応用したグループではインプラント周辺にlamellar bone（層状骨）形成が増進したことが判明した。

結論： ポジティブ/ネガティブElectrical Field発生装置を使用することで、結果的に woven bone（繊維骨梁）を形成した。

キーワード： オッセオインテグレーション, Electrical Field, 骨形成, メタルインプラント

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CHINESE / 中国語

電場對植體周圍骨生成的影響：組織學與組織形態學研究

作者：Graciela Ana Giannunzio, DDS；Rodolfo Carlos Speerli, EEE 及 María Beatriz Guglielmotti, DDS PhD

摘要：目的：傷口癒合期間組織對受傷的反應是非常有系統的生物事件。臨床與實驗研究均已發現，外接電刺激能促進組織修復。針對原位電刺激的影響，已經有針對斷裂治療、骨片切取術、骨生成牽引術和植體的實驗模式研究。

本研究的目的是利用與將金屬板狀植體植入老鼠脛骨相關的實驗模式，評估電場對植體周圍傷口癒合的影響。**資料與方法：**使用 40 隻體重約 100 公克的 Wistar 雄鼠。將一個鈦金屬板狀植體 (6x1x0.1mm) (Implant Vel, Buenos Aires, Argentina) 透過傷口插入並植入髓質間室，然後小心將組織放回並縫合。利用電場產生器 (ECCEL, DAM, Argentina) 傳導電刺激。將電場板放入兩個後腿的皮膚上。假治療組：動物使用相同的手術，但電場板不連接電場產生器。在植入完成後 15 天以過量乙醚殺死所有動物，進行脛骨切除、以 20% 福馬林固定、X 光攝影與處理後，再用甲基丙烯酸甲酯包埋。磨片以 1% 甲苯胺藍染色。評估下列參數：植體周圍骨量與骨整合百分比。利用 ANOVA 進行結果的統計分析 ($p<0.05$)。

結果：與假治療動物比較，利用實驗模式（老鼠脛骨後鈦金屬植體骨治療）來應用外接陽極及／或陰極電場以及在此處載明的條件之下，發現能增進植體周圍板狀骨量。

結論：使用產生陽極／陰極電場的裝置導致出現織網骨。

關鍵字：骨整合、電場、骨生成、金屬植體

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KOREAN / 한국어

임플란트 주위 골생성에 미치는 전기장 효과: 조직적 연구 및 조직계측학적 연구

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초록: 목적: 조직은 상처를 치유하는 동안 손상된 곳에 반응하며, 이는 잘 조직화된 생화학적 사례이다. 임상 및 시험 연구 모두에서 외부 전기 자극이 조직 회복을 강화함을 보여주었다. 정위치(in situ)의 전기적 자극 효과는 골절 치유와 골절제술, 골생성 당김 및 임플란트의 시험 모델로 연구되었다.

본 시험의 목적은 생쥐 경골에 금속 박판의 임플란트를 안착하는 시험 모델을 이용하여, 임플란트 주위 상처 치유에 미치는 전기장의 효과를 평가하기 위함이었다.

재료 및 방법: 대략 100g의 체중이 나가는 위스타(Wistar) 생쥐 41마리가 사용되었다. 구멍을 통해 티타늄 박판의 임플란트(6x1x0.1mm) (아르헨티나, 부에노스 아이레스, 임플란트 Vel)를 삽입하여 수질 구역에 안착하였다. 그 후 조직은 다시 자리를 잡고 조심스럽게 봉합되었다. 전기적으로 자극하기 위해 전기장 발생장치(아르헨티나, DAM, ECCEL)가 사용되었다. 전기장 판은 양쪽 뒷다리의 피부에 부착되었다. 샴(Sham) 군: 동물들을 전기장 발생장치의 판에 연결하지 않은 채 동일한 절차를 거쳤다. 모든 동물들은 이식 후 15일에 과다복용으로 사망하였다. 경골은 절제되어, 20% 포르말린에 고정되어, 방사선촬영과 메틸 메타아크릴레이트(methyl methacrylate)에 임베딩하기 위해 처리되었다. 그라운드 섹션(ground section)은 1% 톨루이딘 블루(toluidine blue)로 염색하였다. 다음의 매개변수들, 즉, 임플란트 주위 골량(BV), 골융합 비율이 평가되었다. 결과의 통계적 분석은ANOVA ($p<0.05$)를 이용하여 실시되었다.

결과: 샴(sham) 치료 동물들과 비교하여, 임플란트 주위의 층판 골량을 강화하기 위해서 시험용 모델(생쥐 경골에 티타늄 임플란트 후 골 치유)을 이용하여 외부의 양극 및/또는 음극 전기장에 적용하는 방법이 이러한 사실에 입각한 상황 하에 발견되었다.

결론: 양극/음극의 전기장을 발생하는 장치를 사용함으로써 무층뼈가 존재하게 되었다.

핵심 단어: 골융합, 골생성, 금속 임플란트

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