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EFFECTS OF SEVEN DAYS OF CONTINUOUS CAPACITIVE ELECTRICAL STIMULATION ON BONE GROWTH AROUND TITANIUM IMPLANTS IN THE RAT TIBIA

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Abstract

The effect of 7 days of continuous capacitive electrical stimulation on bone growth around titanium implants placed in the tibiae of adult male rats, was evaluated in this study. The animals were 'hooked-up' to an external power supply, emitting a symmetrical sinusoidal wave form with an amplitude of 2.0 volt p-p and a frequency of 60 kHz. The current values tested were 20μA, 30μA and 60μA. In surgery I, a head cap was affixed to the animal's skull with stainless-steel screws and an acrylic resin. The head cap was connected to the external power supply by an electrical stimulation wire, encased in a stainless-steel spring. Two electrical leads were subcutaneously tunneled from the head cap to the future tibial implant site, and sutured to the soft tissues. After a one week acclimatization period, a second surgical procedure, surgery II, involved the placement of titanium implants proximal to the tibial tuberosity in each tibiae. Electrodes were then sutured one on either side of the implant, thus 'cuffing' the implant, and the current was then applied to one implant site with the other acting as a control.

After seven days of continuous capacitive electrical stimulation, a biomechanical pull-out test was carried out to measure the force in Newtons (N) of implant extraction. The Student's t-test showed no significant difference between the control (average peak tensile force ± SEM; 47.5 ± 4.25) from that of the stimulated group (38 ± 5.295) for a current of 60μA ($p \leq 0.15$; n=4). A statistically significant difference was seen at a 30μA current level with an average peak tensile force ± SEM for the experimental group (n=13) of 38.38 ± 1.69 versus 31.59 ± 1.72 for the control (n=17). At 20μA there was also a statistically significant difference ($p \leq 0.025$) with 35 ± 3.28 for the experimental group (n=7) versus 26.56 ± 2.097 for the control (n=9), indicating that the force required to extract the titanium implant from rodent tibiae was greater when a capacitive electrical signal of 20 or 30μA was applied compared to sham-stimulated controls.

Résumé

Cette recherche a évalué l'effet d'une stimulation électrique capacitative continue de sept jours sur la croissance osseuse autour d'implants en titane dans les tibias de rats mâles adultes. Les animaux étaient "attelés" à une alimentation électrique externe, qui émettait une onde sinusoïdale symétrique d'une amplitude de 2 volts p-p, à une fréquence de 60 kHz. Les courants testés dans le cadre de cette étude étaient 20μA, 30μA et 60μA. Dans le cadre de la chirurgie I, un "chapeau" a été fixé sur le crâne des animaux au moyen de vis en acier inoxydable et d'une résine acrylique. Le chapeau a ensuite été relié à une alimentation électrique externe au moyen d'un fil de stimulation électrique enchâssé dans un ressort en acier inoxydable. Deux fils électriques ont été reliés, par voie sous-cutanée, du chapeau au futur site d'implantation des implants et suturés aux tissus mous. Après une semaine d'acclimatation, une deuxième procédure chirurgicale (chirurgie II) a permis d'installer les implants en titane sur le bord proximal du tubercule tibial de chaque tibia. Les électrodes ont été suturées de chaque côté de l'implant, 'coiffant' ce dernier, et le courant a ensuite été appliqué sur un implant, l'autre servant de témoin.

Après sept jours de stimulation électrique continue, un test d'arrachement biomécanique a été entrepris pour mesurer la force en Newtons (N) de l'extraction de l'implant. Le t-test de *Student* n'a révélé aucune différence significative entre le groupe témoin (47,5 ± 4,25) et le groupe stimulé (38 ± 5,295), à un courant de 60μA ($p \leq 0,15$; n=4). Une différence significative sur le plan statistique a été observée à un courant de 30μA, moyennant un effort de tension maximal moyen ± ET pour le groupe expérimental (n=13) de 38,38 ± 1,69, contre 31,59 ± 1,72 pour le groupe témoin (n=17). À 20μA, on observe également une différence significative sur le plan statistique ($p \leq 0,025$) avec 35 ± 3,28 pour le groupe expérimental (n=7), contre 26,56 ± 2,097 pour le groupe témoin (n=9), ce qui révèle que l'effort exigé pour extraire l'implant de titane du tibia du rongeur est supérieur lorsqu'on applique un signal électrique capacitatif de 20 ou 30μA, par rapport aux témoins stimulés de manière fictive.

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Table of Contents

Introduction

Endosseous dental implants provide a treatment modality for the replacement of lost dentition. Prosthetic rehabilitation of edentulism is accomplished by the surgical insertion of a relatively inert implant made of titanium into the soft and hard tissue of the jaws. This type of implant provides support and also acts as a mechanism of attachment for a dental prosthetic device.

Healing Responses

A metallic foreign object, such as an implant, when inserted into the body, will elicit an inflammatory response, and cause trauma to the surrounding area. This will, in turn, trigger a healing response. If the implant material is inert, the healing response would follow the same process as a fracture healing and can be broken down into three fundamental phases: inflammation, proliferation and maturation (Albrektsson, 1989a). In the first phase, a fracture leads to the formation of an initial blood clot, and a thin layer of bone surrounding the fracture becomes necrotic. During the second phase, an inflammatory response occurs which includes the proliferation and differentiation of phagocytes and other inflammatory cells. Undifferentiated mesenchymal-like cells from the adjacent periosteum and endosteum will undergo mitosis. At this stage, fracture healing involves the formation of a bony callus that bridges the fracture gap from the periosteal and endosteal surfaces. In animals, a lattice of spongy bone is

formed in about two weeks and in humans it takes about six weeks (Gross, 1988). Vascular invasion of the spongy bone occurs in the third phase providing an oxygenated environment allowing the bone matrix to mineralize and remodel into mature lamellar bone tissue. A lack of oxygen will cause the production of fibrous and cartilaginous tissues. Gradually the callus formed becomes stiffened as the weak woven bone is replaced by stronger lamellar bone matrix, a process called lamellar compaction, completely heals the fracture gap (Behari, 1991). In animal tibiae the time period for the completion of this process is about six weeks, and in human long bone, the process is eighteen weeks (Fleisch, 1993).

Tissue Reaction to Metals

The formation of corrosive products occurs when biological tissues interact with inorganic metals such as stainless-steel. A passive reaction may also take place where metals with high surface energy interact with oxygen to produce a surface oxide layer on the metal. Several different types of metals, such as aluminum, iron and nickel, undergo such a reaction, however, these metals are not beneficial as long-term biomaterials because corrosion of the metals will release metallic ions into the surrounding tissues resulting in adverse local and systemic responses (Worthington, 1988; 1994). The use of titanium as an implant has, on the other hand, been found to produce a good long-term result with respect to the problems alluded to above, as it provides a surface suitable for tissue integration (Hanawa, 1991).

Titanium observation chambers placed in the tibia of rabbits revealed that

bone adhered to it with great "tenacity", since it could not be removed from the surrounding bone once it had healed (Brånemark 1985). The titanium had become completely incorporated in the bone and the mineralized tissues became congruent with the titanium surface. This form of attachment mechanism has been termed, "osseointegration". Ossointegration is a biological concept referring to a direct contact, as an anchoring mechanism, demonstrated at the resolution of the light microscope, between living bone and the surface of an implant (Brånemark 1985). To achieve successful osseointegration, demonstrated by the development of mature differentiated tissue, a proper surgical technique with minimal heat generation using copious saline irrigation, is required (Kasemo, 1983; Worthington, 1994; Albrektsson, 1989a).

Properties of Titanium

Titanium is lightweight and ductile. It can easily be fabricated into useful shapes while still retaining its strength (Worthington, 1994). The special characteristics of titanium, in particular its resistance to corrosion and its biocompatibility, provide the necessary conditions for osseointegration (Hall, 1992). In air, titanium spontaneously forms an oxide surface of three to five nanometers thick at room temperature and has basic chemical properties so that it oxidizes under normal *in vivo* physiologic conditions. When titanium is exposed to blood, water, mineral ions and plasma fluids, it spontaneously forms complex titanium-phosphate and calcium-containing hydroxyl groups on the oxide surface. It was found that the low pH (pH=5.2) at the implant site may

accelerate the formation of calcium-phosphate apatite on the surface of titanium (Hanawa 1991). This oxide surface therefore creates an adaptive interfacial region instead of just a distinct demarcation between the implant and the tissues of the body. As a protective mechanism from the potentially harmful metallic ions, direct contact between the implant and the surrounding tissue is prevented, since it is the surface oxide of the implant that contacts the tissue. It is the reactive nature of this oxide surface and its ability to spontaneously form calcium phosphate minerals that makes titanium biocompatible (Brånemark, 1985). It allows for a normal cellular and tissue healing response to take place on the metal such that the plasma proteins covering the surface of the implant are eventually replaced with deposited mineralizing bone matrix (Hanawa, 1991). The metallic implant must therefore present a mechanically and chemically clean oxidized surface in order for a stable and direct connection between living bone and titanium to transpire.

Titanium Implant-Bone Interface

Scanning electron microscopy and transmission electron microscopy has shown that the interface between the titanium implant and the bone is free of fibrous tissue. There is, however, always a border zone consisting of a proteoglycan layer, a few hundred Ångstroms thick (105Å) (Albrektsson, 1982). This layer is important for adhesion between cells, fibrils and other structures (Albrektsson, 1983b; Hansson, 1983). Any interference of bone integration such as; frictional heat generated at surgery, status of implant bed, implant surface

condition, or loading conditions, may alter the interface zone. This then causes a connective tissue layer of a few Ångstroms thick (106Å) and a layer of disordered bone, characterized by a random organization of its collagen fibers, to develop (104Å thick) (Albrektsson, 1981; Eriksson, 1986). The greater the gap between Haversian bone and the implant, the increase in risk for implant loss with time. The conditions for implantation are therefore a critical factor influencing osseointegration (Albrektsson, 1989a; Kasemo, 1983; Worthington, 1994).

Biological Effects of Titanium Implants Placed into Bone

Evidence of successful dental implantation has been shown in animal models and in clinical studies. Titanium dental implants inserted in the monkey mandible have demonstrated, histologically and microradiographically, bone tissue in immediate apposition to the titanium surface, with no discernible fibrous tissues in the interface (Young, 1979a, b). At the level of the electron microscope, threaded implants placed in the sheep tibia have shown a direct bone-to-implant contact (Rüedi, 1975). In both cases, glycosaminoglycans are seen to fill the space between the implant and the nearest collagen fibrils. These complexes of glycosaminoglycans play a role in mediating adhesion of the mineralizing extracellular matrix to the implant surface.

Clinical reports have documented a direct bone anchorage of titanium implants (Ledermann, 1979; 1982). Four thousand threaded pure titanium implants inserted into mandibular, maxillary, tibial, temporal and iliac bone sites,

were examined. After ninety months, the interface zone between bone and implant was observed using radiology, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and histology. These types of observations demonstrated a close spatial relationship between titanium implant and bone. After ten to fifteen years of placing implants, clinical success rates between 96% and 99% have been demonstrated (Brånemark, 1977).

Factors Influencing the Strength Required to Remove an Implant

Bone Quantity

The degree of contact an integrated implant has with bone is correlated with the relative strength that is required to remove it. The strength of the bone around the implant, which can be measured by a mechanical pull-out test, reflects the amount of bone observed in the tissue threads at a histological level. The unscrewing of titanium implants in the tibia and femur of rabbits after various time periods up to twelve months, demonstrated an increased resistance over time, and histomorphometrically demonstrated more contact between bone and the implant surface (Albrektsson, 1990). Porous calcium aluminate implants placed in a rabbit femur showed extensive ingrowth of bone into implant pores (Park, 1975). In the follow up study, it was demonstrated that the amount of bone which grew into the pores of the implant was directly proportional to the interfacial strength measured by a pull-out test (Park, 1975).

Bone Quality

Another factor that may influence a time-dependent increase in removal torque, or pull-out values of an implant, is a change in the structure of bone adjacent to the implant. After an implant was inserted into the tibia of rabbits, a poorly organized woven bone of weak strength was formed around it, and six weeks later was replaced by lamellar bone of adequate strength (Roberts, 1984). Primary, or woven bone, is characterized by random disposition of fine collagen fibers, and mature lamellar bone contains an organized disposition of collagen, changing the type of bone present. An implants' surface does not have to be completely covered by bone to achieve successful osseointegration; the distribution and quality of bone manifested is just as important as the actual amount of bone (Johansson, 1987). A study performed a removal torque test six weeks after the insertion of titanium implants into rabbit cortical bone of the tibiae metaphysis, and into cancellous bone of the knee joint (Sennerby, 1992). It was found that less torque was required to remove the implant from the cancellous bone, even though more of this bone was found in the threads of the implants compared to the amount of cortical bone. The type of bone differs in these two areas, influencing the torque values. The cortical bone is of higher density, compact lamellar bone, so the tighter packing of collagen fibers and the increase in hydroxyapatite minerals restricts the movement of the implant when being unscrewed by the removal torque test. It was found that between three and twelve months post-implantation, bone contact with the titanium surface did

not increase, but torque values did, thereby reflecting change in bone type.

These findings are significant in a clinical situation, since there seems to be a correlation between bone type and the success rate of an implant. The success rate in the maxilla is lower than that for the mandible, since it is thinner and composed mostly of cancellous and less compact bone (Friberg, 1991). Therefore, not only the amount of bone is important for a successful implantation, but also the type of bone; woven vs. lamellar.

Length of Time for Osseointegration

Osseointegration between bone and an implant surface takes about six weeks in rat tibiae (Roberts, 1984) and six to nine months in human long bones (Leroy, 1987). It would, therefore, be beneficial for clinicians and patients if this time period could be shortened.

Bone is not highly innervated with electrically excitable cells, but according to Wolff's law bone is a source of endogenous electrical current when it is mechanically stressed or injured (Brighton, 1975a). The use of energy sources, such as electric currents, could therefore enhance or even hasten bone formation around dental implants, particularly in areas of poor bone quality.

Wolff's Law

Bone, as an organ, responds biologically to mechanical demands placed on it. "Wolff's Law" relates the dynamic structure of bone and its ability to respond to the forces it is subjected to. Simply stated, tension leads to increased bone formation and pressure leads to bone resorption. It describes

the growth of bone responding to mechanical stress by producing an anatomical structure best able to resist the applied stress, so as to neutralize the effect of the stimulus; a negative-feedback system (Behari, 1991). In a patient who has suffered from a fracture of a weight-bearing long bone that healed with an angulation, each step taken would result in a bending stress with tension on the concave side and compression on the convex side. A repeating mechanical stress, such as this, results in an increased bone growth on the concave side and bone resorption on the convex side. Consequently, remodeling takes place allowing the bone to return to its normal shape (Pienkowski, 1984).

Only a small proportion of bone is composed of cells, approximately 10% of the total mass. Yet it is these few cells that control the maintenance of the physical structure of bone. The exact mechanism of the cellular control is unknown, but the cells are sensitive and receptive to some form of signal, either chemical or physical, that is transduced from the physical pressure placed on the bone (Brighton, 1977). Bone itself may therefore have the property of transduction in its structure.

Characteristics of Bone

Bone is a specialized connective tissue composed of cells and an organic extracellular matrix. The cells found in bone are of three different types; osteoblasts, osteocytes, and osteoclasts. Osteoblasts synthesize and secrete the organic components of the matrix and will eventually become trapped within bone in lacunae and become osteocytes, which are then responsible for the

maintenance of bone matrix. Osteoclasts are multinucleated cells that create an environment that resorbs bone and is therefore associated with the continuous remodeling of bone (Ross, 1995). The organic matrix contains about 95% type I collagen fibrils, long-chained fibrous proteins, and about 5% amorphous ground substance, which contains glycosaminoglycans associated with proteins (Bergman, 1996). Mineral salts are deposited in a very precise fashion onto the collagen fibers and surrounding ground substance. The mineral is composed of microcrystalline calcium phosphate in the form of plate-like crystals of hydroxyapatite with the composition [$Ca_{10}(PO_4)_6(OH)_2$].

The organic matter of bone, the bone matrix, forms about ⅓ of the weight of bone tissue, and the inorganic mineral salts form the remaining ⅔. It is the combination of these two materials that gives bone tissue its characteristic hardness, resistance and its unique mechanical properties (Junqueira, 1992). Each can be isolated from the other. The organic part may be obtained alone by immersing the bone for some time in dilute mineral acid, after which the bone comes out exactly the same shape as before, except it is soft and perfectly flexible. The inorganic part may be isolated from the collagen by calcination, still preserving the shape; however, the bone becomes brittle and will crumble easily when handled. It is the inorganic matter that bestows bone its hardness and rigidity, and the organic matrix its tenacity (Ross, 1995).

Endogenous Electrical Activity in Bone

1. Strain Related Potentials

Piezoelectricity

The application of mechanical stress on inorganic crystalline materials with a nonsymmetrical lattice, results in the displacement of charges within the lattice that can be sensed as a pulse of electricity on the exterior surface of the crystal. If the stress is maintained, the surface potential remains constant, with little decay. When the applied mechanical stress is released, a pulse equal in magnitude to the original, but opposite in polarity is produced. This property is called piezoelectricity, and was found to exist in bone and was postulated to be the stimulus that caused a cellular response that lead to bone growth proposed by Wolff's law (Hall, 1991). Electric potential differences can be measured between one bone surface to another, when bone is deformed, indicating that electrical charges become displaced because of the stresses applied (Borgens, 1984). This was demonstrated with randomly oriented molecular ions and dipoles (Pollack, 1984). In response to an applied stress the charges become evenly oriented to exhibit a net negative charge on one surface and a net positive charge for the opposite surface (Figure 1).

Collagen-Hydroxyapatite Junction theory

Mechanical deformations therefore produce an electrical field in bone and are generally termed, strain-related potentials (SRPs). Initially, it was thought that SRPs were produced by a piezoelectric effect, but Marino and Becker

(1971) identified the organic material of dry bone, mostly collagen, to be the source of these SRPs. From this they proposed the collagen-hydroxyapatite junction theory. Both hydroxyapatite and collagen each have some semiconductive properties, with collagen appearing to be an "N" type material and apatite a "P" type. The junction between the two in bone forms pressure-sensitive PN junctions and is a source of SRPs. A mechanical stress applied to these numerous apatite-collagen PN junctions in the bone matrix, generates an electrical signal, which may then cause new collagen fibers to be oriented in such a way as to resist the applied stress and to structurally change bone. (Vanore, 1992).

Collagen theory

Another proposed theory as to what produces SRPs, is the collagen theory (Bassett, 1968). The molecular ultrastructure of collagen becomes altered in the presence of stress, resulting in a separation of positive and negative charges producing a polarization effect, the flow of electricity (Shandler, 1979; Bassett, 1968).

The charge distribution on the surface of stressed femurs have been measured (McElhaney, 1968). SRPs in bending bone have negative polarities on the concave side versus a positive polarity on the convex side. According to Wolff's law, a net osteogenesis occurs on the concave side and a net bone resorption on the convex side. This suggests that the sign of the potential influences the cellular response, negative potentials stimulating osteoblasts and

osteocytes, while positive potentials stimulate osteoclasts to destroy bone or a lack of stimulation to not produce any bone on the convex side (Shandler,1979).

A constant direct current applied to isolated rabbit osteoclasts and rat osteoblasts demonstrated an effect of electric current on cell migration. The osteoclasts migrated rapidly toward the positive electrode, and the osteoblasts migrated to the negative electrode (Ferrier, 1986).

Streaming Potentials

SRPs have been found in moist or living bone, and are the result of "streaming potentials" rather than pieozoelectric polarization found in dry bone (Gross, 1982). A streaming potential is generated by the flow of a liquid across charged surfaces. Bone matrix may be considered as an amphoteric ion exchanger with both positive and negative fixed charges. The crystallographic structure of apatite contains exposed atoms of oxygen and calcium on the crystals' surface. The negative oxygen atoms will attract cations from the neighboring fluid, and the positive calcium atoms will attract anions. An electrostatic layer, called the "double layer", is thus formed on the surface of bone containing unequal numbers of positive and negative charges. A portion of this "double layer" will be tightly bound to the solid surface. The other portion contains excess ions that can move freely, and is called the "diffuse layer". The boundary between these two layers, the stationary fluid and the moving fluid, is called the "slip plane". The flow of liquid along this double layer carries with it the freely moveable diffuse layer. The movement of this charged liquid produces

an electric current resulting in a "streaming potential", which may then stimulate bone production from osteoblasts (Burny, 1978; Eriksson, 1974; Pollack, 1984; Vanore, 1992).

2. Bioelectric potentials

Another source of electrical potentials can be seen in resting, non-stressed bones. Bioelectric potentials are generated by the activity of bone cells and represent the metabolic state of the tissue. Areas with high cellular turnover and increased blood supply are electronegative, whereas areas with less metabolically active cells are electropositive (Brighton, 1975a; 1977). *In vivo,* a constant potential difference exists between the periosteum and the endosteum, with a negative potential always at the endosteum and a positive potential at the periosteum. When fractured, the bone will generally become more electronegative, with the greatest amount being at the fracture site (Figure 2). Bone forming cells are thus stimulated by these bioelectric potentials generated at the fracture site (Digby, 1966).

The summation of all these types of electrical activities represents the naturally occurring endogenous electrical signals found in bone. These signals are important because of the possible role they may play in transducing mechanical energy into biological responses. An applied extrinsic electrical stimulation functions as an imitator of the endogenous electrogenerative properties of living bone increasing its capacity to heal (Pienkowski,1994).

Methods of Electrical Stimulation

There are several methods that have been developed for the application of electric energy to bone. Currently the three main methods of electrical stimulation are: direct current, inductive coupling (pulsed electromagnetic field) and capacitive coupling. All three share the fact that they can stimulate bone healing, but they differ in the method of application (Bassett, 1974a;1974b; Brighton, 1981; Friedenberg, 1970).

Direct Current

Direct Current requires the surgical implantation of electrodes directly into the bone. The cathode or negative electrode is placed into the medullary canal, and the anode or positive electrode is situated in the neighboring soft tissue (Lavine, 1969). By virtue of it requiring an operative procedure, this type of stimulation runs the risk of causing infections and a possible toxic build up of ions at the cathode or anode by electrolysis. During the treatment period, breakage or displacement of the electrodes may occur. The electrodes are connected to an external power unit that generates a high local potential gradient that requires a precise placement for optimum effect. The high current utilized in this method can also cause tissue necrosis at the anode, and bone formation at the cathode (Vanore, 1992; Kubota, 1995). The fact that the whole unit is implanted renders it a portable system and therefore does not require significant patient or animal compliance.

Inductive Coupling or Pulsed Electromagnetic Field

The attempt to create a method that did not require an invasive surgical procedure, resulted in a method termed inductive coupling or pulsed electromagnetic field. In a clinical situation, specific coils are mounted on the external surface of the skin overlying the area of bone that required stimulation (Bassett, 1974b). These coils are termed Helmholz coils and are tightly wound wire around a circular bar that is attached to a large sized power source. The large size of this generator is due to the fact that it consumes a lot of electrical power since it has to produce a specific waveform parameter. The power source generates primarily a magnetic field, and a secondary electric field in the tissues between the coils (Leroy, 1987). The power unit emits a low frequency signal, which allows minimal tissue penetration, so the placement and orientation of the magnetic coils is extremely critical for an optimal effect. This technique is non-portable and requires full patient or animal compliance

Capacitive Coupling

Capacitive coupling has been developed as a treatment modality with numerous advantages over direct current or inductive coupling. In a typical clinical application, externally placed capacitor plates are located on the skin overlying bone and attached to a voltage source (Brighton, 1985b). This method therefore is non-invasive as it does not require a surgical procedure. The primary effect of capacitive coupling is to induce an electrical field in bone, and a minor magnetic field over a larger area than for the other methods (Kubota,

1995). The signal applied to the electrodes is of high frequency and low voltage which allows a more effective penetration of tissues. The placement of the electrodes is therefore not as critical (Brighton 1985a). The low voltage causes minimal tissue necrosis. Electrolysis at the electrodes does not occur. The stimulus signal allows for a small, light weight, portable stimulation device. The only minor disadvantage of this method is that it requires patient compliance as the stimulator uses a 9V battery that has to be replaced daily (Leroy, 1987).

Electrical parameters

The three stimulation systems described above employ a variety of wave forms from simple sinusoidal to complex waves. It is, however, not yet known what constitutes an effective wave form for bone healing to occur. The electrical parameters typically manipulated are current, voltage, frequency and current density.

Current

"Electric current is defined as the rate of flow of charge through some region of space." (Serway, 1990). The SI (Systéme Internationale) unit of current is the Ampere (A). One amperage of current is equivalent to one coulomb of charge passing through a surface in one second (1A=C/S). In practice, smaller units of current are often used, such as the microampere ($1\mu A=10^{-6}$ A).

Voltage

The SI units of voltage is Volts (V). One volt is the electric potential at a

point in an electric field if one joule (J) of work is required to move one coulomb (C) of charge from infinity to that point (Serway, 1990). (1V= 1J/C) In practice, just a few volts are used.

Frequency

The frequency of a symmetrical sine wave is defined as the number of crests and troughs, or complete cycles, that pass a given point in the medium per unit time (usually 1s) (Serway, 1990). (Frequency = Cycles/Time). The SI unit for frequency is Hertz (HZ). In practice, larger units of frequency are used, such as kilohertz ($1kHz = 10^3Hz$).

Current Density

Current density (J) is defined to be the current per unit area (Serway, 1990). (J=I/A). The SI units of current density are A/m^2. In practice, between $0.01\text{-}1mA/mm^2$ are used.

Stimulus Characteristics

These signals emitted by the stimulation devices to produce an electric field that is postulated to enhance bone formation. These parameters vary depending on the impedance and geometry of the tissues as well as the location of the electrical source.

With inserted electrodes, the electric potential and electrode material influence the electrochemical reactions that occur on the bone and surrounding tissues. A stainless-steel electrode emitting a current larger than $10\mu A$ causes anode necrosis by toxic reaction products, and currents exceeding $100\mu A$

results in tissue destruction around the cathode (Brighton, 1975; Friedenberg, 1970; Hassler,1977). The electrode position can effect the current density required for stimulation (Behari, 1991). With surface electrical stimulation the effect of the electric current (the current density) diminishes with depth of tissue. The highest success have been with current densities in the range of 0.01-1mA/mm^2 (Spadaro, 1982a).

Application of Electrical Stimulation

Animal Studies

Animal models were developed in order to study various parameters and methods of applying electricity and have confirmed the phenomenon of electrically induced osteogenesis (Baranowski, 1983). Direct current, inductive coupling, and capacitive coupling techniques have been examined in many different model systems.

Application of Direct Current

Fractured bones stimulated with direct current have exhibited enhanced cell proliferation, differentiation and calcification in the repair zone (Connolly, 1974). Autoradiographic studies using ^{3}H-thymidine carried out on injured rat femurs stimulated by direct current demonstrated the proliferation of osteoprogenitor cells, as well as the differentiation of these cells into osteoblasts and then into osteocytes (Ohashi, 1982).

The administration of direct current to the mandible of mature Beagle dogs showed an increase in osteogenesis at the cathode (negative electrode)

when compared to sham-stimulated controls (Zengo, 1976).

Direct current applied to a fracture site was found, via macroscopic, radiologic, and histopathologic methods, to accelerate fracture healing in the rat tibia (Zorlu, 1998). A statistical difference between the sixteen stimulated rat tibiae and the sixteen control tibiae was observed ($p<0.05$). In a study where mongrel dogs underwent direct current electrical stimulation on lumbar spinal fusions, all stimulated facet joints showed solid bony fusion after twelve weeks, whereas, none of the control showed bridging of the fusion site (Kahanovitz, 1990).

Application of Inductive Coupling or Pulsed Electromagnetic Field

Inductive coupling or pulsed electromagnetic field stimulation has also demonstrated increased calcification and enhanced radiographic and mechanical measures of healed bone (Bassett, 1974b; 1979). Pulsed electromagnetic field stimulation has indicated in histomorphometric studies a promotion of endochondral calcification and maturation of bone trabeculae (Aaron, 1989).

Non-invasive electromagnetic stimulation has been applied to sites of alveolar resorption after the extraction of premolar teeth in Beagles. The resorption of the alveolar ridge was reduced by up to 50 percent (Vingerling, 1979). In another study where a pair of Helmholz coils placed medially and laterally to the hind limb of a dog with an osteotomized fibula, were subjected to an alternating current for 28 days, the osteotomized fibula was significantly

stiffer than that of the control (Bassett, 1974a).

Application of Capacitive Coupling

Capacitive Coupling stimulation was reported to improve mechanical aspects of experimental fractures and healing osteotomies, as well as reverse an induced osteoporosis (Brighton, 1981). A dose response study on rabbits was developed where the current, voltage, and signal frequency's were varied (Brighton, 1985c). After 14 days of constant stimulation, researchers examined the strength of the healed rabbit fibula fracture by a 3 Point-Bending test. A load applied onto the fibula by this test measured the stiffness of the healed fracture. X-rays and histological analysis were also done. All three methods showed that in the midphase of the healing cycle, the capacitively coupled electrical field stimulated fracture healing in the rabbit fibula in a dose response manner, the best dose being 250A- 220mV-60kHz.

Castration induced osteoporosis in rat vertebra was assessed by a capacitively coupled electrical signal which reversed the castration induced osteoporosis (Brighton, 1989). The electrodes were placed paraspinally on the skin, and emitted a signal for six and eight weeks. The vertebrae were measured by taking the dry and ash weights per unit volume. The results at eight weeks denoted that a 60kHz, 100 A signal significantly reversed the induced osteoporosis in the rat lumbar vertebrae and restored bone mass per unit of volume. In a related study that produced disuse osteoporosis in a rat tibia via sciatic denervation, an applied capacitively coupled electrical signal of

60kHz, 0.5volt peak-to-peak was also capable of reversing an induced disuse osteoporosis (Brighton, 1985a) .

Clinical Studies

Application of Direct Current

Twenty microamperes of constant direct current were applied for twelve weeks on 178 non-unions of various long bone types. A non-union is a fracture that has either failed to unite after 9 months, or radiographic evidence shows no further healing over 3 months (Vanore, 1992). Results indicated that 83.7% achieved solid bone union. In an additional study of eighty non-unions, 72.5% achieved bony healing (Brighton, 1981). Twenty-four acute tibial fractures treated with pulsating asymmetric direct current reported a 30 percent faster healing rate (Jørgensen, 1972). Invasive direct current applied to delayed unions and non-unions of long bone fractures showed union rates of approximately 70 to 90 percent (Black, 1987). Low intensity direct current stimulation, in the nanoampere range, applied to the long bones of patients with non-unions or pseudoarthroses resulted in a 77 per cent success rate (Becker, 1977). Stimulation of a human congenital pseudoarthroses of the tibia by direct electric current formed new bone in the defect area as detected by X-ray, and LM and EM leading to bony union (Lavine, 1972).

Application of Inductive Coupling or Pulsed Electromagnetic Field

Low-frequency pulsing electromagnetic fields used on 32 patients with femoral intertrochanteric osteotomy in the hip showed a statistically significant

difference between for the stimulated patients compared to the controls ($p \leq 0.01$) when roentgenographic evaluations and callus density measurements were performed (Borsalino, 1988).

A double-blind study with a placebo treated group and an inductive coupling stimulated group of tibial fractures demonstrated bone union after 12 weeks of treatment in 10 of 20 stimulated fractures compared with 2 of 25 in the control group ($p \leq 0.002$) (Sharrard, 1990).

Of 1007 patients who received pulsing electromagnetic field treatment on non-union fractures, 82 percent of tibial fractures healed successfully (Bassett, 1982a). Fractures of the femur, humerus, radius-ulna, and scapula had a lesser success rate.

Application of Capacitive Coupling

Capacitive coupling has been used in a clinical situation, to treat non-union fractures (Brighton, 1984a). Capacitor plates were placed on the skin of a plaster casted human tibia that contained an established non-union fracture (Brighton,1984a). An electrode gel was applied to the capacitor plates to lower the electrode-to-skin impedance. Capacitively coupled electrical stimulation was applied for 24 weeks with a 5V peak-to-peak ,8mA and a 60kHz symmetrical sine wave signal. After 12 weeks, the non-union began to heal, as assessed by roentgenograms, and after 24 weeks the non-union fracture had healed. A non-union was considered healed when bone trabeculae were present across the full width of the fracture line (Brighton, 1985b).

Six out of ten patients who received electrical capacitive coupling for six months showed healing of the tibial non-unions. In the placebo group none of the patients had healed tibial non-unions. There was a significant difference ($p \leq 0.004$) in the rates of healing between the stimulated group and the placebo group (Scott, 1994).

Capacitive coupling technique treated on 22 tibial non-unions reported successful union in 77.3 percent of the cases (Brighton, 1985b).

The treatment of twenty-five lower-limb stress fractures in twenty-one athletes using capacitively coupled electric fields has promoted bone formation (Benazzo, 1995). Twenty-two fractures were healed, one did not heal and two showed improvement.

Electrical Stimulation on Titanium Implants

Animal studies have been done with the application of electrical stimulation to titanium implants in order to accelerate bone growth. A study was developed to determine an optimal current that will form bone in titanium implants inserted into the tibia of a rabbit using direct current (Buch, 1984). The titanium implant consisted of three plates with two canals running through them. Electrodes were then screwed in near the implant site and were attached to a direct current generator that applied a variety of current levels for three weeks. A direct current stimulation with 5 or 20 μA showed to be the most effective dose to stimulate bone ingrowth into these canals.

Several other studies using direct current on animal tibiae also found the

optimum current to be within the range of 5-20 μA at the cathode for successful osteogenesis and an increase in interfacial strength of experimental implants (Baranowski, 1983; Friedenberg, 1970; Park 1975, 1978).

Porous titanium implants placed in femurs of mongrel dogs demonstrated a greater interfacial strength than that of controls, and the quantity and rate of bone ingrowth showed enhancement by electrical stimulation (Colella, 1981).

Four guinea pigs were implanted with intra-osseous implants in the femur, and were electrically stimulated with 10μA direct current for twenty-eight days (Moriya, 1990). A large amount of bone formation occurred around and on the surface of the implant.

A magnetic titanium screw implanted in the temporal bone of adult goats, became well integrated with the application of inductive transcutaneous coupling (Dormer, 1986). A nuclear magnetic resonance head scan was performed to test the level of integration of the implant with the surrounding bone.

Current Proposal

We propose that electrical stimulation can be used to increase the rate of osseointegration of titanium implants placed in bone, and therefore increase the speed and efficiency of healing. Clinically, it would be useful to decrease the six to nine month waiting period it takes in humans for the titanium implant to become fully osseointegrated prior to placement of a prosthetic device. A rodent model was therefore developed, based on clinical experiences, to accelerate osseointegration by capacitive coupling. In this model, a

continuous capacitively coupled electrical signal of 2.0 V peak-to-peak, varying currents of 20μA, 30μA or 60μA RMS, and 60 kHz was applied for seven days to a titanium implanted rat tibia. The peak force required to extract the titanium implants was carried out by a biomechanical pull-out test to assess the level of osseointegration.

Materials and Methods

Animals and Housing

Adult male Sprague-Dawley rats weighing between 250-300g were purchased from Charles River Ltd., St. Constant, Quebec. They were kept in individual wire mesh cages and were fed Purina Rat Chow and tap water, *ad libitum*. The animals were weighed daily (08:00) and kept on a 12 hour light and 12 hour dark cycle (lights on at 07:30) throughout the study.

Description of the Stimulation Device

The electrical stimulation apparatus (Orthopak II electrical stimulation pack; Bioelectron Inc., Hackensack, NJ) generated a signal of 2.0 V peak-to-peak, 20.0, 30.0, or 60.0 μA RMS at 60.0 kHz from a 9 Volt alkaline battery, via a commutator-containing swivel (Figure 3). The swivel contained two plastic encased, stainless-steel female coupling devices. One of which accommodated the external power supply, and the other fitted to the male leads of the electrical stimulation wire/tethering spring combination. This stimulation wire measured 35.0 cm in length and was composed of a vinyl insulated stainless-steel stimulation wire, that was contained within a tightly coiled bronze silver-plated tethering spring. Both the swivel portion of the commutator and the tethering spring of the stimulation wire, allowed the animals free and unrestrained movement when hooked-up.

The electrode housing unit forming a 'head cap', (9 mm in overall height x

5 mm in width, Plastics One, Roanoke, VA) was configured to receive the male leads of the electrical stimulation wire. From its base, protruded two silicone covered electrical stimulation wires. Each lead measured 20.0 cm in length and terminated as two stainless-steel electrodes. Both electrodes were rectangular shaped (1.9 X 0.4 X 9.0 mm in length) and contained two, 1.0 mm diameter suture holes.

Placement of the Electrode Housing Unit (Surgery I)

Sprague Dawley rats were anesthetized with an intra peritoneal injection of 60 mg/kg and 10 mg/kg of ketamine (Rogarsetic, Montreal, Quebec) and xylazine (Rompun, Etobicoke, Ontario), respectively. The animals were then shaved on the top of their heads and on the mid section of their backs, and the shaved sites were wiped with isopropyl alcohol. A longitudinal incision, 2 cm in length was then made along the mid-sagittal region of the skull and the underlying connective tissue and periosteum were gently scraped away to expose the bony surface of the skull cap. Bone wax was spread on the surface of the exposed skull to stop bleeding. Under saline irrigation, two trans-cortical holes were then drilled through both the frontal and parietal bones. Four stainless-steel screws (3.2 X 0.8 mm diameter) were then screwed into place (Figure 4).

A two cm incision was made in the mid section of the back. The electrical leads were immersed in 5% solution of erythromycin (Erythrocin, Abbott Lab., Qc) and were tunneled subcutaneously from the skull incision through the dorsal

subcutaneous tissue to the back incision.

The electrode housing unit was then placed within the margins of the four stainless-steel screws (Figure 5) and permanently fixed to the skull by an acrylic resin forming a structure referred to as the 'head cap' (Figure 6). The skin surrounding the head cap was sutured using Silk sutures 4.0 (Cyanamid Canada Inc., Qc). The subcutaneous wires in the back were then sutured into a coil and left within the underlying soft tissue. The wound was flushed with two ml of 5% erythromycin solution and was closed with wound clips. The head and back wounds were further treated with an antibiotic Opsite spray (Smith and Nephew Inc., Qc). The animals were then placed in a terry-cloth wrap and were observed during the recuperation period.

Week 1

Following surgery the animals were acclimatized to the 'hook-up' procedure for 4, 8, 8, 12, 12 and 24 hours on post-surgical days 1 through 6, respectively. During the acclimatization period, the animals are weighed daily, and the wounds were monitored for presence of infections and open wounds. On the seventh day all tibiae were implanted with titanium implant screws.

Description and preparation of implants

Commercially pure Grade IV titanium implants (98.821%) were used (National Titanium Fabricators, Montreal, Qc). The implant consisted of an intra-osseous segment and an extra-osseous segment separated by a circular titanium collar. The intra-osseous end, designed to be screwed into the bone,

consisted of four threads and measured 0.18 cm in length and 0.16 cm in diameter. The titanium collar, designed to lie flush with the bone, was 0.24 cm in diameter and 0.20 cm thick. The extra-osseous segment of the implant, designed to fit the titanium cap, consisted of five threads measuring 0.22 cm long and 0.16 cm in diameter. The upper surface of the extra-osseous portion was slotted to facilitate screwing of the implant into the tibia. The titanium cap, designed to prevent tissue growth into the threads of the extra-osseous segment of the implant, measured 0.24 cm in diameter and 0.22 cm in height and had a hole diameter of 0.16 cm.

The titanium implants and caps were cleaned in an ultrasonicator cleaner for 10 minutes in a glass container containing hydrated n-butanol and then in 99% ethanol for 10 minutes. The implants and caps were air dried prior to being placed in a titanium container and then steam autoclaved for sterilization.

Placement of the Implants (Surgery II)

Seven days after the first surgery, surgery II was performed. The animals were unhooked, weighed and anaesthetized as previously described. Any new hair growth around the wound site on the back was shaved, as well as the hair on the anterior portion of both right and left tibiae. Isopropyl alcohol was swabbed onto shaven areas.

A 2.0 cm longitudinal incision was then made along the antero-medial aspect of each tibia. The skin and underlying muscle were carefully separated by dissection to expose the periosteum, which was then incised and reflected to

expose the tibial implantation site, just proximal to the tibial tuberosity. A trans-cortical hole was made in each tibia using a 1.4 mm round carbide burr (0.014 size) at 1000 RPM fitted to a dental handpiece. During the drilling, saline was constantly applied to irrigate the area. Using titanium tipped forceps, the implant was placed over the hole and then screwed into place using a jeweler's screwdriver until the collar of the implant lay flush with the surface of the bone. The titanium cap was then screwed onto the extra-osseous component of the implant.

The wound in the back was then reopened and the electrical leads were uncoiled and cleaned with a 5% erythromycin solution. The titanium implants were placed in both the right and left tibiae, however the right tibia was selected as the experimental side to which it received the electrical leads. The skin was then undermined to create a tunnel, in which the electrical leads were brought to the right tibial implantation site. Both the terminals and the leads were sutured to the soft tissue adjacent to the implant, thus cuffing the implant (Figure 7). After having received 5% erythromycin solution, the wounds on the back and right tibia were closed with wound clips. The animals were monitored and wrapped in terry clothes until awake.

Electrical Stimulation Model and Dosage (Week II)

All rats were hooked up and the right experimental tibia was given capacitive electrical stimulation within 2-3 hours after the tibial implantation (surgery II), and the left tibia acted as its own control. The Orthopack II electrical

stimulation pack provided a capacitively coupled electrical signal. The power generated by the 9.0 Volt alkaline battery was adjusted by the stimulation pack to deliver a 2.0 V peak-to-peak, and various current values ranging from 20.0 μA, 30.0 μA and 60.0 μA RMS at 60.0 kHz. The power pack was also equipped with an audible or light alarm system that would indicate when a 20% or more reduction in current or a disconnection somewhere along the circuit occurred. Each day the batteries were changed, and twice a day the flow of current was verified via an oscilloscope. All animals were 'hooked-up' and electrically stimulated or sham-stimulated continuously for seven days prior to sacrifice for biomechanical testing.

Pull-Out Technique

Animals were sacrificed by lethal injection of ketamine and xylazine, followed by cervical dislocation. The tibial implantation site was opened and the electrode connections were checked. The implant was then freed of the surrounding muscles and connective tissue. The tibia was amputated at the knee and dissected of all soft tissues (Figure 8).

After the removal of the titanium cap, the extra-osseous segment of the implant was then screwed onto the metal shaft of a digital force gauge of a Chatillon Tension/Compression recorder (Figure 9; Chatillon Tension/Compression Tester model TCM 201-ss, Digital Measurement Metrology, ON). The tibia was positioned perpendicular to the longitudinal axis of the metal shaft (Figure 10) and rigidly secured in a custom-designed serrated

tooth grip. The pull-out machine was set at an extraction rate of 2.54 millimeters per minute, and the peak tension of the breaking force in Newtons (N) of the implant extracted from the tibia was recorded.

Statistical Analysis

Statistical analysis on animal weight data was done using a repeated measure trend analysis. The differences in force required from the experimental and control groups were compared using a one-tailed Student's t-test with a required level of significance set at $p \leq 0.05$. All three groups were analyzed using the t-test for unpaired mean samples, assuming unequal variances.

Results

Macroscopic Observations

After 7 days of continuous capacitive electrical stimulation or sham-stimulation of the tibial implant sites, there were no signs of infections. There were four loose implants, 3 experimental and 1 control, and these were removed from the analysis. The subcutaneous electrodes, were observed to be well positioned, one on either side of the implant site, and they remained sutured to the underlying soft tissue.

Growth Curves

The mean weight of the animals at the time of surgery I (day 0) was 313±10.55 grams (mean weight ± SD) (Figure 11). On the day of sacrifice, fourteen days later, the mean body weight increased to 368±11.68 grams. This represented a mean weight gain of 17.6 %. A weight loss averaging 3.8 and 7.4 grams, respectively, was demonstrated in the animals the next day following the first (day 0) and second (day 7) surgical procedures. Animal weights recovered within 24 hours, and exhibited a steady weight gain averaging 5.58 grams per day, thereafter (Figure 11).

N-Values

Included in the results were 9 of 10 control values and 7 of 10 experimental values in the 20μA group; 17 of 24 control values and 13 of 24 experimental values in the 30μA group; and 4 of 5 control values and 4 of 5

experimental values in the 60μA group. To be accepted for data analysis, the placement of the titanium implants had to be proximal to the tibial tuberosity, and the electrodes had to have maintained a 'cuffing' position, one electrode on either side of the implant site. Of the 78 tibiae involved in this study, 54 tibiae were included in the results. Ten tibiae were eliminated from analysis because of adherence to the aforementioned prerequisites; 4 loose implants and 6 poorly placed implants. Seven animals were sacrificed during the 'hook-up' period and therefore another 14 tibiae were omitted from the results; and of these one animal swallowed a wound clip, and 6 animals had open wounds with electrode disconnections.

Biomechanical Pull-Out Test

A greater peak force was required to extract the titanium implants from the bone of the experimental tibiae compared to that of controls for both the 20μA and 30μA groups. The reverse was observed for the 60μA group but this difference was not statistically significant (Figure 12).

After one week of capacitively coupled electrical stimulation at 20μA, the tibiae of the experimental group (n=7) required 35±3.28 Newtons to extract the implant compared to 26.56±2.097 Newtons for controls (n=9) (Table 1). The Student's t-test indicated that this was a statistically significant difference ($p \leq 0.025$ one-tailed).

The experimental group (n=13) of tibiae for the 30μA group required 38.38±1.69 Newtons to pull-out the implant compared to 31.59±1.72 Newtons for

controls (n=17) (Table 1). The Student's t-test indicated that this was a statistically significant difference ($p \leq 0.004$ one-tailed).

The experimental group (n=4) of tibiae for the 60μA group required 38±5.295 Newtons to extract the implant compared to 47.5±4.25 Newtons for controls (n=4) (Table 1). The Student's t-test indicated that this difference was not statistically significant ($p \geq 0.05$).

Discussion

Growth Curve

An animal model was developed to assess bone strength around titanium implanted tibiae after seven days of continuous capacitive electrical stimulation or sham-stimulation. The growth curve represents the health status of the animals during the study period, and demonstrated an average weight gain of 5.58 grams per day. The net weight gain was equivalent for both experimental and control tibiae, since the tibiae were from the same animal. An expected weight loss, averaging 6.1 grams, occurred the day following the two surgical procedures, however each animal recovered their weight loss within the next 24 hours.

Other research using animals tethered via head caps and fitted with chronic indwelling atrial catheters have produced normal growth curves with normal ultradian rhythms of growth hormone secretion (Tannenbaum, 1976).

In previous studies of fracture healing where the electrical signal required a portable power source contained within a custom made vest, the rabbits exhibited an average weight loss of 4.3% over fourteen days of treatment and 2.3% of the animals entered into the study, died (Brighton, 1985c). Moreover, 10.3% of adult rodents restrained with Elizabethan collars and a velcro secured jacket containing a portable stimulator unit suffered from skin breakdown under the electrodes after twelve days of stimulation, and 3.3% of the animals died

(Brighton, 1985a). A study with a similar animal model demonstrated more serious weight losses averaging 18% after six weeks, and 21% after eight weeks of administration of a low voltage high frequency signal to castrated adult rats (Brighton, 1989).

The formation of an animal head cap, subcutaneous tunneling of the stimulation wire, and an acclimatization period together permitted the healthy development of the animals and therefore created a reliable animal model that can assess the effects of continuous capacitive electrical stimulation on bone growth around titanium implants.

Biomechanical Pull-out Test Results

Statistical analysis of the pull-out test results in our study demonstrated that a significant acceleration of bone formation around the titanium implants required more force to break from the bone when stimulated for one week by a capacitively coupled signal of 20μA and 30μA ($p \leq 0.025$; $p \leq 0.004$, respectively), when compared to sham-stimulated controls. A negative trend was established for the tibiae that received a 60μA signal ($p \leq 0.15$), showing less or no difference in bone strength between the test and control groups.

Electrical Stimulation of Implants

Several investigators subsequently confirmed the phenomenon of electrical osteogenesis around or into the pores of implants using various animals models. One experiment used a direct current generating 10μA to stimulate bone growth into porous ceramic and metallic implants placed in

canine femoral medullary canals, where a significant increase in interfacial shear strength occurred between two to four weeks (Weinstein, 1976). Another study used a similar current model and applied it to porous calcium aluminate implants in the femurs of rabbits for four weeks (Park, 1975). The tensile strength of the electrically stimulated implants were twice the value of the non-stimulated ones, indicating that electrical stimulation increased the rate of new bone formation under the experimental conditions. A study using titanium cathodes implanted in rabbit medullary canals has shown a significant increase in new bone formation by 46-48%, over control implants (Spadaro, 1982). Another research experiment using porous PMMA dental implants implanted in canine mandibles were stimulated by a 4-6μA constant direct current for two to six weeks. The results of x-ray and scanning electron micrographs showed enhanced bony growth into pores of the implants, and a higher interfacial strength for the electrically stimulated samples than for the controls. These findings indicate that alveolar bone osteogenesis is affected by electrical stimulation (Young, 1978).

One of the few studies conducted on the application of an electromagnetic field on porous coated titanium implants placed into the humerus of Japanese albino rabbits, indicated significantly more bone growth into the implant, by measuring the circumference of grown bone (Ijiri, 1996). A similar study also showed, by means of microradiography and densiometry, an increase in bone ingrowth and a constant high osteogenetic activity with electromagnetic stimulation around porous titanium implants (Buch, 1993).

The employment of a capacitively coupled electrical signal around a titanium implant placed in bone has, to date, not been documented. The results obtained from our rodent model demonstrates that a 20 and 30 μA capacitively coupled electrical signal (2 V p-p, 60kHz) has the ability to enhance bony healing around titanium implants. The 60μA group has, however, shown a negative effect, perhaps causing inhibition of bone formation or an increase in bone resorption. Smaller samples produce statistics with more variability and a larger margin of error, therefore the small sample size (n=4 for experimental and control groups) for the 60μA group may have affected the results.

Other studies have also found that currents above about 50μA (depending on the animal model and electrical circuitry) resulted in necrosis and gross tissue destruction (Marino, 1971). At 40μA osteonecrosis occurred in four of six rabbit tibiae that were stimulated, and all animals in the groups that received 50μA and 100μA showed bone destruction (Friedenberg, 1974b). A direct current stimulation with 50μA, applied to a dividable titanium implant inserted in the tibial metaphysis of rabbits caused a clear decrease of bone volume, averaging 48% less bone than control implants (Buch, 1984; 1986).

Healing process of Osseointegration

The optical titanium chamber permits direct microscopic investigations between living bone and implants (Albrektsson, 1986a). With this method, it is possible to study bone healing and remodeling around a titanium implant. When an implant is placed into a prepared site, the body immediately responds to the

"trauma". The cellular elements of blood and non-cellular elements of the fibrin network produces a hematoma in the closed cavity bordered by the implant surface and bone. Next to the blood clot is a thin (about 5 mm) layer of damaged bone caused by thermal and mechanical trauma, which lies next to the original undamaged bone. The adsorption and desorption of proteins occur, as well as a minor inflammatory response. During an unloaded healing period, the hematoma becomes transformed into new bone through callus formation.

Initially, woven bone is formed, characterized by a random organization of its collagen fibers. The layer of necrotic bone also heals by undergoing revascularization, demineralization, and remineralization. The woven bone becomes replaced by mature, lamellar bone, composed of successive layers each of which has a highly organized infrastructure. After the healing period, bone tissue is in close contact with the implants surface, without any other intermediate tissue. Some studies show that not all osseointegrated titanium implants have a direct bone contact but may have an intervening nonmineralized or organic layer (Collier, 1988; Soballe, 1991). Degenerated debris of multinucleated giant cells and osteoblasts have been found in the intervening layer at the interface, suggesting that bone remodeling steadily took place around the implant (Ohtsu, 1997).

Types of Implant Integration

Endosseous implants can assume two types of integration with the surrounding bone; osseointegration, or a fibroosseous integration.

Osseointegration is described as a direct bone-implant contact, biologically fixating titanium implants. The extent of bone contact can be measured by histomorphometry (Worthington, 1994). The degree of trabecular bone contact with the implant varies between 30% and 70%, with a mean of 50% (Johansson, 1987). The amount of bone contact varies greatly depending on where the measurements are taken, and the degree of bone contact increases with progressive bone remodeling, and can eventually amount to over 90% bone contact.

In unsuccessful implants, fibroosseous integration occurs forming a nonmineralized fibrous layer at the bone-implant interface, resulting in an improper anchoring tissue, because of its inadequate mechanical and biologic capacities (LeGeros, 1993). The lack of mechanical strength would result in lower peak force values obtained from the pull-out machine used in our study, with less force required to remove a fibrous integrated implant as compared to an osseous integrated implant.

There are many factors that can affect the healing process, resulting in either successful or unsuccessful implantation of titanium implants in bone.

Factors Affecting Healing

Implant Design

The geometric design of an implant affects osseointegration. The length of an implant statistically correlates with mechanical results, and the diameter of an implant does not (Block, 1990). Irregular cylinders, plates and screws of

titanium were compared, and it was found that screw shaped implants demonstrated the highest degree of bony contact (Carlsson,1986). The screw design makes a threaded socket in bone, providing immobilization immediately after installation and during the initial period. The initial stability facilitates the organization of the overlying connective tissue and allows the differentiation of bone cell proximal to the implant surface (Albrektsson, 1989a). In our study the screw shaped implants used permitted an immediate stable area for the healing process to occur. Bone growth between the threads of a screw shaped implant causes mechanical interlocking of the bone and the implant. The threaded structure allows a larger surface area for integration. Clinically, screw-shaped oral implants have become directly bone-anchored (Brånemark, 1977b; Albrektsson, 1988; 1989b).

Implant Surface

A material surface that is chemically nonreactive to the surrounding tissues and body fluids allows for a direct bone contact at the interface. (Kasemo, 1983). Titanium falls under this category as a bioinert material that is biocompatible, therefore this was the material used in our study. Titanium is an osteoconductive material, providing a scaffold or template for new bone growth, however, the titanium metal does not become directly attached to the bone, instead the oxide layer does. In the air and under normal *in vivo* physiologic conditions titanium quickly forms an oxide thickness of 3 to 5 nm, mainly composed of TiO_2 (Kasemo, 1983). This oxide layer is highly protective and

prevents a direct contact between the potentially harmful metallic ions and the tissue. Several chemical interaction forces occur between the oxide surface and bone, such as van der Waal's interaction , hydrogen bonding, dipole interaction, and covalent and ionic interaction, with force fields of $\leq$ 1 nm (Alberktsson, 1983b). A number of chemical processes occur at the implant interfaces that also influence the integration of an implant in bone, such as corrosion, denaturation of proteins, adsorption and fragmentation of biomolecules (Parsegian, 1983). The condition of the metallic implant at the time of implantation must be a mechanically and chemically clean passivated surface, since the outermost atomic layers of the implant are key factors in the integration process (Adell, 1981).

The preparation procedure used in our study, produces a clean surface by ultrasonically cleaning the titanium implants in different solvents for about 10 minutes followed by autoclaving at 130-140°C for about 20 minutes (Kasemo, 1983). During production and implantation, no surface of the final implant is touched by anything other than titanium-coated instruments (Brånemark, 1985).

In addition to surface chemistry, the physical properties of an implant surface influence the retentive strength of an osseointegrated implant (Thomas, 1987). Implants with smooth finishes have histologically demonstrated a fibrous encapsulation at implant interfaces (Carlsson, 1988; 1989; Thomas, 1985). Roughened or grit-blasted finishes have a significantly higher level of bone contact with titanium surfaces than implants that are polished smooth (50%

verus 20%) correlating with greater pullout strengths (Buser, 1991).

Status of Implant bed

Bone of poor-quality or inadequate volume has been associated with implant failures (Friberg, 1991). Bone tissue is organized macroscopically into cortical and medullar or trabecular structures. Cortical (compact) bone forms the dense surface layer of the skeletal bones. Trabecular or medullar (spongy or cancellous) bone forms the three-dimensional network within the skeletal bones.

A 5 year retrospective study classified bone quality into four types based on cortical thickness and density of the trabecular bone (Jaffin, 1991). A total of 1054 implants were placed into various bone types. Of the 102 implants placed in type IV bone, the category with thinnest cortices and the least trabecular density, 35% failed to osseointegrate, whereas only 3% of implants placed in bone types I to III failed. The percent of bone contact, assessed at the light microscopic level, of osseointegrated implants is largely related to the character of bone at the implant site (Albrektsson, 1990). Cortical compact bone has been shown to cover as much as 90% of the implant surface, and considerably less was found in medullary cancellous bone.

In our study the placement of implants must be consistently located in the same area to obtain a valid comparison. Titanium implants placed above or below the position proximal to the tibial tuberosity, the location of choice in our study, are located in an area of different bone density, thus influencing the pull-out value. All implants from our study that were located away from the tibial

tuberosity were eliminated from the results. Electrode placement may be just as vital, with larger distances between the electrodes differing in bone formation than electrodes placed closer to the implant site.

Dental endosseous titanium implants have reported higher levels of success when placed in the mandible than in the maxilla (Jemt 1991; Van Steenberghe, 1990). The mandible possesses an alveolar arch of cancellous bone supported by a foundation of dense basal bone. In contrast the maxilla is entirely composed of cancellous bone lying inferiorly to the nasal floor and the maxillary sinus. (Heimke, 1980; McKinney, 1985). The poorer quality and quantity of bone in the maxilla may allow implant movement during the early stage of healing, resulting in increased rates of implant failures. (Van Steenberghe, 1990; Adell, 1990). Progressive bone loss of the alveolar ridge complicates implant placements and prosthesis retention due to sites of thin cortices and poor trabeculation (Jaffin, 1991; Jemt, 1991).

Surgical Technique

The preparation of a surgical site is considered to be traumatic to the host tissues, however the degree of trauma determines whether healing will progress towards a fibrous or osseous integration. Necrosis of surrounding differentiated and undifferentiated cells at the implant-bone interface occurs due to the cutting of blood vessels, frictional heat generated from drilling, and vibrational trauma (Albrektsson, 1983b).

Surgical instrumentation of bone produces heat that raises the local

temperature of bone. The average temperature measured at a distance of 0.5 mm away from the preparation site during drilling procedures was 89°C (Eriksson, 1984b). Exposure time of only a few seconds at this temperature permanently prevented any bone healing around the screws. Threaded titanium implants heated to 500°C for one minute when inserted in the rabbit tibia prevented implant incorporation (Eriksson, 1984a). Even during a follow-up period of four weeks, no osseointegration took place. Heating to 47°C for one minute significantly reduced the amount of bone that grew into porous implants, whereas, heating to 44°C for one minute showed no reduction of bone formation (Clarke, 1953; Eriksson, 1984a). The critical threshold temperature for bone necrosis and impaired bone regeneration is between 44°C to 47°C applied for one minute (Lavelle, 1980).

Excessive trauma leads to decreased osseointegration, therefore to optimize success rates surgical trauma must be minimized (Brånemark, 1969). This was controlled in our study by using a gentle surgical technique using sharp drills that run at moderate speeds (<2000 rpm) with constant copious amounts of saline irrigation in order to prevent the local bone temperature from exceeding the crucial threshold (Eriksson, 1986; Lavalle, 1980). Debris is removed from the bur flutes by saline irrigation, which minimizes frictional heat. It was found that screws inserted with a 'strong hand' (average insertion torque around 35 Ncm) became poorly osseointegrated, whereas screws inserted with a 'gentle hand' (around 10 Ncm) required much higher torque values for removal after 4

and 12 weeks of implantation (Johansson, 1991a;b). Reinsertion of implants in animals responded by a poor implant incorporation and a negative tissue response , even after they were thoroughly cleaned and sterilized (Sennerby, 1988). Therefore, in our study an implant was never reinserted. Permanent bone damage, where bone will not heal as highly differentiated tissue but as low differentiated scar tissue, will result from surgical trauma, therefore minimal tissue injury is necessary for successful bone healing (Eriksson, 1984c). Great caution was taken in our study to ensure that minimal surgical trauma occurred.

Surgical Fit

Ideally, implants placed in bone should have a perfect microscopic contact, however this is virtually impossible to obtain. A space between the residual bone and the implant surface is bound to be created where bone contacts only portions of the implant (Uhthoff, 1973). Bone must be deposited in this space for implant support and successful integration. A lack of initial bone contact increases the potential for micromotion between the implant and bone, disturbing bone cell differentiation thus promoting a fibrous tissue interface (Pillar, 1981; Uhthoff, 1973). Bone generation around titanium implants were studied at interfacial gaps of close to 0, 0.35, and 0.85 mm (Cameron, 1976). After six weeks the only implants to achieve significant osseous integration were the ones with nearly no interfacial gap.

Biomechanical Testing

The force that is required to unscrew an implant may be influenced by the

amount and/or the type of bone formed in the threads and at the bone-implant interface. Previous investigations have suggested that mechanical testing, geometry, and densitometry predict the strength of a screw type implant-bone system (Coe, 1990; Daftari, 1994). Measures of bone mineral density by dual photon absoptiometry and DEXA (dual energy x-ray absoptiometry) has been shown to describe vertebral body strength and implant-bone mechanical strength (Myers, 1994; Yamagata, 1992). Similarly, peak torque and bone mineral density measured during screw insertion correlates with screw pull-out strength (Daftari, 1994). An axial pull-out test of pedicle screws in human cadaveric lumbar spine provided a measure regarding the strength of the screw-bone interface. Multiple regression analysis identified insertional torque, and equivalent mineral density determined by quantitative computed tomography, as the strongest predictive method of pull-out force (Myers, 1996). Several variables correlate with mechanical pull-out strength, including morphometric analysis, thus suggesting that a removal pull-out test is a useful parameter when studying osseointegration of screw-shaped implants. The strength of bone around an implant, which is assessed by a mechanical pull-out test, reflects the amount or type of bone observed in the tissue threads at a histological level (Park, 1975; 1976). The quality of interfacial bone has been reported to correlate with the amount of bone quantified by morphometric protocols (Steflik, 1996). Thus our results demonstrated that electrical stimulation applied to titanium implants caused greater pull-out values compared to sham-stimulated

controls; indicating that a greater amount of bone was formed and/or more lamellar bone was formed within the threads of the implant.

A bottle brush type of implant made of titanium was inserted into cancellous bone of rabbit femurs and were removed by a pull-out test and histologically examined four months later (Krysander, 1997). A correlation was seen in all fifteen animals between pull-out values and histological observations. The higher the pull-out test value the greater the quantity and quality of bone integration around titanium implants.

Quantity of bone

It has been shown that the interfacial strength increases as the ratio of bone material volume increases at the interface (Robertson, 1976). Torsion tests and pull-out tests on osseointegrated titanium implants were performed on 26 rats, and showed significant ($p \leq 0.01$) correlations between torque values and percentage of bone contact with the implant surface, and between pull-out load and bone thickness around the implant ($p \leq 0.001$) (Branemark, 1997). The increase in bone volume resulted in an increase in mechanical capacity.

Direct electrical current applied to cylindrical porous metallic implants in canine femurs underwent tensile tests and histological observations (Salman, 1980). The electrical stimulation substantially increased the anchorage strength of implant to bone. Histological observations found a larger amount of bone formation within the pores of the stimulated implants, thus increasing the required strength for implant removal.

A related study using electrical stimulation on porous calcium aluminate implants in the femurs of rabbits, also required greater force and energy to pull out the stimulated implants (Park, 1976). The total amount of bone grown into the implant pores was directly proportional to the interfacial tensile strength.

Another similar study measured the effect of direct current electrical stimulation on the interfacial strength of porous titanium implants implanted in the midfemoral diaphysis of mongrel dogs (Colella, 1981). Scanning electron microscopy evaluated a larger quantity of bone ingrowth by electrical stimulation, but no difference was found in the quality of bone between control and stimulated implants. This increase in bone volume was responsible for the increased interfacial strength represented by the higher pull-out values for the stimulated group.

In overdenture implant patients, a 100 percent success rate occurred among 53 implants placed in an area of bone with minimal resorption, and a bone quality composed of a thin cortex with dense cancellous bone. Among 33 implants placed in bone of the same quality, however, lesser quantity with some to extreme resorption of bone showed a 77.4% success rate (Smedberg, 1993).

Quality of Bone

Not only the quantity of bone formation, but also the quality, i.e. morphology of surrounding bone, may influence pull-out values when unscrewing a titanium implant. It has been suggested that an increase in removal torque with time for threaded titanium implants inserted in the tibia of

rabbits and in human mastoid bone, is not only a result of progressive bone formation but also bone remodeling around the implant (Johansson, 1987; Tjellstrom, 1988). As bone remodels the weaker woven bone is replaced by stronger lamellar bone, anchoring the implant with greater tenacity.

A three year follow-up study on 70 overdenture implant patients showed that jaws with failed implants had a statistically significantly lower mean bone quality compared to jaws with no failed implants (Jemt, 1993).

Cellular mechanisms

Despite the success and extended therapeutic use of implants, little is known about the biological mechanism that leads to the osseointegration process occurring between bone and the titanium surface. The cellular mechanisms associated with any form of electrical stimulation and its relation to bone formation have been hard to identify and are as of yet still only hypothesized. The following are some of the several hypotheses in the literature:

Micro-environmental changes in tissues elicited by a reaction at a stainless-steel cathode was studied (Brighton, 1975a) . Direct current stimulation resulted in consumption of dissolved oxygen and the production of hydroxyl radicals increasing the pH in the vicinity of the cathode (Renooij, 1983). Previous studies provide support for the concept that the low oxygen tension in tissue and a slightly alkaline environment are favorable for bone formation (Brighton, 1974).

An experiment showed that calcification is enhanced near the cathode and inhibited at the anode (Jahn, 1968) . Bone was modeled as an amphoteric ion exchanger, and that a continuous potential caused accumulations of positive charges such as calcium and phosphate ions at the cathodic bone surfaces where they deposit in an alkaline environment to produce apatite crystals.

The effect of electric current on galvanotaxis (cellular migration) and an applied constant direct current on freshly isolated rabbit osteoclasts and on rat osteoblast-like cells was investigated (Ferrier, 1986) . The osteoclasts migrated rapidly toward the positive electrode, and the osteoblast-like cells migrated in the opposite direction, toward the negative electrode.

The rate of bone formation is primarily determined by the number of osteoblasts as opposed to osteoblast activity (Fritzsimmons, 1992). An *in vitro* model was developed that showed mesenchymal cell proliferation with the application of capacitively coupled electric current to human bone cells.

It has been observed that electric stimulation caused differentiation of undifferentiated mesenchymal cells into osteoblasts and osteocytes (Matsunaga, 1993). The stimulation of proliferation, measured by tritiated thymidine incorporation and DNA content, has been observed in bone cell cultures treated with a form of electrical stimulation (Rodan, 1977). A concept developed from *in vitro* studies suggested a relationship between the stimulation of cells by electrical fields and the position of stimulated cells in the cell cycle (Janssen, 1979) . Electrical stimulation may have the ability to increase either cell

proliferation or matrix synthesis, depending on what event in the cell cycle is stimulated. However, stimulation can not alter the cell cycle itself and can not cause matrix synthesis in a cell population undergoing active proliferation, or stimulate proliferation in a cell population actively synthesizing matrix (Brighton, 1979).

A group of researchers proposed that the growth and remodeling process in bone is mediated by a potent local hormone, prostaglandin E_2 (Somjen, 1980) . An applied electrical current acts as a first messenger to stimulate the synthesis of prostaglandin E_2 to activate adenylate cyclase, which activates cyclic AMP synthesis to induce a series of physiologic responses in osteoprogenitor cells to induce differentiation of these cells to form bone.

The effects of capacitive coupling on cyclic AMP levels in osteoblasts grown in monolayer were investigated (Brighton, 1988) . This work showed that a small capacitively coupled electrical field extensively decreased cyclic AMP production by the bone cells in response to parathyroid hormone. Normally, osteoblasts would dramatically increase cyclic AMP levels in response to parathyroid hormone, however applied electrical current showed no such increase, therefore the current has induced the bone cells to become desensitized to parathyroid hormone. Since, the effect of parathyroid hormone is to increase bone resorption, desensitizing its effect at the osteoclast level could result in a net increase in bone.

Although, the exact mechanism is unknown in how an electrical signal

acts on the cells, the final common pathway is the formation of bone, or a lack of its breakdown.

Clinical Relevance

Osseointegration's documented success, allowing bone and mucosal tissue to tolerate a titanium implant, has dramatically expanded the possibilities of dental care. A dental titanium implant supported prosthesis requires a two-stage surgical procedure (Brånemark, 1985). Stage I surgery involves the placement of commercially-pure titanium implant cylinders within the jaw bone (endosseous) and covering them with mucosa. During a healing period of six to nine months the initial osseointegration response is established. Stage II surgery involves uncovering the implants from the overlying gingival tissues followed by several months of healing once the implants are stable within the jaw bone. A prosthesis, generally involving a transmucosal abutment, is then attached to the implants.

The ability to shorten the time period between the two surgical procedures would be beneficial to the clinical field of dentistry. Capacitive electrical stimulation has demonstrated accelerated osseointegration, thus potentially improving current clinical implant procedures. This electrically mediated method will have the advantage of shortening the fixation time for permanent attachment of prosthesis by shortening the period required for osseointegration.

Conclusion

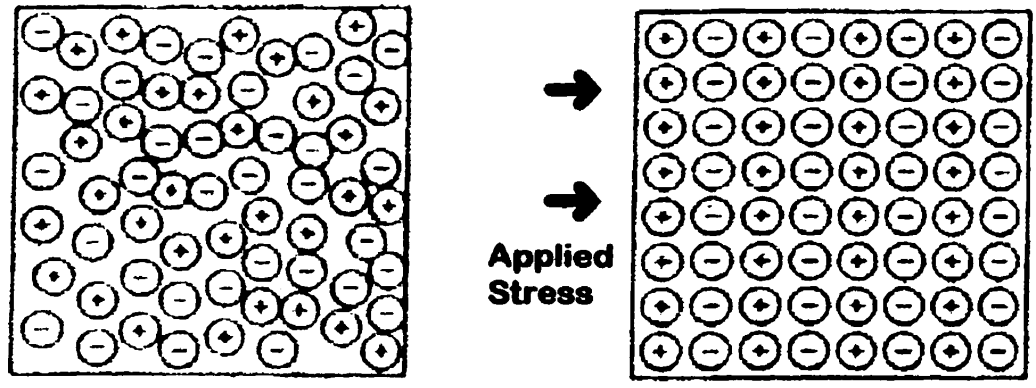
Biomechanical assessments of the effects of 7 days of continuous capacitive electrical stimulation (2.0 V p-p, 20 and 30μA RMS, 60kHz) on bone formation around titanium implants, has demonstrated a positive effect in our study.

Appendix A

List of Figures

Figure 1. Piezoelectricity. This schematic representation shows the formation of a piezoelectric signal. *A*. An electric potential exists as a result of applied stress. Charge separation has occurred and the ions become evenly oriented to exhibit a net negative charge on one surface and a net positive charge for the opposite surface. *B*. An electrical potential exists as a result of the alignment of dipoles in the material when a stress is applied.

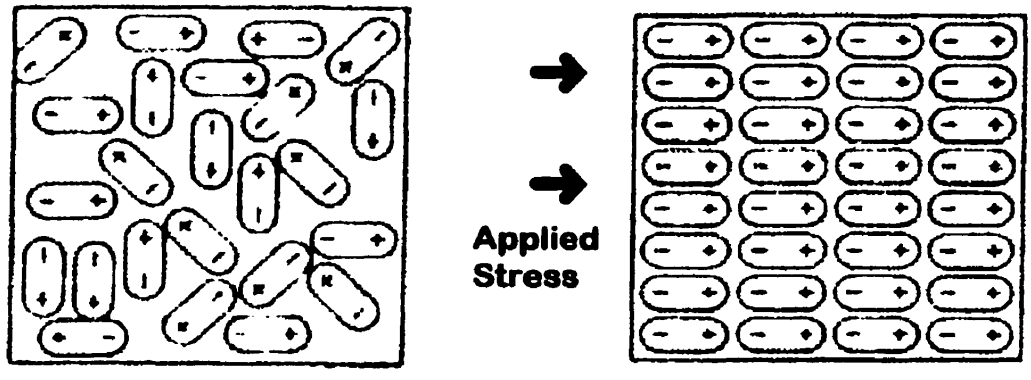
A.



Randomly oriented molecular ions in material when no stress is applied.

Evenly oriented molecular ions in material when stress is applied. A net positive charge on one surface, and a net negative charge on the other surface.

B.



Randomly oriented molecular dipoles in material when no stress is applied.

Evenly oriented molecular dipoles in material when stress is applied. A net positive charge on one surface, and a net negative charge on the other surface.

Figure 2. Charge distribution in a fractured bone. *In vivo,* a constant potential difference exists between the periosteum and the endosteum. The endosteum always has a negative potential with respect to the periosteum. A fractured bone will become more electronegative, with the greatest amount being at the fracture site.

Fracture

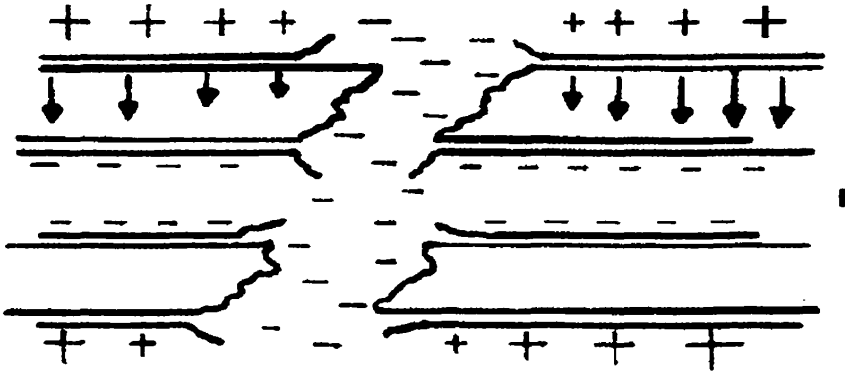


Figure 3. Electrical stimulation apparatus. A schematic illustration of the electrical stimulation apparatus (Orthopack II electrical stimulation pack, Bioelectron, N.J.), generating a 2.0 V p-p, 20, 30 or 60μA RMS at 60kHz capacitive electrical signal to the commutator-swivel, both of which are attached to the top of the rodents' cage. Connected to the swivel is the tethering spring, within which the electrical stimulation wire is contained. This unit attaches to the electrode housing unit of the head cap. The electrical leads are subcutaneously tunneled from the head cap to the tibial implant site, terminating as two stainless-steel electrodes, which are sutured onto the adjacent subcutaneous tissue.

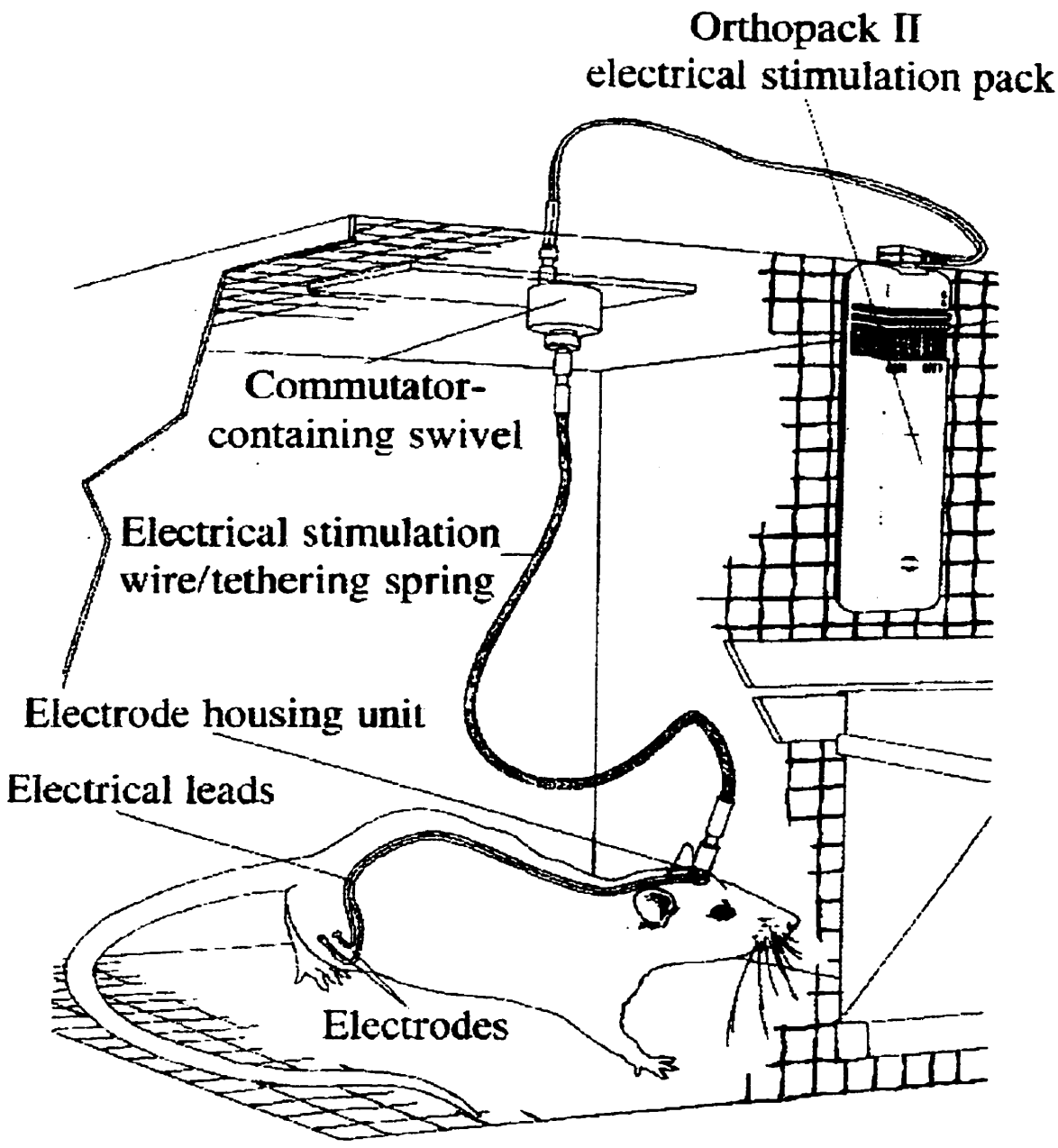

Orthopack II
electrical stimulation pack
Commutator-
containing swivel
Electrical stimulation
wire/tethering spring
Electrode housing unit
Electrical leads
Electrodes

Figure 4. Surgery I, Placement of the stainless-steel screws. The exposure of the bony surface of the skull cap, with the placement of four stainless-steel screws, two in the frontal and two in the parietal bones.

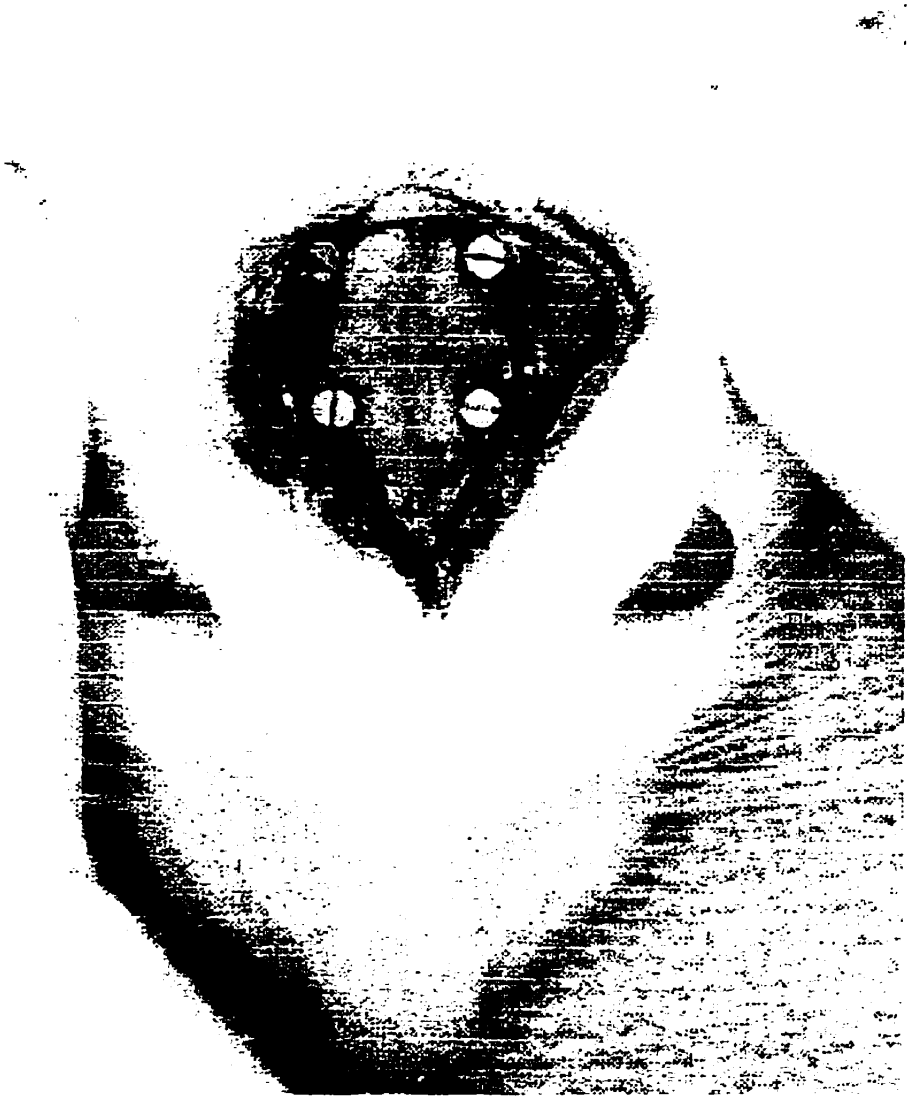


Figure 5. Surgery I, Placement of the electrode housing unit. The electrode housing unit placed within the margins of the four screws.

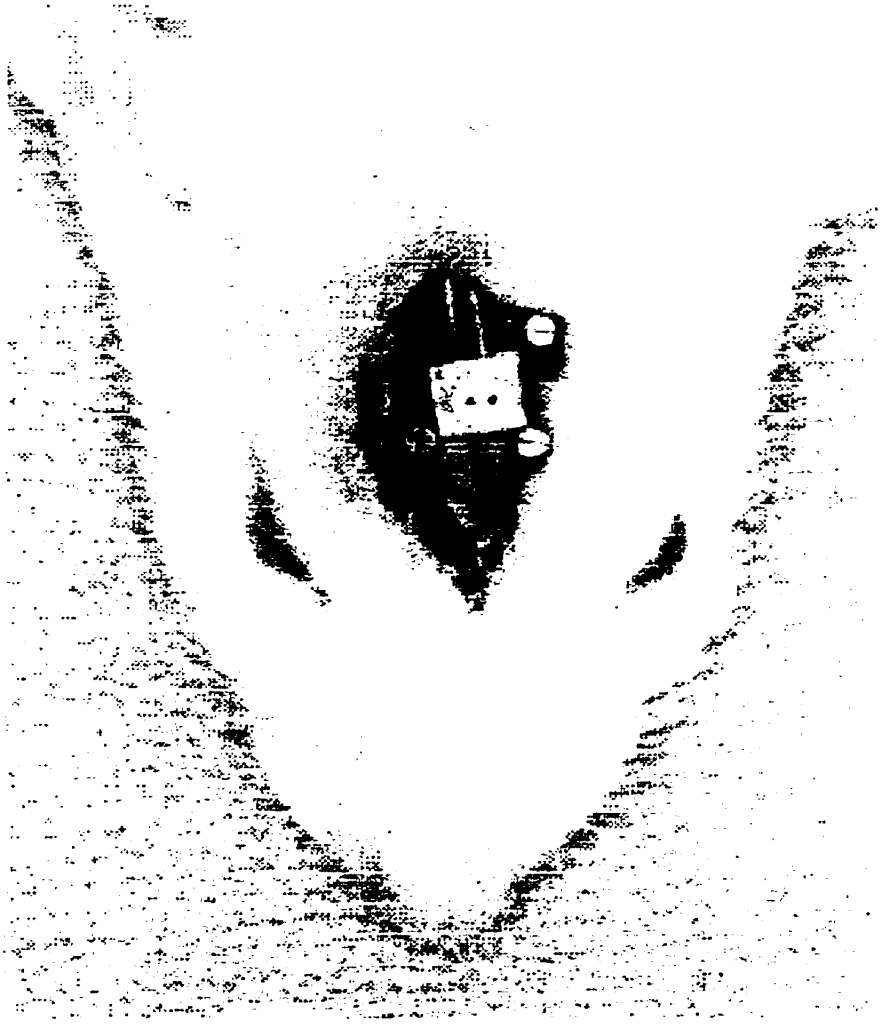


Figure 6. Surgery I, Placement of the acrylic resin. The electrode housing unit permanently fixed to the skull by an acrylic resin forming a 'head cap'.

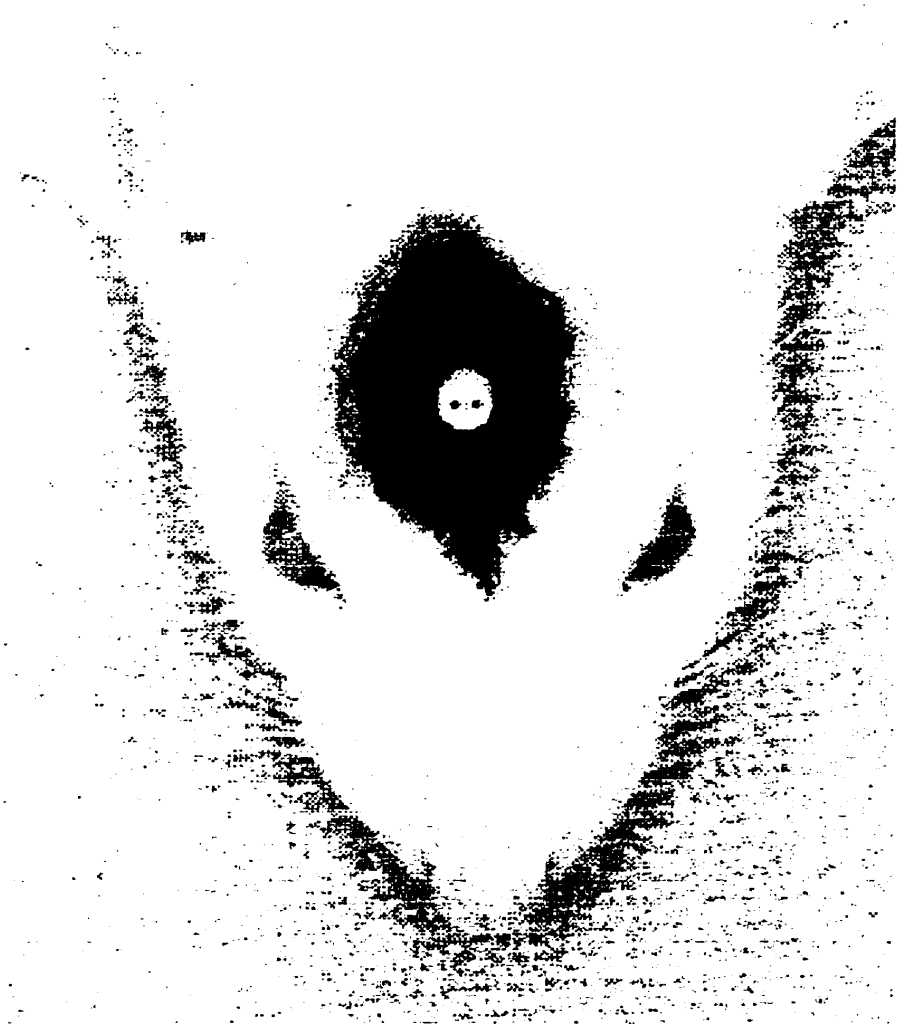


Figure 7. Surgery II, Electrical leads "cuffing" the implant. Both the electrical terminals and the leads sutured to the subcutaneous soft tissue adjacent to the implant, thus "cuffing" the implant.

Figure 8. Titanium implanted Tibia. A rat tibia dissected of all soft tissues with a titanium implant and cap still in place.

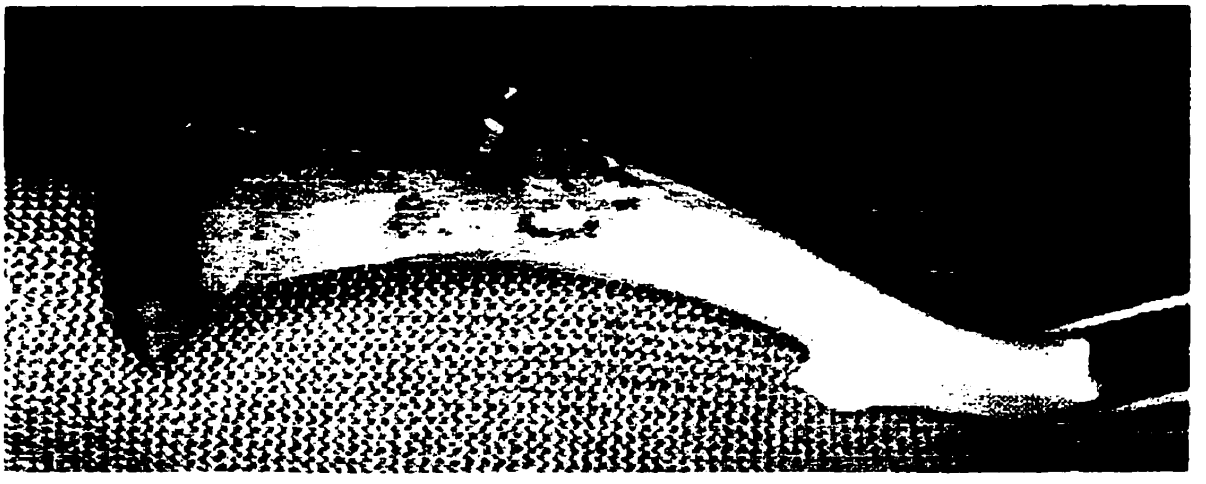


Figure 9. Chatillon Tension/Compression recorder. The Chatillon Tension/Compression Tester model TCM 201-ss, Digital Measurement Metrology, ON, set at an extraction rate of 2.54 millimeters per minute, recorded the peak tension of the breaking force in Newtons (N) of the implant extracted from the tibia, once rigidly secured in a custom-designed serrated tooth grip.

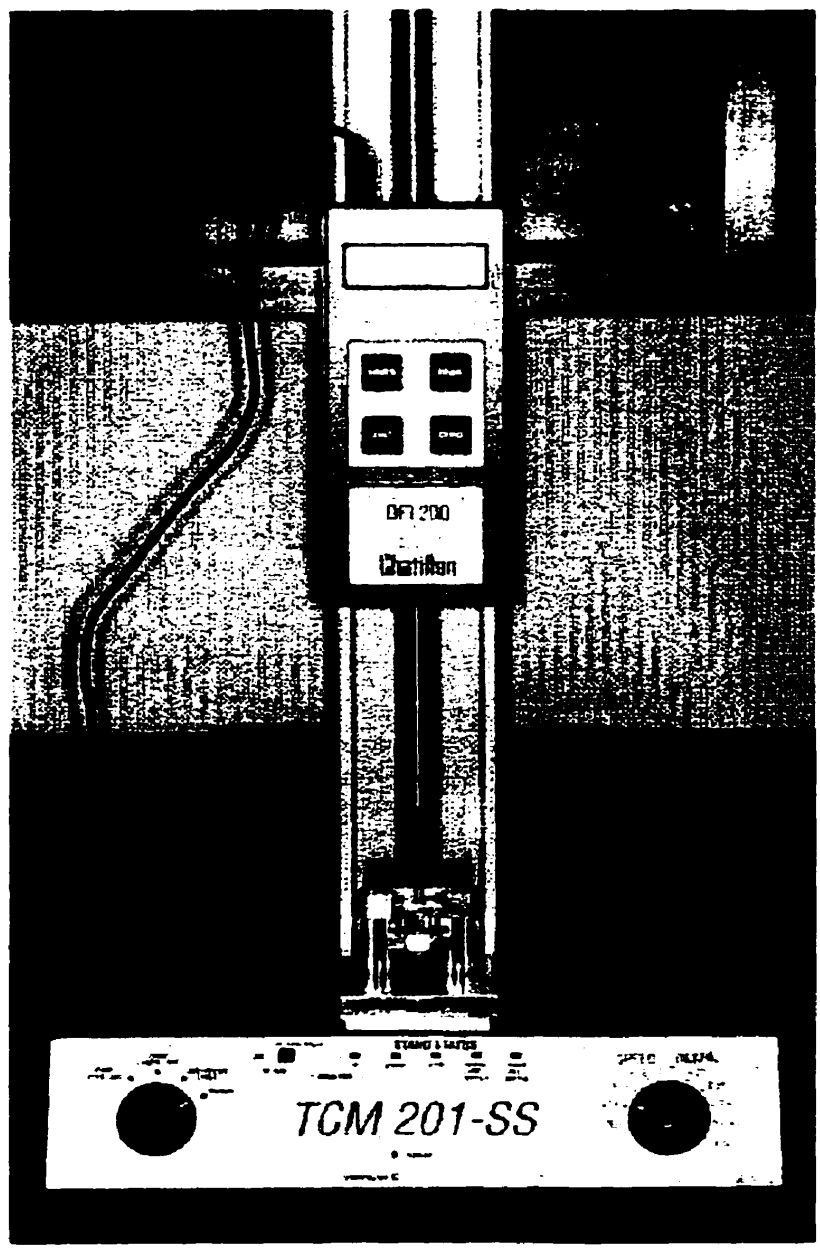

DFI 200
TCM 201-SS

Figure 10. Tibia on metal shaft of tension recorder. A rat tibia is shown attached to the metal shaft of the tension recorder, being prepared for the pull-out test.

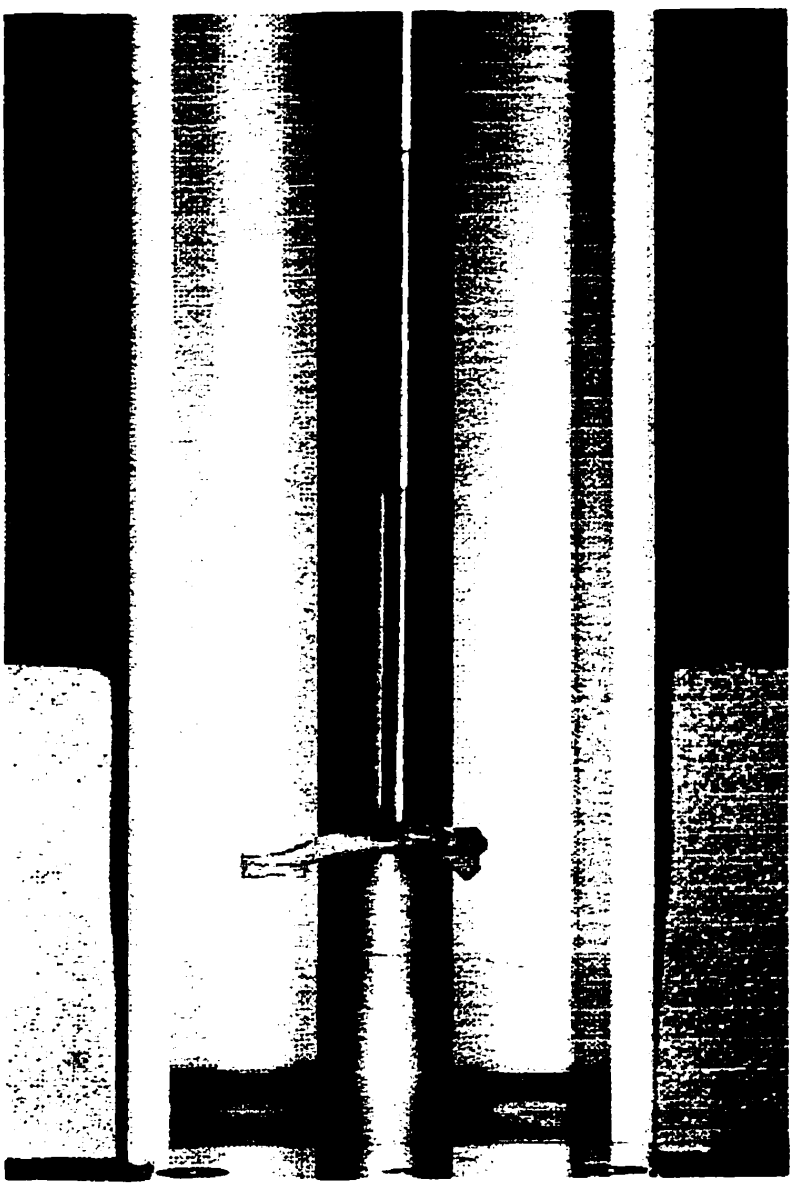


Figure 11. The Growth Curve. The growth curve follows the average growth of the animals, each acting as their own control, though out each experimental groups : 20, 30, and 60 μA. The day following each surgery (day 1 and day 8) an average weight loss of 3.8 grams at day 1 and 7.4 grams at day 8 was observed. The weight of the animals recovered within twenty-four hours following the surgeries, and the animals gained weight averaging 5.58 grams per day, thereafter.

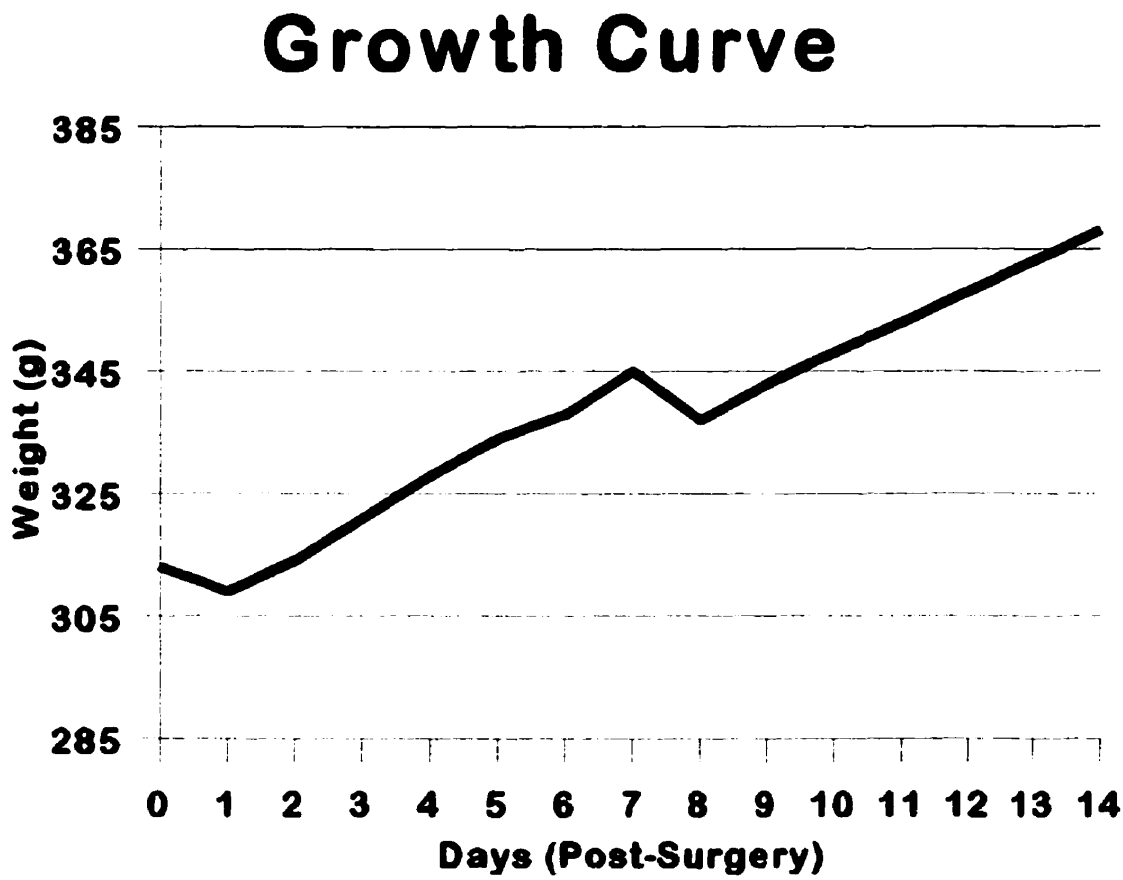
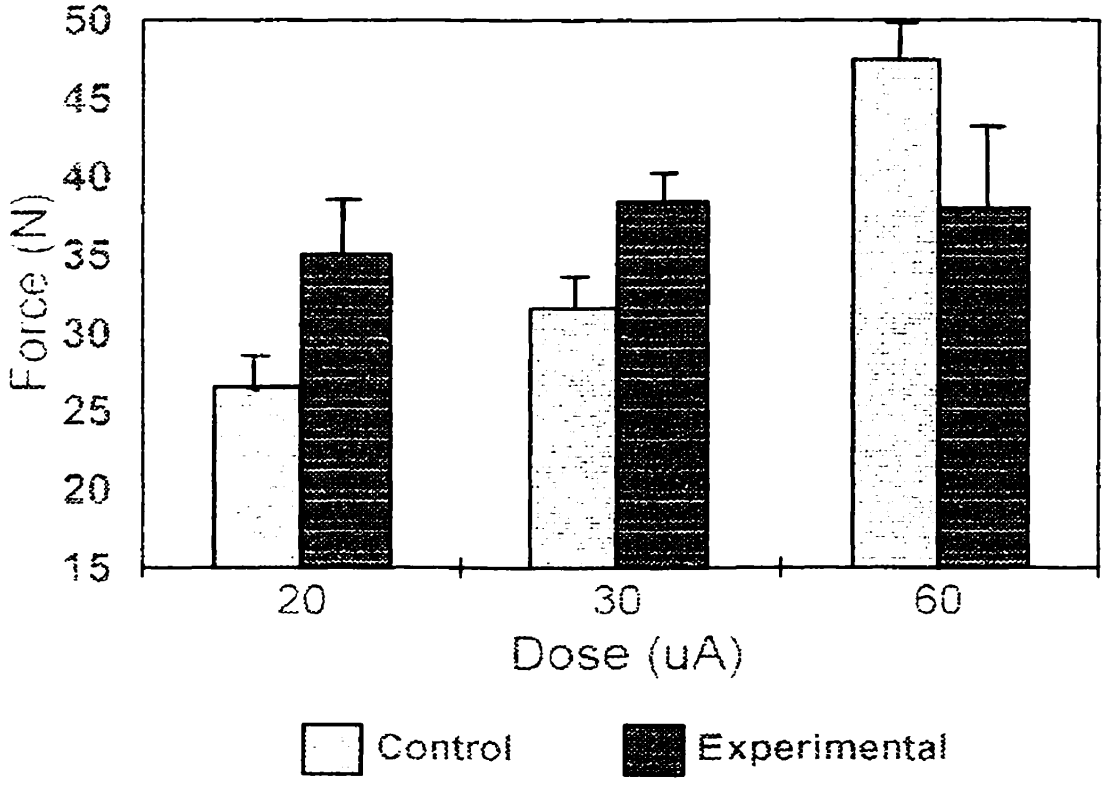

Growth Curve
385
365
345
325
305
285
Weight (g)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days (Post-Surgery)

Figure 12. Biomechanical Pull-out Test. Biomechanical pull-out peak tensile forces in Newtons (N) following 7 days of continuous capacitive electrical stimulation (2.0 V p-p, at 20μA, 30μA or 60μA RMS, and 60 kHz) as compared to non-stimulated control tibiae (contra lateral, sham-stimulated tibiae). Each value indicated the mean peak tensile force (n)±SEM. Using a Student's t-test, a significant healing effect was observed when animals were given a dosage of 20μA ($p \leq 0.025$) and at 30μA ($p \leq 0.004$). A dosage of 60μA did not produce a significant difference between the stimulated and sham-stimulated tibiae ($p > 0.05$).

Biomechanical Pull-Out Test



Appendix B

List of Tables

Table 1. T-Test. Biomechanical mean pull-out peak tensile forces in Newtons (N) following 7 days of continuous capacitive electrical stimulation (2.0V p-p, 20μA, 30μA and 60μA at source and 60 kHz) as compared to non-stimulated controls (controlateral, sham-stimulated tibiae).

T-Test: Unpaired Two Sample for Means

Treatment	Peak Tensile Force (N)
Control (n=9)	26.56±2.097
Continuous Electrical stimulation at 20μA (n=7)	35±3.28*
Control (n=17)	31.59±1.72
Continuous Electrical stimulation at 30μA (n=13)	38.38±1.69**
Control (n=4)	47.5±4.25
Continuous Electrical stimulation at 60μA (n=4)	38±5.295***NS

* $p \leq 0.025$

** $p \leq 0.004$

***$p > 0.05$; NS: Not Significant

Each value represents the mean peak tensile force ± SEM. One-tailed Student's t-test was used to statistically analyze the results. It was determined that there is a significant difference between the control and experimental groups for the 20μA and 30μA groups. No significant difference was determined for the 60μA group.

BIBLIOGRAPHY

Aaron RK, Ciombor DMcK. Electrical Stimulation of Bone Induction and Grafting. J Bone Miner Res 1989;4:192-205.

Adell R, et al. A 15-year study of osseointegrated implants in the treatment of edentulous jaw. Int J Oral Surg. 1981:10:387-416.

Adell R, Lekholm U, Brånemark PI, Jemt T. A long-term follow-up study of osseointegrated implants in the treatment of the totally edentulous jaw. Int J Maxillofac Surg. 1990;5:347-359.

Albrektsson T, et al. Osseointegrated titanium implants. Requirements for ensuring a longer lasting direct bone-to-implant anchorage in man. Acta Orthop Scand. 1981;52:155.

Albrektsson T, et al. Ultrastructural analysis of the interface zone of titanium and gold implants. Adv Biomater. 1982;4:167-177.

Albrektsson T. Direct bone anchorage of dental implants. J prosthet Dent. 1983a;50:255-261.

Albrektsson T, et al. The interface zone of inorganic implants *in vivo*: Titanium implants in bone. Am Biomed Eng. 1983b;11:1.

Albrektsson T, et al. Osseointegration of dental implants. Dent Clin North Am. 1986a;30:151-174.

Albrektsson T, Hansson HA. An ultrastructural characterization of the interface between bone and sputtered titanium or stainless-steel surfaces. Biomaterials. 1986b;7:201-205.

Albrektsson T, et al. A multicenter study of osseointegrated oral implants. J Prosthet Dent. 1988;60:75-84.

Albrektsson T, Zarb GA. The Brånemark osseointegrated implant. Quintessence Pub Co, Inc. 1989a.

Albrektsson T, Lekholm U. Osseointegration current state of the art. Dent Clin North Am. 1989b Oct;33(4):537-54.

Albrektsson T, Sennerby L. Direct bone anchorage or oral implants: Clinical and experimental considerations of the concept of osseointegration. Int J Prosthodont. 1990;3:30-41.

Andrew C, Bassett L. Pulsing Electromagnetic Fields: A New Method to Modify Cell Behavior in Calcified and Noncalcified Tissues. Calcified Tissue Int 1982; 34:1-8.

Baranowski T, Black J, Brighton CT, Friedenberg ZB. Electrical osteogenesis by low direct current. J Orthop Res. 1983;1:120-128.

Bassett CA. Biologic significance of piezoelectricity. Calcif Tissue Res. 1968;1:252-272.

Bassett CA, Pawluk RJ, and Pilla AA. Acceleration of fracture repair by electromagnetic fields- a surgically non-invasive method. Ann NY Acad Sci. 1974a; 238:242.

Bassett CA, Pawluk RJ, and Pilla AA. Augmentation of bone repair by inductively coupled electromagnetic fields. Science 1974b; 184:575-577.

Bassett CA, et al. The effect of pulsing electromagnetic fields on cellular calcium and calcification of

non-unions. In Brighton CT. Electrical properties of bone and cartilage. New York:Grune & Stratton. 1979;427-440.

Bassett CA, Mitchell SN, Gaston SR. Pulsing electromagnetic field treatment in ununited fractures and failed arthrodeses. J Am Med Assoc. 1982a;247:623-628.

Bassett CA. Pulsing electromagnetic fields. A new method to modify cell behavior in calcified and noncalcified tissues. Calcif Tissue Int. 1982b;34:1-8.

Becker RO, Spadaro JA, Marino AA. Clinical experiences with low intensity direct current stimulation of bone growth. Clin Orthop. 1977 May;(124):75-83.

Behari J. Electrostimulation and bone fracture healing. Biomed Eng. 1991;18(4):235-254.

Benazzo F, et al. Use of capacitive coupled electric fields in stress fractures in athletes. Clin Orthopaedics Related Res. 1995 Jan;310:145-50.

Bergman RA, Afifi AK, Heidger PM. Histology. WB Saunders. 1996.

Binderman I, Shimshoni Z, Somjen D. Biochemical Pathways involved in the Translation of Physical Stimulus into Biological Message. Calcif Tissue Int 1984; 36:S82-S85.

Black J. Electrical stimulation-It's role in growth, repair, and remodelling of the musculoskeletal system. New York: Praeger, 1987.

Block MS, Delgado A, Fontenot MG. The effect of diameter and length of hydroxyapatite-coated dental implants on ultimate pull out force in dog alveolar bone. J Oral Maxillofac Surg. 1990;48:174.

Block MS, Kent JN, Guerra LR. Implants in dentistry. WB Saunders Co. 1997.

Borgens RB. Endogenous ionic currents traverse intact and damaged bone. Science. 1984;225:478.

Borsalino G, et al. Electrical stimulation of human femoral intertrochanteric osteotomies. Clin Orthop Rel Res. 1988 Dec;237:256-63.

Bourguignon, L. Y. W. Electric stimulation of proteins and DNA synthesis in human fibroblasts. Cell Biol Int Rep 1987;8:398-402.

Brånemark PI, et al. Intra-osseous anchorage of dental prostheses. Scand J Plast Reconstr Surg. 1969;3:81-100.

Brånemark PI, et al. Osseointegrated fixtures in the treatment of the edentulous jaw; experience from a 10-year period. Scand J Plastic Reconst Surg. Suppl. 1977;16:1-132.

Brånemark PI, et al., Osseointegrated implants in the treatment of edentulous jaw. Scand J Plast Reconstr Surg. 1977a;11(Suppl.16).

Brånemark PI, Zarb GA, Albrektsson T. Tissue-integrated prostheses: osseointegration in clinical dentistry. Chicago: Quintessence. 1985.

Branemark R, Ohmell LO, Nilsson P, Thomsen P. Biomechanical characterization of osseointegration during healing: an experimental in vivo study in the rat. Biomaterials. 1997 Jul;18(14):969-78.

Branham GB, Triplett RG, Yeandle S, Vieras F. The effect of electrical current on the healing of mandibular freeze-dried bone allografts in dogs. J Cra Maxillofac Surg. 1985;40:403-407.

Brighton CT, Friedenberg ZB. Electrical stimulation and oxygen tension. Ann NY Acad Sci. 1974;238:314-320.

Brighton CT, Adler S, Black J. Cathodic oxygen consumption and electrically induced osteogenesis. Clin Orthop. 1975a;107:227-289.

Brighton CT, et al. Direct-current stimulation of non-union and congenital pseudarthrosis. J Bone Joint Surg. 1975b;57-A(3):368-377.

Brighton CT, Friedenberg ZB, Mitchell EI, Booth RE. Treatment of nonunion with constant direct current. Clin Orthop Rel Res. 1977 May;124:106-123.

Brighton CT, Black J, Pollack S, eds. Electrical properties of bone and cartilage. New York:Grune & Stratton, 1979:491-517.

Brighton CT, Black J, Friedenberg ZB, Esterhai JL, Day LJ, connolly JF. A multicenter study of the treatment of non-union with constant direct current. J Bone Joint Surg. 1981 Jan.; 63A:2-13.

Brighton CT, Pfeffer GB. In vivo growth plate stimulation in various capacitively coupled electrical fields. J Orthopaedic Res. 1983;1(1):42-9.

Brighton CT, Pollack SR. Treatment of nonunion of the tibia with a capacitively coupled electrical field. J Bone and Joint Surg. 1984A;67-A:557-585.

Brighton CT. The semi-invasive method of treating nonunions with direct current. Orthop Clin North Am. 1984B;15:33-45.

Brighton CT, Tadduni GT, Pollack Sr. Treatment of sciatic denervation disuse osteoporosis in the rat tibia with capacitively coupled electrical stimulation: dose response and duty cycle. J. Bone and Joint Surg. 1985a; 67-A:1022-1028.

Brighton CT, Pollack SR. Treatment of recalcitrant non-union with a capacitively coupled electrical field. . J. Bone and Joint Surg. 1985b; 67-A:557-585.

Brighton CT, Hozack WJ, Brager MD, Windsor RE, Pollack SR, Vreslovic EJ, Kotwick JE. Fracture healing in the rabbit fibula when subjected to various capacitively coupled electrical fields. J. Orthop. Res. 1985c;3:331-340.

Brighton CT, McCluskey WP. Response of cultured bone cells to a capacitively coupled electric field: inhibition of cAMP response to parathyroid hormone. J. Ortho Research. 1988;6:567-571.

Brighton CT, Luessenhop CP, Pollack SR. Treatment of castration-induced osteoporosis by a capacitively coupled electrical signal in rat vertebrae. J. Bone and Joint Surg. 1989;71-A:228-236.

Brunski JB. Biomechanical factors affecting the bone-dental implant interface. Clin Mater 1992;10:153-201.

Buch F, Albrektsson T, Herbst E. Direct current influence on bone formation in titaniun interface. Biomat. 1984;5:341-346.

Buch F, Albrektsson T, Herbst E. Effects of direct currents on bone growth into delrin implants. Scand J Plast Reconstr Surg. 1985;19(3):223-30.

Buch F, Albrektsson T, Herbst E. The bone growth chamber for quantification of electrically induced osteogenesis. J Orthop Res. 1986;4(2):194-203.

Buch F, Jonsson B, Mallmin H, Kalebo P. The quantification of bone tissue regeneration after electromagnetic stimulation. Arch Orthop Trauma Surg. 1993;112(2):75-8.

Burny F, Herbst E, Hinsenkamp M. Electrical stimulation of bone growth and repair. Springer-Verlag. New York. 1978.

Buser D, et al. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. J Biomed Mater Res. 1991;25:889-902.

Cameron Hu, et al. The rate of bone ingrowth into porous metal. J Biomed Mater Res. 1976;10:295-302.

Carlsson L, Röstlund T, Albrektsson B, Albrektsson T, Brånemark PI. Osseointegration of titanium implants. Acta Orthop Scand. 1986;57:285-289.

Carlsson L, Röstlund T, Albrektsson B, Albrektsson T. Removal torques for polished and rough titanium implants. Int J Oral Maxillofac Impl. 1988;3:21-24

Carlsson L, Albrektsson T, Berman C. Bone response to plasma cleaned titanium implants. Int J Oral Maxillofac Impl. 1989;4:199-204.

Chang WH, Hwang I-M, Liu H-C. Enhancement of fracture healing by specific pulsed capacitively-coupled electric field stimulation. Frontiers Med.Biol.Engng. 1991;3:57-64.

Clark EGC, Hickman J. An investigation into the correlation between the electric potential of metals and their behavior in biological fluids. J Bone Joint Surg. 1953;35B:467.

Clokie CML, Warshawsky H. Morphologic and radioautographic studies of bone formation in relation to titanium implants using the rat tibia as a model. Int.J Oral Maxillofacial Implants. 1995;10:155-165.

Coe JD, Warden KE, Herzig MA, McAfee PC. Influence of bone mineral density on the fixation of thoracolumbar implants: A comparative study of transpedicular screws, laminar hooks, and spinous process wires. Spine. 1990;15:902-7.

Colella SM, Miller AG, Stang RG, Stoebe TG, Spengler DM. Fixation of porous titanium implants in cortical bone enhanced by electrical stimulation. J Biomed Mater Res. 1981 Jan;15(1):37-46.

Collier JP, Mayor MB, Ohae JC, Surprenant VA, Daupninais LA. Macroscopic and microscopic evidence of prosthetic fixation with porous-coated materials. Clin Orthop. 1988;235:173-180.

Connolly J, Ortiz J, Price R, Bayvzick R. The effect of electrical stimulation on the biophysical properties of fracture healing. Ann NY Acad Sci. 1974;238:519-528.

Daftari TK, Horton WC, Hutton WC. Correlations between screw hole preparation, torque of insertion, and pull-out strength for spinal screws. J Spinal Disord. 1994;7:139-45.

Davidovitch Z, Shanfeld J.L., Montgomery P.C., Lally E, Laster L, Furst L, Korostoff E. Biochemical mediators of the effects of mechanical forces and electric currents on mineralized tissues. Calcif Tissue Int. 1984;36:S86-S97.

Davidovitch Z, Steigman S, Finkelson M.D., Yost R.W., Montgomery P.C., Shanfeld J.L., Korostoff E.

Immunohistochemical evidence that electric currents increase periosteal cell cyclic nucleotide levels in feline alveolar bone *in vivo*. J. Oral Biol. 1980;25:321-327.

Digby PSB. Mechanism of calcification in mammalian bone. Nature. 1966; 212, 1250-1252.

Dormer KJ, Richard G, McGee M, Hough JV, Shew RL. An implantable hearing device: osseointegration of a titanium-magnet temporal bone stimulator. Am J Otol. 1986 Nov;7(6):399-408.

Eriksson C. Streaming potentials and other water dependent effects in mineralized tissues. Ann. NY Acad sci. 1974;238,321.

Eriksson RA, Albrektsson T. The effect of heat on bone regeneration. An experimental study in the rabbit using the bone growth chamber. J Oral Maxillofac Surg. 1984a;42:705-711.

Eriksson RA, et al. Heat caused by drilling cortical bone. Acta Orthop Scand. 1984b;55:629-631.

Eriksson RA. Albrektsson T. Magnusson B. Assessment of bone viability after heat trauma. A histological, histochemical and vital microscopic study in the rabbit. Scandinavian Journal of Plastic & Reconstructive Surgery. 1984c;18(3):261-8.

Eriksson RA. Albrektsson T. The effect of heat on bone regeneration: an experimental study in the rabbit using the bone growth chamber. Journal of Oral & Maxillofacial Surgery.1984d Nov; 42(11):705-11.

Eriksson RA, Adell R. Temperatures during drilling for the placement of implants using the osseointegration technique. J Oral Maxillofac Surg. 1986;44:4-7.

Esterhai J.L., Friedenberg Z.B., Brighton C.T., Black J. Temporal course of bone formation in response to constsnt direct current stimulation. J. Orthopaedic Research. 1985;3:137-139.

Ferrier J, Ross S.M., Kanehisa J, Aubin J.E. Osteoclasts and osteoblasts migrate in opposite directions in response to a constant electrical field. J. of Cellular Physiology. 1986;129:283-288.

Fleisch H. Bisphosphonates in bone disease: from laboratory to the patient. Fleisch, Bern, Switzerland. 1993.

Friberg B, Jemt T, Lekholm U. Early failures in 4641 consecutively placed Branemark implants: A study from stage 1 surgery to the connection of completed prostheses. Int J Oral Maxillofac Implants. 1991;6:142-146.

Friedenberg ZB, Andrews ET, Smolenski BT, Pearl BW, Brighton CT. Bone reaction to varying amounts of direct current. Surg Gynecol Obstet. 1970;131:894-899.

Friedenberg Z.B., Harlow MC, Brighton CT. Healing of nonunion of the medial malleolus by means of direct current : A case report. J Trauma. 1971;11:883-885.

Friedenberg Z.B., Brighton CT. Electrical fracture healing. Ann NY Acad Sci. 1974a;238:564-574.

Friedenberg Z.B., Zemsky LM, Pollis RP, Brighton CT. The response of non-traumatized bone to direct current. J. Bone and Joint Surg. 1974b;56-A:1023-1030.

Friedenberg ZB, et al. The effects of demineralized bone matrix and direct current on an *in vivo* culture of bone marrow cells. J Orthop Res. 1989;7(1):22-27.

Fritzsimmons R.J., Farley J.R., Adey W.R., Baylink D.J. Frequency dependence of increase cell

proliferation, in vitro, in exposures to a low-amplitude, low-frequency electric field: Evidence for dependence on increased mitogen activity released into culture medium. J. of Cellular Physiology. 1989;139:586-591.

Fritzsimmons R.J., Strong D.D., Mohan S., Baylink D.J., Low-amplitude, low-frequency electric field-stimulated bone cell proliferation may in part be mediated by increased IGF-II release. J. of Cellular Physiology. 1992;150:84-89.

Fukada E, Yasuda I. On piezoelectric effect of bone. J Physical Soc Jap. 1957;12:1158-1162.

Gross D, Williams WS. Streaming potentials and the electromechanical response of physiologically moist bone. J Biomech. 1982; 15:277.

Gross UM, J Dent Educ 52, 798, 1988.

Hagert CG. Brånemark PI. Albrektsson T. Strid KG. Irstam L. Metacarpophalangeal joint replacement with osseointegrated endoprostheses. Scandinavian Journal of Plastic & Reconstructive Surgery. 1986;20(2):207-18.

Hall BK. Bone. Volume 3. CRC Press, Inc. 1991;109-133.

Hall BK. Bone. Volume 5. CRC Press, Inc. 1992;153-199.

Hanawa T, Ota M. Calcium phosphate naturally formed on titanium in electrolye solution. Biomaterials. 1991 Oct,;12(8):767-74.

Hansson HA, Albrektsson T, Brånemark PI. Structural aspects of the interface between tissue and titanium implants. J Prosthet Dent. 1983;50:108.

Hassler CR, et al. Studies of enhanced bone healing via electrical stimuli. Clin Orthopaed Rel Res. 1977 May;124:9.

Haupt HA. Electrical stimulation of osteogenesis. South Med J. 1984 Jan;77(1):56-64.

Heimke G. Dental implants. Carl Hanser Verlag. 1980.

Hobos S, Ichida E, Garcia LT. Osseointegration and occlusal rehabilitation. London, Quintessence Publishing co, Inc. 1989;33-54.

Hulth A. Fracture healing. Acta Orthop Scand. 1980.51:5-8.

Ijiri K, Matsunaga S, Fukuyama K, Maeda S, Sakou T, Kitano M, Senba I. The effect of pulsing electromagnetic field on bone ingrowth into a porous coated implant. Anticancer Res. 1996 Sep-Oct;16(5A):2853-6.

Jaffin RA, Berman CL. The excessive loss of Brånemark fixtures in type IV bone: A five year analysis. J Periodontol. 1991;62:2-4.

Jahn TL. A possible mechanism for the effect of electrical potentials on apatite formation in bone. Clinical Orthopaedics & Related Research. 1968 Jan-Feb; 56:261-73.

Janssen J, Akkermans L, Wittebol P. The effect of electrical stimulation on embryonic rat calvaria in vitro. In Brighton C, Black J, Pollack S, eds. Electrical properties of bone and cartilage. New York:Grune & Stratton, 1979:491-517.

Jemt T. Failures and complications of 391 consecutively inserted fixed prostheses supported by Brånemark implants in edentulous jaws: A study of treatment from the time of prosthesis placement to the first annual check-up. Int J Oral Maxillofac Surg. 1991;6:270-276.

Jemt T. Implant treatment in resorbed edentulous upper jaws: A three-year follow-up study on 70 patients. Clinical Oral Implants Research. 1993;4:187-194.

Johansson C, Albrektsson T. Integration of screw implants in rabbit: A 1 year follow-up of removal torque of titanium implants. Int J Oral Maxillofac Implants. 1987;2:69-75.

Johansson C, Lausmaa J, Ask M, Hansson HA, Albrektsson T. Ultrastructural differences of the interface zone between bone and Ti6Al4V or commercially pure titanium. J Biomed Eng. 1989 Jan;11(1):3-8.

Johansson CB. Sennerby L. Albrektsson T. A removal torque and histomorphometric study of bone tissue reactions to commercially pure titanium and Vitallium implants. International Journal of Oral & Maxillofacial Implants. 1991a; 6(4):437-41.

Johansson CB. Albrektsson T. A removal torque and histomorphometric study of commercially pure niobium and titanium implants in rabbit bone. Clinical Oral Implants Research.1991b Jan-Mar; 2(1):24-9.

Jørgensen TE. The effect of electric current on the healing time of crural fractures. Acta Orthop Scand. 1972;43:421-437.

Junqueira LC, Carneiro J, Kelley RO. Basic histology. Appleton & Lange. 1992; 7th ed.

Kahanovitz N, Arnoczky SP. The efficacy of direct current electrical stimulation to enhance canine spinal fusions. Clin Orthop 1990 Feb;(251):295-9.

Kasemo B. Biocompatibility of titanium implants: Surface science aspects. J Prosthet Dent. 1983;49:832.

Krauser JT. Hydroxylapatite-coated dental implants. Biologic rationale and surgical technique. Dent Clin North Am. 1989;33:879-903.

Krysander L, Hellem S, Berggren A, Klinge B. The mini bottle brush--a new fastening device in cancellous bone. Clin Oral Implants Res. 1997 Dec;8(6):506-11.

Kubota K, Yoshimura N, Yokota M, Fritzsimmons RJ, Wikesjo UME. Overview of effects of electrical stimulation on oseogenesis and alveolar bone. J.of Periodont. 1995;66:2-6.

Lalor PA, et al. Sensitivity to titanium. J Bone Surg. 1991;73:25-8.

Lavelle C, et al. Effect of internal irrigation frictional heat generated from bone drilling. J Oral Surg.1980;38:499-503.

Lavine LS, Lustrin I, and Shamos MH. Experimental model for studying the effect of electric current on bone *in vivo*. Nature. 1969;224:1112-1113.

Lavine LS, Lustrin I. Electric enhancement of bone healing. Science. 1972 March;175:1118-21.

Ledermann PD. Schroeder A. Sutter F. Single tooth replacement with the aid of the ITI (International Team fur Implantologie) type F hollow-cylinder implant (late implant). [German] SSO: Schweizerische Monatsschrift fur Zahnheilkunde. 1982 Dec.; 92(12):1087-98.

Ledermann PD, et al. Bar-prosthetic management of the edentulous mandible by means of plasma-coated implantation with titanium screws. [German]. Deutsche Zahnarztliche Zeitschrift. 1979 Dec; 34(12):907-11.

LeGeros RZ, Craig RG. Strategies to affect bone remodeling: osteointegration. J Bone Miner Res. 1993 Dec;8 Suppl 2:S583-96.

Leroy S, Lavine MD, Grodzinsky AJ. Current concepts review electrical stimulation of repair of bone. J of Bone and Joint Surg. 1987;66-A:626-630.

Lee RC, Rich JB, Kelley KM, Weiman DS, Mathews MB. A comparison of in vitro cellular responses to mechanical and electrical stimulation. The American Surgeon. 1982;48:567-574.

Marino AA, Becker RO. Origin of the piezoelectric effect in bone. Calcif Tissue Int. 1971; 8:177-180.

Masureik C, Eriksson C. Preliminary clinical evaluation of the effect of small electrical currents on the healing of jaw fractures. Clinical Ortho. 1977;124:84-91.

Matsunaga S, Ijiri K, Sakou T. Immunohistochemical study of activating osteoblasts from undifferentiated mesenchymal cells by electrical stimulation. J Jpn Orthop Ass. 1993;14:327-332.

McElhaney JH, Stalmaker R, and Bullard R. Electric fields and bone loss of disuse. J Biomech. 1968;1,47.

McKinney RV, Lemons JE. The dental implant. PSG Pub Co, Inc. 1985.

McLeod KJ, Lee RC, Ehrlich HP. Frequency dependence of electric field modulation of fibroblast protein synthesis. Science. 1987;236:1465-1468.

Melcher AH, Accursi GE. Osteogenic capacity of periosteal and osteoperiosteal flaps elevated from the parietal bone of the rat. Arch Oral Biol. 1971;16:573-580.

Moriya M, Tanaka H. Experimental study on the application of direct current to the intra-osseous implant. Nippon Hotetsu Shika Gakkai Zasshi. 1990 Apr;34(2):309-17.

Myers BS, et al. Improved assessment of lumbar vertebral body strength using supine lateral dual energy X-ray absorptiometry. J Bone Miner Res. 1994;9:687-93.

Myers BS, Belmont PJ Jr, Richardson WJ, Yu JR, Harper KD, Nightingale RW. The role of imaging and *in situ* biomechanical testing in assessing pedicle screw pull-out strength. Spine. 1996 Sep 1;21(17):1962-8.

Noda M, Sato A. Appearance of osteoclasts and osteoblasts in electrically stimulated bones cultured on chorioallantoic membranes. J Jpn Orthop. 1985;193:288-298.

Norton LA, Moore RR. Bone growth in organ culture modified by an electric field. Growth. 1971;51:1492-1499.

O'Connor Bt, Charlton MH, Currey JD, Kirby DRS, and Woods C. Effects of electric current on bone *in vivo*. Nature. 1969;222:162-163.

Ohashi T. Electrical callus formation and its osteogenesis. Nippon Seikeigeka Gakkai Zasshi. 1982 Jul;56(7):615-33.

Ohtsu A, Kusakari H, Maeda M, and Takano Y. A Histological Investigation on Tissue Responses to Titanium Implants in Cortical Bone of the Rat Femur. J Periodontol. 1997;68:270-283.

Park JB, and Kenner KH. Effects of electrical stimulation on the tensile strength of the porous implant and bone interface. Biomater Med Devices Artif Organs 1975;3(2):233-43.

Park JB, Kenner GH. Effect of electrical stimulation on the interfacial tensile strength and amount of bone formation. Biomater Med Devices Artif Organs. 1976;4(2):225-33.

Park JB, et al. Dental implant fixation by electrically mediated process. Biomater Med Devices Artif Organs. 1978;6:291.

Parsegian JA. Molecular forces governing tight contact between cellular surfaces and substrates. J Prosthet Dent. 1983;49:838.

Paterson CI, Holmer NG, and Johnall O. Electrical stimulation of osteogenesis studies of the cathodic effect on rabbit femur. Acta Orthop Scand. 1982;55:727.

Peltier LF. A brief historical note on the use of electricity in the treatment of fractures. Clin Orthop. 1981;161:4-7.

Piattelli A, Paolantonio M, Corigliano M, Scarano A. Immediate loading of titanium plasma-sprayed screw-shaped implants in man: a clinical and histological report of two cases. J Periodontol. 1997 Jun;68(6):591-7.

Pienkowski D, Pollack SR, Brighton CT, Griffith NJ. Low-power electromagnetic stimulation of osteotomized rabbit fibulae. J. Bone and Joint Surgery. 1984;76-A:489-501.

Pilliar RM, et al. Radiographic and morphologic studies of loadbearing porous-surfaced structured implants. Clin Orthop. 1981;156:249.

Pollack SR. Bioelectrical properties of bone. Orthopedic Clin North Am. 1984;15(1):3-14.

Renooij W, Janssen LWM, Akkermans LMA, Lagey CLRS, Wittebol P, Electrode-oxygen consumption and its effects on tissue-oxygen tension. A study by mass spectrometry. Clin. Orthop. 1983;173-239-244.

Rijal KP, Kashimoto O, Sakurai M. Effect of capacitively coupled electric fields on an experimental model of delayed union of fracture. J Orthop Res. 1994;12(2):262-267.

Roberts RW, Smith RK, Zilberman Y, Mozsary PG, Smith R. Osseous adaption to continuous loading of rigid endosseous implants. Am J Orthod 1984;86:95-111.

Robertson D, St.Pierre L, Chahal RL. J Biomed Mater Res Symp. 1976;10:335-344.

Robinson KR. The responses of cells to electrical fields: A review. J. of Cell Biology. 1985;101:2023-2027.

Rodan G, Bourret L, Norton L. Electric field stimulation of DNA synthesis in chondrocytes mediated by membrane ion fluxes. J Dent Res. 1977;56:B98.

Ross MH, Romrell LJ, Kaye GI. Histology. A text and atlas. Williams & Wilkins. 1995; 3rd ed.

Rüedi TH. Titan und Stahl in der Knochenchirurgie. Hefte Unfallheilkd. 1975;123:1.

Salman NN, Park JB. The effect of direct electrical current stimulation on the bone/porous metallic implant interface. Biomaterials. 1980 Oct;1(4):209-13.

Schutzer SF, Jasty M, Bragdon CR, Harrigan TP, Harris WH. A doulble-blind study on the effects of a capacitively coupled electrical field on bone ingrowth into porous-surfaced canine total hip prostheses. Clinical Orthopaedics and related research. 1990;260:297-303.

Scott G, King JB. A prospective, double-blind trial of electrical capacitive coupling in the treatment of non-union of long bones. J bone Joint Surg. 1994;76-A(6):820-26.

Sennerby L, Thomsen P, Ericson LE. A morphometric and biomechanic comparison of titanium implants inserted in rabbit cortical and cancellous bone. Int. J. of Oral & Maxillofacial Implants. 1992;7:62-71.

Sennerby L, Lekholm U, Ericson LE. Soft tissue response to clinically retrieved titanium implants reinplanted in the rat's abdominal wall. Soc biomater symp retrieval and analysis surg. Implants and Biomater. 1988;27.

Serway RA. Physics for scientists & engineers with modern physics,1990;3rd ed.

Shafer DM, Rogerson K, Norton L, Bennett J. The effect of electrical perturbation on osseointegration of titanium dental implants:A preliminary study. J of Oral & Maxillofacial Surgery. 1995;1063-1068.

Shandler HS, Weinstein S, Nathan LE. Facilitated healing of osseous lesions in the canine mandible after electrical stimulation. J Oral Surgery. 1979;37:787-792.

Sharrard W. A double blind trial of pulsed electromagnetic fields for delayed union of tibial fractures. J Bone Joint Surg. 1990;72:347-355.

Smedberg JI, Lothigius E, Bodin I, Frykolm A, Nilner K. A clinical and radiological two-year follow-up study of maxillary overdentures on osseointegrated implants. Clinical Oral Implants Research. 1993;4:39-46.

Soballe K, Gotfredsenk, Brockstedt-Rasmussen, Nielsen PT, Rechnagel K. Histologic analysis of retrieved hydroxyapatite-coated femoral prosthesis. Clin Orthop. 1991;272:255-258.

Somjen D. Binderman I. Berger E. Harell A. Bone remodelling induced by physical stress is prostaglandin E2 mediated. Biochimica et Biophysica Acta. 1980 Jan; 627(1):91-100.

Spadaro JA. Bioelectric stimulation of bone formation: methods, models and mechanisms. J Bioelectricity. 1982a;1:99.

Spadaro JA. Electrically enhanced osteogenesis at various metal cathodes. J Biomed Mater Res. 1982b Nov;16(6):861-73.

Spadaro JA, Mino DE, Chase SE, Werner FW, Murray DG. Mechanical factors in electrode-induced osteogenesis. J of Orthopaedic Research. 1986;4:37-44.

Spadaro JA. Mechanical and electrical interactions in bone remodeling. Bioelectromagnetics. 1997;18(3):193-202.

Stefan S, Sansen W, and Mulier JC. Experimental study on the electrical impedance of bone and the effect of direct current on the healing of fractures. Clin Orthop. 1976;120:264-267.

Steflik DE, Lake FT, Sisk AL, Parr GR, Hanes PJ, Davis HC, Adams BO, Yavari J. A comparative investigation in dogs: 2-year morphometric results of the dental implant--bone interface. Int J Oral Maxillofac Implants. 1996 Jan-Feb;11(1):15-25.

Steiner M, Ramp WK. Electrical stimulation of bone and its implications for endosseous dental implantation. J of Oral Implantology 1990;XVI:20-27.

Tannenbaum GS, Martin JB. Evidence for an endogeneous ultradian rhythm governing growth hormone secretion in the rat. Endocrinology. 1976; 98:562-570.

Ten Bruggenkate CM, van der Kwast WAM, Oosterbeek HS. Success criteria in oral implantology. Int J Oral Implant. 1990a;7:45-51.

Ten Bruggenkate CM, van der Kwast WAM, Oosterbeek HS. Patient selection in oral implantology. Int J Oral Implant. 1990b;7:53-60.

Thomas KA, Cook SD. An evaluation of variables influencing implant fixation by direct bone apposition. J Biomed Mater Res. 1985;19:875.

Thomas KA, Kay JF, Cook SD, Jarcho M. The effect of surface macrotexture and hydroxylapatite coating in the mechanical strengths and histologic profiles of titanium implant materials. J Biomed Mater Res. 1987;21:1395.

Thomsen P, Ericson LE. Light and transmission electron microscopy used to study the tissue morphology close to implants. Biomaterials. 1985;6:421-424.

Tjellström A, Jacobsson M, Albrektsson T. Removal torque of osseointegrated craniofacial implants: A clinical study. Int J Oral Maxillofac implants. 1988;3:287-289.

Uhthoff K. Mechanical factors influencing the holding power of screws in compact bone. J Bone Joint Surg. 1973;55B:633.

Van Steenberghe D, Lekholm U, Bolender C, Folmer T, Henry P, Herrmann I, Higuchi K, Lancy W, Linden U, Astrand P. The applicability of osseointegrated oral implants in the rehabilitation of partial edentulism: A prospective multicenter study of 558 fixtures. Int J Oral Maxillofac Surg. 1990;5:272-281.

Vanore JV, Jacobs AM. Electrically induced osteogenesis. Podiatry Tracts. 1992;5(3):117-133.

Vingerling PA, van der Kuij P, DeGroot K, Sillevis Smitt PAE. Non-invasive treatment of alveolar wounds. In: Electrical properties of bone and cartilage. Brighton CT, Black J, Pollack S. Eds, New York. Grune and Stratton. 1979;341-346.

Weinstein AM, Klawitter JJ, Cleveland TW. Electrical stimulation of bone growth into porous A12O3. J Biomed Mater Res. 1976 Mar;10(2):231-47.

Worthington P. Current implant usage. Int J Oral Implantology. 1988;5(2):49-51.

Worthington P, Lang BR, LaVelle WE. Osseointegration in dentistry. Quintessece Pub Co, Inc. 1994.

Yamagata M, Hiroshi K, Shohei M, et al. Mechanical stability of pedicle screw fixation systems for the lumbar spine. Spine. 1992;17:S51-4.

Yamanaka E, Tjellstrom A, Jacobsson M, Albrektsson T. Long term observations on removal torque of directly bone-anchored implants in man. Transplants and implants in Otology II. 1992;1-6.

Yasuda I. Piezoelectricity of living bone. J Kyoto Pref Univ Med. 1953;53:325.

Yasuda I. Mechanical and electrical callus. Ann NY Acad Sci. 1974;238:457-563.

Yonemori K, Matsunaga S. Early effects of electrical stimulation on osteogenesis. Bone. 1996 Aug;19(2):173-80.

Young SO, Park JB, Kenner GH, Moore RR, Myers BR, Sauer BW. Dental implant fixation by electrically mediated process. I. Interfacial strength. Biomater Med Devices Artif Organs. 1978;6(2):111-26.

Young FA, Spector M, and Kresch CH. Porous titanium endosseous dental implants in rhesus monkeys: Microradiography and histological evaluation. J Biomed Mater Res. 1979a;13:843.

Young FA, Spector M, and Kresch CH. Porous titanium tooth roots: Clinical evaluation. J Prosthet Dent. 1979b;41:561.

Zengo AN, Bassett CA, Prountzos G, Pawluk RJ, Pilla A. In vivo effects of direct current in the mandible. J Dent Res. 1976 May-Jun;55(3):383-90.

Zorlu U, Tercan M, Ozyazgan I, Taskan I, Kardas Y, Balkar F, Ozturk F. Comparative study of the effect of ultrasound and electrostimulation on bone healing in rats. Am J Phys Med Rehabil. 1998 Sep-Oct;77(5):427-32.