Bioelectric Perturbations of Bone

Research Directions and Clinical Applications

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A review of the clinical uses of bioelectric perturbation of hard tissue in medicine and dentistry is presented, along with some current research directions in the field. The authors present some of the hypotheses about the cellular mechanisms of this phenomenon.

In orthopedic pedagogy, the example of teeth moving through bone in response to applied force is the classic paradigm for mechanical stresses causing clinical changes. The way these forces are mediated to the cellular component of the tissue is still not well understood.

The observation that mechanical loading of bone produces electrical changes has led to speculation that these electrical perturbations might influence the biological processes of hard tissue cells.

On the basis of this assumption, potentials and currents were applied to bone in an attempt to influence its growth. Within certain limits, this proved to be successful in affecting the creation of new bone tissue and stimulating healing in recalcitrant fractures of long bones.

Research in the area of electrical

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stimulation has developed in three main directions.

First, there has been a great deal of effort in the clinical area to develop methods for treating nonunions or related problems in bones and to elucidate the molecular mechanisms by which these events occur.[1] Modest attempts have also been made to solve dental problems by application of these principles.

A second area for investigation has been the effects of electrical perturbations on living systems or organs, attempting to elucidate the properties of tissue regeneration and to ultimately control regeneration itself.[2]

The third topic of interest has been electromechanical properties of hard tissue.[3] These studies focus on the physics of bone, tooth or cartilage as a composite material, and are not directly concerned with physiology. The methods used are bench tests for strain-dependent potentials, relating them to the ferroelectric character of bone and its osteonic structure.

Bioelectric perturbation has become a tool for probing the interrelationships among the mechanical, chemical and electrical properties of tissue and its cellular response to external stimuli. The focus in this report is on the more clinically relevant information.

A Brief Historical Review

Bone healing via electrical means was used as early as the 19th century.[4–8] The application was empirical, and although reports indicate clinical success, the scientific community of the time showed little interest in pursuing this phenomenon.

Current interest in electrical effects in bone dates from 1957, when Fukada and Yasuda[9] demonstrated that stress induced a region in bone which was electronegative in relation to a region which was not stressed. They attributed this effect to the piezoelectric nature of the crystalline structure of bone.

Working independently in 1962, Bassett and Becker[10] also showed that bone produced electrical potentials when deformed. Their experiments indicated that the amplitude of the electrical potentials created in the stressed bone was dependent on both the rate and the magnitude of the deformation. Polarity was dependent on the direction of bending; areas under compression were electrically negative in relation to other areas.

They deduced, as did Fukada and Yasuda, that there is a relationship between piezoelectricity and callus formation. They also proposed that stress-induced bioelectric potentials were the command signals in the operation of Wolff's Law, and that these signals controlled both bone cell activity and the orientation of their macromolecular byproducts.

Bassett[11,12] also stated that bone formation occurred in areas of negative charge, while bone remodeling took place in areas of positive charge. These results captured the imagination of many researchers working in the field of calcified tissue, and led many investigators to study the effects of electrical charges and currents on bone formation.

Bassett *et al*[13] induced significant bone formation around surgically implanted cathodes in the middle-lateral area of femora of adult beagle dogs. Tissue response around the anodes in these dogs was similar to that seen around surgically implanted nonactive electrodes in contralateral control femora.

O'Connor *et al*[14] repeated these ex-

periments and found a similar, though not identical, response to that reported by Bassett. They cautioned that even though the response appeared to be real, it seemed to vary considerably within species, and caution should be used in the clinical application of electrical fields.

Lavine *et al*[15] applied 2-4 μAmperes of direct electric current to surgical osteotomies in rabbit femora for periods of one to three weeks. They found that rabbits exposed to an electric current of 2.5-3.5 μAmperes for three weeks showed the greatest amount of healing compared to control osteotomy sites, actually showing more healing than was seen in six-week non-electrically treated control osteotomies.

Stefan[16] exposed fractures of tibiae and femora in rabbits to continuous and oscillating DC currents, evaluating their efforts radiographically and histologically. He stated that a 10 μA interrupted DC current reduced the period of healing from around 40 days to 15-20 days. Uninterrupted DC currents were less effective. Thus, it appears that the fresh fracture healing rate could be enhanced by a very specific electrical input.

Martin and Gutman[17] exposed immobilized rat femora to 30Hz perturbations 200V peak to peak, delivered via insulated copper electrode plates twice a day.[17] After 28 days in the cast, disuse osteoporosis had not developed in the electrically stimulated bones. Indeed, these bones were shown to have increased cortical area, bone mass, density and percent ash weight.

Clinical applications of these principles have shown much success. The greatest success has been seen in patients with nonunion fractures, and to a lesser degree in congenital pseudoarthrosis which had been unresponsive to more conventional treatment modalities.

Lavine *et al*,[18] using an apparatus similar to the one used to increase healing of osteotomies in rabbits, showed the beginning of bony union after only four months treatment in a patient with a congenital pseudoarthrosis.[18] Histological examination of the healing area showed deposition of normal bone and collagen. This patient had not responded to the more conventional treatment of surgery and stabilization, and was a candidate for amputation.

Jorgensen[19] treated crural fractures with an electrical stimulator mounted on a Hoffman apparatus. The stimulator delivered a 1Hz alternating potential and a direct current of 20-100 μA. He found that healing time decreased 30% for those patients with the electrical stimulator.

Further clinical trials of direct electric currents have also met with success. Healing has been 81% with direct current stimulation of fractures of long bones which have gone over 9 months with nonunion.[20] The success varies from bone to bone, the tibia healing 86% of the time, the fibula 100%, the radius 93% and the humerus uniting successfully at a rate of only 54%. The major reason for these variations appears to be the variability in immobilization.

Surgical procedures are required for implantation and removal of electrodes at the fracture site for the use of direct electric current. (Fig. 1) Such invasive procedures have obvious risks and drawbacks. This led investigators to search for a nonsurgical method of delivering an electric current to a fracture site. There are two possible alternatives—capacitive cou-

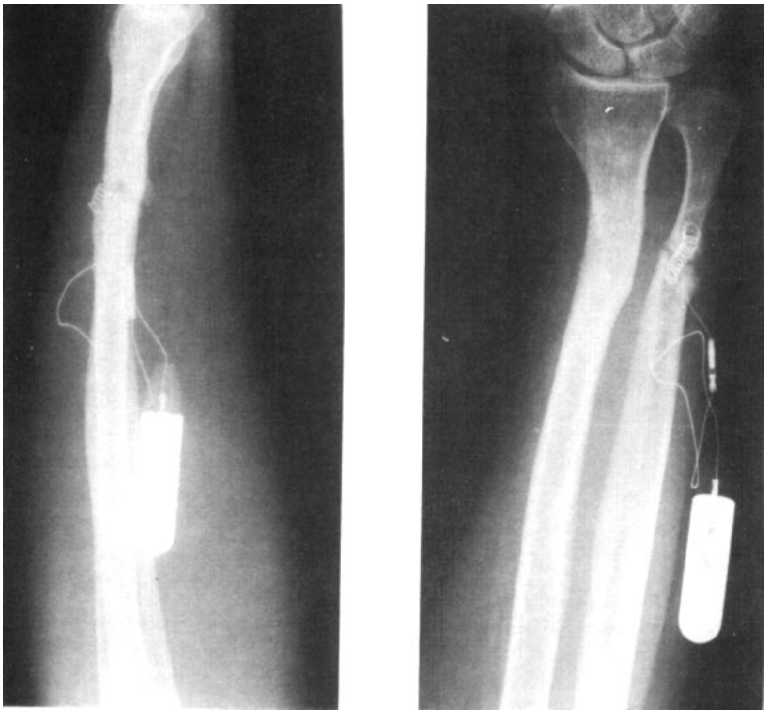

Fig. 1 Two x-ray views of an implanted direct current device for stimulation of healing in a fractured ulna which had a nonunion for 1½ months.

pling of electrostatic and electrodynamic fields and inductively-coupled pulsed electromagnetic fields.

Capacitive-coupled electrostatic and electrodynamic fields were tested experimentally but rejected as being potentially hazardous to the patient because of the relatively large voltages required to achieve the desired results. However, recent studies indicate that this technique may still hold clinical promise.[20a]

Other investigators have studied the effects of inductively-coupled pulsed electromagnetic fields on surgically treated osteotomies in adult beagle dogs. They were also used later to correct pseudoarthroses and nonunion fractures.[21-24]

In humans, inductively-coupled pulsed electromagnetic fields (PEMF) have shown an overall success rate of 75% in the treatment of pseudoarthroses and nonunion fractures. These have ranged from 86% success on tibial fractures down to 60% on the hemerus. Unlike the direct current devices, the inductively-coupled apparatus can be used in the presence of bone infection[25] (Fig. 2).

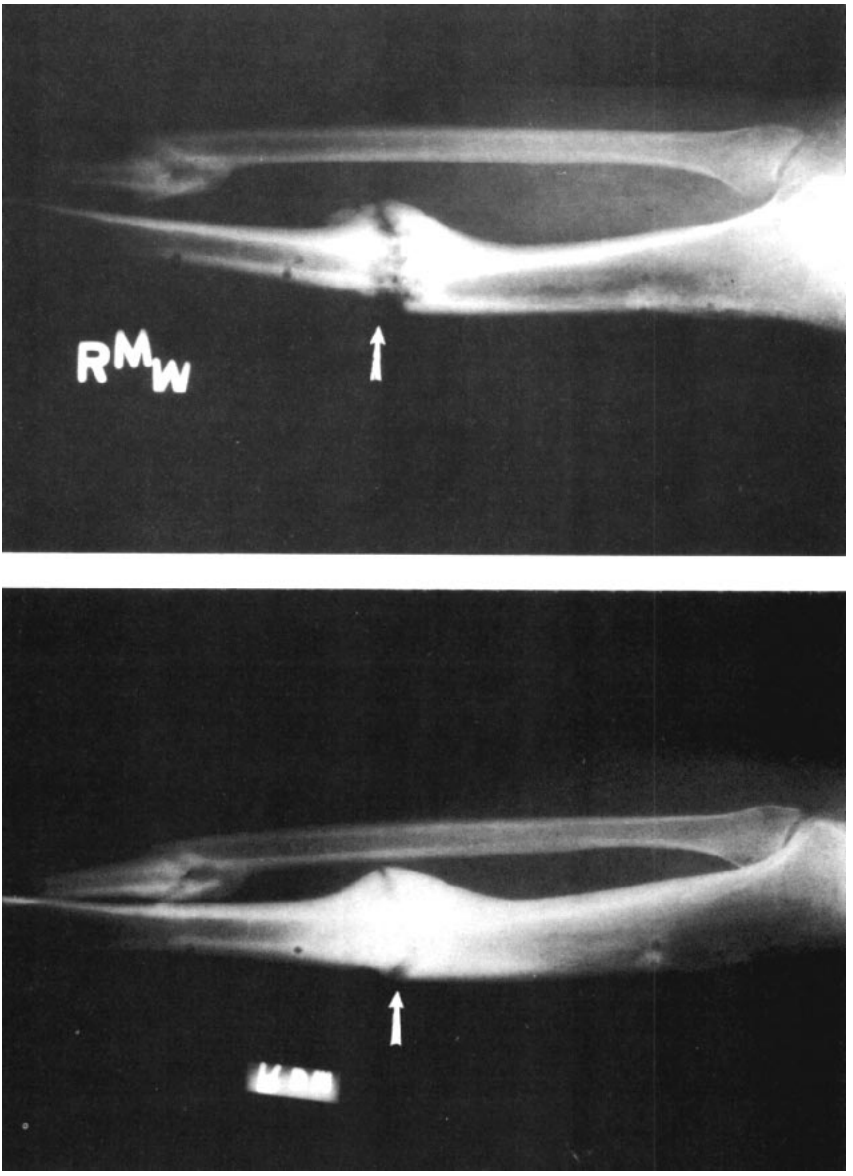

Fig. 2 Top, radiograph of a two-year hypertrophic nonunion in the midshaft of the tibia. The fibula, although appearing to be in pieces, was healed. The circular radiolucent areas are bone screw holes.

Bottom, the healed fracture after six months of treatment with pulsed electromagnetic fields applied without surgical invasion. There is a small radiolucent "scar" at the old fracture site, but the bone is rigid and pain-free. Such old fracture lines usually remodel and often disappear in time.

Applications in Dentistry

Application of bioelectric perturbation to clinical dental problems has been very limited.

Cochran, Pawluk and Bassett[26] recorded electrical potentials in bovine bone sections in response to calibrated axial and sagittal loads on teeth at various reference points in the cortex near the teeth. This led them to the hypothesis that electrical currents may be generated in bone during mastication, deglutition and through hemodynamic action within the bone. They felt that this may account for normal physiologic tooth and bone relationships as well as bone response to orthodontic forces.

Soon after, Gillooly *et al*[27] reported differences in electrical potentials when known forces were applied to the dentition in dried canine alveolar bone. The authors concluded that the polarity and magnitude of the peak voltage response related to the direction and magnitude of force applied to individual teeth. They stated that monitoring the bioelectrical changes in alveolar bone in response to force application might be a useful research tool for measuring the relationships between forces and hard tissue response

Zengo, Pawluk and Bassett[28,29] investigated stress-induced electrical potentials generated in the alveolar process. When nonvital teeth were loaded with controlled forces, a transient biphasic voltage was recorded in dried alveolar bone. This led the authors to speculate that there may be a piezoelectric control for bone apposition or resorption as a response to orthodontic forces, suggesting that Wolff's Law may be mediated by bioelectrical phenomena. DiAngelis[30] and Norton[31] have both expounded on these observations.

Zengo *et al*[32] next examined the same phenomenon in vivo. They were able to show voltage variations along the alveolar bone opposite the root. The data corresponded roughly to the variation that one might expect in the stress on the alveolar bone as a result of dental tipping around a midroot center of rotation. This work combined and correlated biomechanical theory with biophysical measurements.

Since many in vitro studies indicated that new bone growth could be stimulated by bioelectric perturbation, Jacobs and Norton[33,35] and Kopczk *et al*[34] attempted to replace lost alveolar bone in periodontally diseased beagle dogs using two different direct current devices. Some increase was seen in endosteal bone, but the studies were inconclusive.

In oral surgery, Masuriel[36] used a direct-current device to enhance healing of fresh mandibular fractures anterior to the mental foramen. Electrical stimulation enhanced primary healing as measured by mobility, but no difference was observed between control and stimulated groups at the end of the usual intermaxillary fixation time.

Vingerling *et al*[37] and Van der Kuij[38] did an extensive study on control of residual ridge reduction by bioelectrical means. They placed an inductive-coupled device adjacent to the alveolar ridge of partially edentulous beagle dogs in an attempt to decrease ridge resorption secondary to the loss of buccal teeth. The ridge reduction was lessened by this treatment at statistically significant levels, but the effect was clinically small. In time, without continued electrical perturbation, the ridge reduction on the two sides became similar.

It is interesting to note that the mucosa on the electrically stimulated side appeared healthier than comparable control tissue. The composition of organisms in the microbiological flora of the mucosa also differed between control and experimental dogs. Withdrawal of stimulation resulted in a shift of the microflora composition toward the control profile.

Finally, this inductive-coupled device apparently radiated energy affecting biologic processes over a long distance. The control ridge on the side opposite an experimentally stimulated ridge showed less resorption than similar controls in other animals that did not have an active bioelectrical device. These control animals were even caged far from the electrically perturbed animals.

Few studies have been made on the response of bone resorption and remodeling to anodal stimulation. This area has some exciting clinical implications. Davidovitch *et al*[39-42] have investigated the effects of DC current at the anode on bone resorption and tooth movement in cats. Using immunohistochemical as well as physical measurements, they showed an increase in bone remodeling, a slight enhancement of speed of tooth movement and increased periodontal nucleotide levels over the experimental period of two weeks.

Beeson *et al*[43] did a similar experiment over a longer period of time, using a different electrical perturbation and measuring only rate of tooth movement. They found an increase in the rate of tooth movement near the anode in the first two weeks, but no difference over a longer period of time.

Shapiro *et al*[44] described a patient who received a pulsating force theoretically designed to act as a piezoelectric stimulus to bone in order to enhance tooth movement over a short time. It was never determined whether a measurable piezoelectric current was induced in the patient by the device. No biological measurements were made to determine whether the observed tooth movement was bioelectrically induced or merely caused by the complex controlled intermittent force.

The important point is that orthodontists and others are testing many hypotheses related to these bioelectrical phenomena in an effort to find clinical applications.

Current Mechanistic Hypotheses

The important underlying question is exactly how bioelectric perturbation actually affects hard-tissue cells. The mechanism is not known, but many hypotheses have been proposed and new evidence is being found.

Clusters of charged molecules of proteins, lipids, lipoproteins and cholesterol form receptor sites in the cell wall. These molecules of the membrane flow at different rates. For example, it has been calculated that lipids move approximately three times faster than the proteins.[45,46] There are complex charge patterns as the molecules interact with each other and with the plasmas surrounding them inside and outside the cell.

These charges may be altered by the superimposition of direct or induced current flow on the membrane surface through the electrolyte environment in which it dwells. Thus, ions may move across the cell membranes.

Critical chemical concentration gradients may change, turning on (activating) various biological pump mechanisms.

Receptor sites may be activated by the change in charge so that low con-

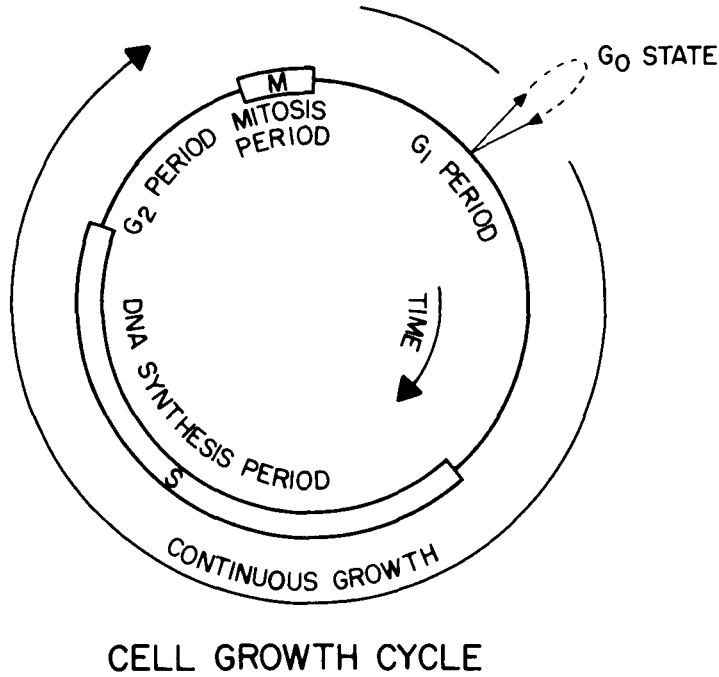

Fig. 3 A classic diagrammatic representation of the cell cycle. G_0 state is the normal waiting subcycle where cells are recruited to divide. Once the cell swells into the G_1 phase, it soon enters the DNA synthesis of S phase. There is a short wait in G_2 while microtubules are synthesized and assembled. Then the cell enters M for mitosis, division and production of daughter cells.

centrations of normal circulating hormone-like molecules may cause an enzymatic cellular response.

Alternately, inhibitors of some important biochemical process such as the calcium-dependent current on the cell membrane may temporarily lose their ability to bind or dam a potential effect, setting a cascade of events into motion.

Data collected from various laboratories using many systems allow us to speculate about the sequence of events that may take place.[47-58] It is likely that within milliseconds, cation flux changes involving Na^+ and K^+ take place. Changes produced by ATP*ase* are detectable within minutes after perturbation. Changes in cyclic AMP concentration and Ca^{++} flux have been noted at about the same time.

The charge apparently changes the physical characteristics of the cell surface, because changes in cell adherence have also been detected. This may be due to changes in the charge on the cell surface or to changes in matrix production with an increase in fibronectins or other nectin-like products.

There are changes or shifts in the cell cycle (Fig.3). For example, cells in G_2 are recruited into the M phase of the cell cycle. Cells are also mobilized from G_0 and G_1, expressed as increased numbers of cells entering the

DNA synthesis phases as measured by ^{3}H-thymidine uptake studies.

Thus, cell proliferation may be enhanced by bioelectric perturbation. This has been independently observed in many laboratories using various stimulation techniques.[51, 52, 54, 59, 60, 61] Since cell proliferation is enhanced, one might be concerned about induction of neoplasia from bioelectric perturbation. This has not been demonstrated clinically or in a repeatable fashion in the laboratory. The predominant effect appears to be at the cell membrane through activation of cytosolic processes leading to increased proliferation, without affecting the process of DNA replication itself. This makes it unlikely that a neoplastic event could be caused by this modality.

The quantity of the products of cell differentiation may also be affected by bioelectric stimuli. For example, it was recently shown that components of the cartilaginous matrix and possibly the matrix's biochemical geometry was altered by pulsed electromagnetic fields[62,63,64] (Fig. 4). The changes in metabolic products were dependent on the growth state of the cells within the matrix.

The quantitation and integration of these components of the matrix are often interdependent. A change in a highly charged molecule may eventually affect all the others. In addition, changes in proteoglycan structure lead to local changes in pH, pO_2, and ultimately in calcification.[57,65] These observations have long been associated with the hypothesis that bioelectric perturbation ultimately enhances calcification in a nonunion.[12]

Empirically, one very successful clinical orthopedic treatment modality involves intermittent stimulation with a pulsating electromagnetic field (PEMF) applied via coils precisely placed in relation to the unhealed fibrous defect.

One study indicated that a rest period following PEMF stimulation may be an important factor in the success of electrical perturbation.[63] Continuous PEMF stimulation failed to enhance collagen synthesis more than shorter intermittent exposure periods followed by rest periods outside the field.[62] This observation that PEMF effects continue well after the pauses suggests that in vivo PEMF may affect an early critical biochemical event in the osteogenic process leading to the observed proliferation or differentiation.

A biphasic effect with continuous exposure to PEMF has also been noted.[66,67,68] Depending on cell state (G_0 or G_1), PEMF may or may not be effective. This biphasic susceptibility to stimulation has precedence in pharmacology, as in cyclic AMP control of proliferation or differentiation.

In orthodontics, the principle of intermittent application of an external perturbation above a certain threshold value to provide maximum bony response is used clinically for tooth movement, and current evidence shows that intermittent force application which provides the tissue with rest periods results in the most physiologic tooth movement with the minimum of undesired sequelae.

A detailed look at the electrical perturbations produced by PEMF in vitro is helpful in understanding the PEMF-induced effects seen clinically. PEMF is capable of generating a current in any conductive material. This includes the electrolytic extracellular environment as well as any intracellular charged molecules. Measurement of the current generated by electromagnetic fields used in vitro has shown

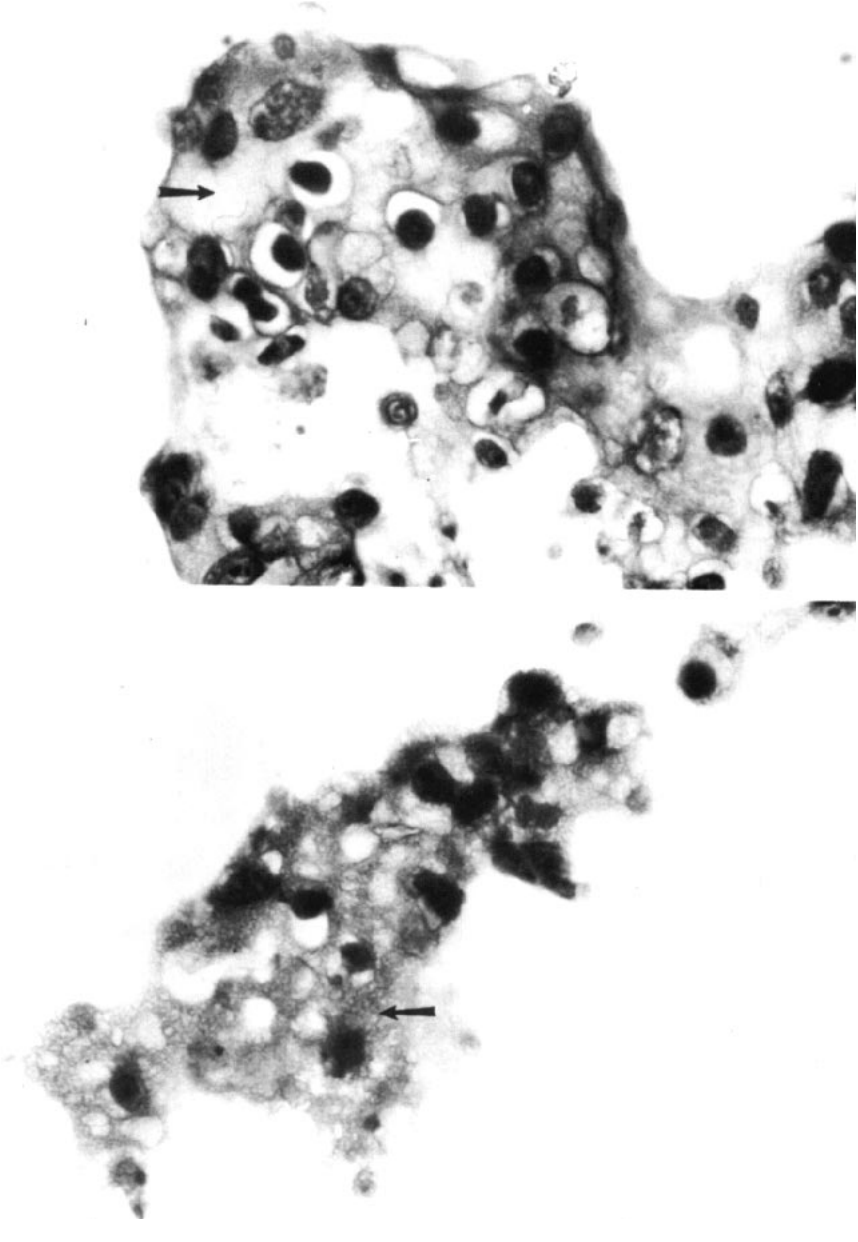

Fig. 4 Top, control specimen of cartilage tissue culture stained with hematoxalin and eosin. Note the relatively smooth nature of the extracellular matrix (arrow).

Bottom, similar specimen of cartilage tissue culture after being subjected to a pulsed electromagnetic field for 48 hours. Notice how the extracellular matrix appears to have a moth-eaten appearance (arrow).

that in a saline environment, they produced an induced electrical field of approximately lmV/cm and current from 2-8μA/cm$_2$, depending on the radius of the culture dish.[69]

The effect of direct current on tissue development and repair has been extensively reviewed.[70] The data show that a DC current affecting a biological system such as the cell membrane can be inferred from the current passing through the extracellular medium. Oscillating DC currents were shown to produce biological effects such as alkaline phosphatase inhibition or prostaglandin stimulation similar to those produced by PEMF.[72,73]

Since fracture repair has been achieved with two types of DC and with pulsating inductive currents, we must consider whether they both act through similar responses in the target cell or tissue.

It has been hypothesized that continuous DC currents do not penetrate the cell membranes, but cause electrochemical changes in the cell membrane from ionic redistribution and translocation of charged molecules on the cell surface.[71] This type of response is similar to the changes in membrane permeability subsequent to hormonal binding by surface receptors. It may also involve a second messenger such as cyclic AMP or a cytoskeletal rearrangement for transduction of stimuli.[74,75]

On the other hand, based on the impedence properties of cell membranes, PEMF-produced pulsating currents are capable of penetrating the cell membrane.[47] Therefore, these stimuli could act either at the level of the cell membrane or directly affect intracellular organelles. They may, in fact, be more efficient in eliciting a biological response.

In terms of initiating physiological fracture repair, bioelectrical perturbation may exercise control at several levels:[76]

- by promoting proliferation of osteogenically competent cells
- by increasing the probability of expression of differentiated properties in a precursor cell population
- by controlling the expression of the differentiated phenotype (matrix formation or calcification) through activation of competent cells.

These concepts have been addressed with regard to skeletal tissue containing osteogenic precursor cells that can differentiate only in bone formation, and inductible osteogenic precursor cells which require the presence of an inducing agent to trigger the differentiated state.[77] It is possible that bioelectric stimulation is involved at several of these levels.

Some effects of PEMF on control of cartilage matrix differentiation have been demonstrated recently.[63,64]

It is known that calcified cartilage matrix contains fewer anionic glycosaminoglycans, which are components of proteoglycans, than noncalcified cartilage.[78] In general, the sulfate content and size of proteoglycan aggregates is higher in a noncalcified cartilage matrix than in calcified cartilage. Thus, a decrease in either proteoglycan aggregate size, acidity or charge appears to promote calcification.[78]

It has also been demonstrated that application of hyaluronidase to aspirate of rat hypertrophic cartilage leads to mineral deposition.[79] In addition, the relative concentration of lysozyme, an enzyme localized in the extracellular matrix of cartilage, is elevated in areas undergoing calcification. PEMF alters the sulfation of the glycosaminoglycan molecule and liberates hyaluronic acid from cartilage in vitro, and

the activity of lysozyme found in this matrix is greatly enhanced. The decrease in sulfation from PEMF perturbation can be enhanced by the addition of exogenous lysozyme or blocked by the addition of lysozyme inhibitor.

Thus, bioelectrical control of cell function can modify specific cell products and lead to changes in tissue state.

Bioelectrical stimulation may also be a general multifaceted stimulus. Ultimate effects may be dependent on the responsiveness of the cell, its threshold to stimulation and its state of differentiation.

Summary and Conclusion

Bioelectric perturbation of living hard tissue produces clinically useful effects. It is being used effectively in the treatment of intractable orthopedic problems such as nonunion and avascular necrosis.

Dental applications of this phenomenon are still in the early stages of research and development. Barring untoward circumstances, this form of local growth control may be used as an adjunct to localized bone induction therapy such as in the treatment of periodontal diseases, bone grafting or implantation of biocompatible products. It also appears to show promise in enhancing the rate of tooth movement or the stability of anchor teeth.

The mechanism for these perturbation-induced changes in cells has not been explained. This is an active area of research with many implications for helping the clinician understand the molecular biology of hard-tissue cell proliferation, migration and differentiation.

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