IEEE 1981
FRONTIERS OF ENGINEERING IN HEALTH CARE

B. A. Cohen, Editor

September 19-21, 1981
Shamrock Hilton Hotel
Houston, Texas

"Frontiers of Engineering in
Health Care"
with
"Frontiers of Computers in Medicine"

The 3rd Annual Conference
of the
Engineering in Medicine
and Biology Society
of the
Institute of Electrical
and Electronics Engineers

81CH1621-2

ELECTRET-INDUCED BONE FORMATION IN RATS

A. A. Marino, Ph.D.*, J. M. Cullen, Ph.D., R. O. Becker, M.D.
E. Fukada, D.Sc.**
*Department of Orthopedic Surgery, Louisiana State University Medical Center, Shreveport, Louisiana 71130
Upstate Medical Center, Syracuse, New York 13210
**The Institute of Physical and Chemical Research, Saitama, Japan

Abstract

Electrical osteogenesis at 1-50 μa may be explained in terms of a non-specific irritative response such as occurs following trauma, heat, or other diverse stimuli. But the osteogenic responses observed at substantially lower currents appear to arise from a different mechanism -- possibly such low currents more faithfully mimic naturally-occurring electrical signals. Electrically charged polymer films (electrets), one class of low-current sources, have previously been found to stimulate bone growth, and this study was conducted to further explore the phenomenon. We found that 50-μ thick teflon electrets with an initial surface charge of 12.3 mucoulomb/cm^2 stimulated bone growth in the rat femur 3-6 weeks after implantation. The electret-stimulated bone appeared to differ from its sham control only in amount, but not in organization or cellularity. We suggest that the piezoelectric polarization produced when the electret was flexed (by the animal's movement)was the likely mechanism of the observed effect.

Introduction

Many reports describe osteogenic effects of electromagnetic energy at currents of about 1-50 μa; above this range, tissue necrosis is frequently observed. The 1-50 μa currents are at least several orders of magnitude above the range of naturally-occurring electrical signals -- from piezoelectricity for example -- and it has been proposed that their mechanism of action involves a non-specific irritative response similar to that found with other common stimuli (1)(Figure 1).

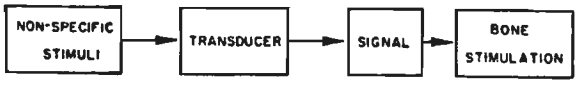

Figure 1: Many non-specific stimuli such as heat, chemicals, electromagnetic energy, and trauma are transduced into a common biological signal which is capable of initiating an osteogenic response.

There are also reports of growth stimulation at current levels that can, at least arguably, occur naturally. Biogalvanic current sources, for example, consist of two dissimilar wires joined together and insulated from the tissue except at their tips; they can produce about 1-10 mua (2, 3), and they have been reported to be effective in stimulating limb growth in frogs and rats (4, 5, 6). Another novel low-current source is the electret -- a material which exhibits an external electric field because of its specific thermal and electrical history. Electrets made from the polymer polytetrafluoroethylene (teflon)have been found to stimulate bone growth in rabbits (7), rats (8), and humans (9).

Electret-induced bone growth appears to involve a mechanism quite different from that associated with 1-50 μa currents. We have undertaken a co-operative study to further explore the nature of the influence of polymer electrets on growth. Reported here are the results of our pilot study on the consequences of implanting teflon electrets in rats.

Methods

Teflon electrets (4x5 cm, 50 μ thick)were supplied by H. Yamagami, Rion Company, Japan. They were prepared by subjecting the plastic to a high electric field at 160°C and then cooling to room temperature while continuously applying the field. Control implants were prepared by similarly heating the teflon in the absence of the field. The surface charge density, measured by the method of electrostatic induction (10)(Figure 2), was 12.3 ± 2.3 coulomb/cm^2.

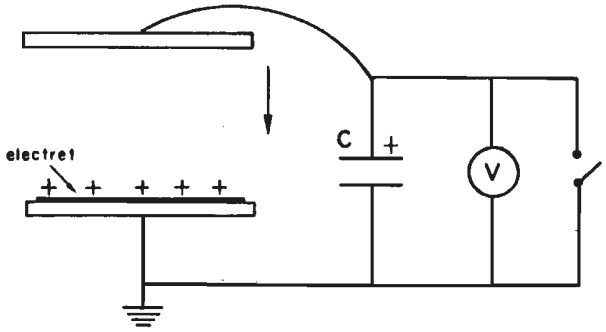

Figure 2: With the upper electrode raised, the shorting switch is opened and the electrode is lowered onto the electret. The surface charge density, σ, is given by σ = CV/A where A is the electret surface area, and V is the potential across C.

The surface charge was stable indefinitely when the electret was stored between grounded metal plates or in a low-humidity environment.

Seven-millimeter strips of the teflon were implanted in young, adult, male, Sprague-Dawley rats as follows. The rats were anesthetized with

3 1/2% chloral hydrate. For each animal, the skin on both hind limbs was opened along the lateral margin of the thigh. The fascia lata was cut longitudinally at mid-thigh and the flexor and extensor muscles were separated to expose the periosteum of the femur. Using curved-typed forceps, muscle was gently removed from the femoral shaft in a one-centimeter wide cuff around the bone. One femur of each animal was wrapped with the electret so that the ends overlapped, and the opposite femur received the control teflon. With the implants in place, the muscles were returned to their original configuration. The fascia lata and overlying skin were sutured with 3-0 silk sutures. The electrets were implanted in the right femurs of half the animals and in the left femurs of the other half: The negative and positive faces contacted the bone in approximately equal number of animals.

The rats were x-rayed weekly; at 3-6 weeks, they were sacrificed, and the femurs were excised and processed for routine staining with hematoxylin and eosin.

Results

Bone growth was seen in the portion of the shaft that contacted the teflon, regardless of the treatment regimen. The new growth was irregularly distributed on the external margin, and was generally limited to 1/4-3/4 of the circumference -- in no case did it completely circumscribe the margin (Figure 3).

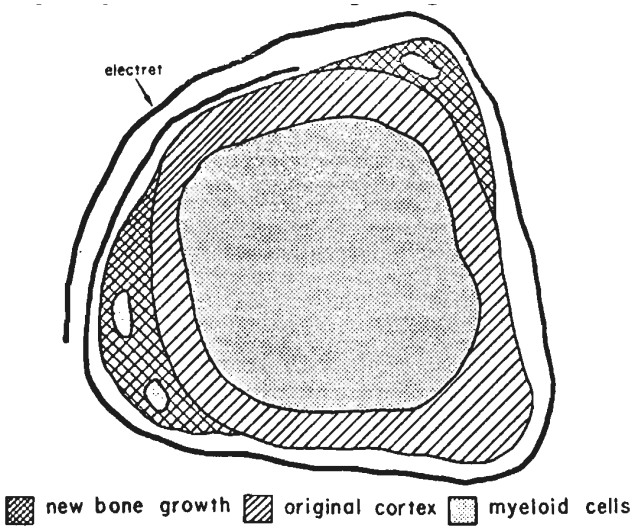

Figure 3: Relation between electret and bone growth.

No relation was found between bone surfaces on which growth occurred and the location of specific muscle groups. For the 3-week group, the new bone was predominantly cancellous and only sparse areas of new- growth compact bone were seen. In 2 of the 3 animals, there was greater bone deposition on the electret femur -- judged by the thickness of the bone cortex -- but no differences were seen with regard to the organization, vascularity, or cellularity of the new growth, and there was no signs of active osteogenesis such as are present in a typical fracture callus.

In the 6-week group, the new subperiosteal bone was composed exclusively of compact bone (Figure 4).

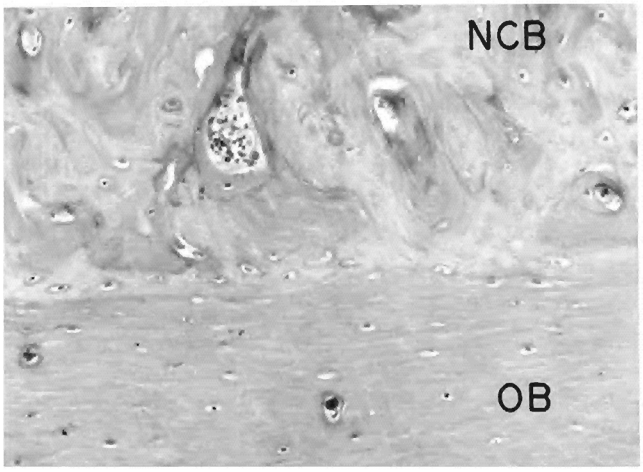

Figure 4: Bone growth 6 weeks after implantation of a teflon electret, lOOx.OB, original bone; NCB, new compact bone.

In 3 of 4 animals, the experimental side exhibited greater bone deposition than the corresponding control side. In both the 3- and 6-week groups, islands of medullary tissue containing myeloid cells were interspersed among the haversian systems. We observed no growth-stimulation effects attributable to the sign of the surface charge in contact with the bone.

At four and five weeks after implantation, an intermediate histological picture was presented consisting of both cancellous and compact new-bone growth. Again, there seemed to be greater bone deposition on the electret side (3 of 4 animals at four weeks, 2 of 4 at five weeks). In no instance did the bone growth in the control limb exceed that seen in the electret limb. Because of the relatively small size of the rat femur, we found that the x-ray data was not useful in evaluating the cortical growth.

In most instances the implanted electret was recovered intact, and we found no measurable residua surface charge.

Discussion

Implanting 7-millimeter strips of untreated teflon around the femurs of mature rats caused some bone growth on the portion of the shaft adjacent to the teflon after 3-6 weeks (the amount of growth was significantly greater that which occurred in response to the surgery alone.) When the teflon was made in an electret, the amount of new bone growth usually exceeded that which occurred in response to the non- electret material in the contralateral femur. The electret-induced bone appeared to differ from its control in size, but not in organization or in other anatomical characteristics. Thus, a definite conclusion concerning the physiological effect of the electret must await more detailed studies involving area measurement of the new bone growth: such measurements could not be done in the present study because the level of the traverse sections were not identical in the experimental and control side. Also, since the bone growth was not labelled chemically or radiographically, it was not always possible to establish that areas of growth were

associated with events that occurred subsequent to implantation.

Assuming that future studies substantiate the efficacy of teflon electrets in producing bone growth, there are at least two mechanisms that may be responsible. One possibility is that the bone cells detect and respond to weak currents produced by the electret as its charge decays. If the loss of surface charge is viewed as an actual current flow (7), then, over 3-6 weeks, it would correspond to a current of $2\text{-}4 \times 10^{-15}$ a. Inoue et al. manufactured electrets by subjecting teflon to the ion flux produced by corona discharge in air. They found that when the electrets were implanted in rats, the decay process was almost complete after about four days (8). If this were also true of the electrets studied here, it would correspond to a current of about 2.5×10^{-14} a. But the electrets studied by Inoue et al. consisted of a surface layer of free charge. In contrast, allowing a polymer to cool in a strong electret field produces a true volume electret whose surface contains fixed charges resulting from the ordered distribution of dipoles throughout its volume (11). Previously, we found that teflon volume electrets (poled at 288°C) exhibited no measurable surface charge after only several hours exposure to saline at room temperature. Since the frozen-in dipole moment of teflon volume electrets has an indefinite lifetime in air, we interpret our inability to measure a surface charge following immersion in saline as due to the neutralization of the fixed surface charges by free charges from the solution. Thus, it is difficult to interpret a volume electret, such as was studied here, as a current source in analogy with the action of a surface-charged electret.

A piezoelectric theory (7, 12) is a more likely explanation of our results. Teflon electrets exhibit bending piezoelectricity, and it is possible that the current produced when the teflon was periodically flexed (by the animal's motion)was osteogenic. Such piezoelectric currents would be produced almost continuously and would probably have a magnitude greater than that calculated on the basis of the electret hypothesis (7, 12). This idea is supported by the report that an osteogenic response occurs in the rat femur following the implantation of poly-γ-methyl-L-glutamate), polymer (8). Further, evidence has previously been presented that the natural piezoelectric polarization in bone -- or the neutralization kinetics that occur in response to it -- were controlling factors for bone remodeling (13), and this also seems to suggest that at least some cells are piezoelectrically sensitive. On the other hand, we implanted 1-millimeter diameter x-cut quartz (its piezoelectric constant is comparable to that of PMLG)plates in pouches in the muscles surrounding the femurs in rats, and observed no stimulation of the normally-present mesenchymal cells. This is probably some evidence against the piezoelectric hypothesis for the origin of the teflon-electret-induced bone growth because, as Urist has shown (14), the mesenchymal cells in muscle do have the capability of producing bone if appropriately stimulated.

In conclusion, our results indicated that teflon piezoelectric electrets induced bone growth in rats that differed only in amount from that induced by control teflon films. Further studies -- preferably on larger animals -- will be required to quantitatively establish these observations.

References

1. A. A. Marino, and R. O. Becker: Electrical Osteogenesis: An Analysis. Clin. Orthop., 123, 280, 1977.

2. J. A. Spadaro: Simple Biogalvanic Current Sources For Growth Control. Bioelectrochem. & Bioenerg., 1, 266, 1974.

3. J. A. Spadaro: Bimetallic Current Sources As Stimulators of Osteogenesis. Trans. Orthopedic Research Society, 6, 13, 1981.

4. R.O. Becker: Stimulation of Partial Limb Regeneration in Rats. Nature, 235, 109, 1972.

5. S. D. Smith: Effects of Electrode Placement On Stimulation of Adult Frog Limb Regeneration. Ann. N.Y. Acad. 238, 500, 1974.

6. R. M. Libbin, T. Person, S. Papiermen, D. Shah, D. Nevid, and H. Grob: Partial Regeneration of the Above-Elbow Amputated Rat Forelimb. J. Morphol. 159, 439, 1979.

7. E.Fukada, T. Takamatsu, and I. Yasuda: Japan. J. Appl. Phys. 14, 2079, 1975.

8. S. Inoue, T. Ohashi, E. Fukada, T. Ashihara: Electric Stimulation of Osteogenesis in the Rat: Amperage of Three Different Stimulation Methods. In Electrical Properties of Bone and Cartilage, C. T. Brighton, J. Black, S. R. Pollack, Grune & Stratton, New York, 1979, 199.

9. Y. Suzuki: Bone Proliferation Induced By Electret in Tibia Fracture. Proc. Second Inter. Conf. Mechanical Behav. Materials, Federation of Materials Societies, 1428, 1976.

10. H. H. Wieder, and S. Kaufman: Plastic Electrets. J. App. Phys., 24, 156, 1953.

11. R. N. Varney, and H. T. Hahn: Electrets and Electrostatic Measurement. Am. J. Phys. 43, 509, 1975.

12. E. Fukada: Piezoelectricity of Bone and Osteogenesis by Piezoelectric Films. In Proceedings Of the Symposium On Mechanisms of Growth Control, Charles C. Thomas, Springfield, in press.

13. A. A. Marino, and R. O. Becker: Piezoelectric Effect and Growth Control in Bone. Nature 228, 473, 1973.

14. M. R. Urist: Bone Morphodifferentiation and Tumorigenesis. Persp. Biol. Med. 22, S89, 1979.