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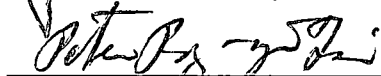
I am submitting herewith a thesis written by Robbye NyCole Grier entitled "Development and Use of a Polylactic Acid Electret to Stimulate Bone Growth." I have examined the final paper/electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Engineering Science.


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Accepted for the Council:


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**DEVELOPMENT AND USE OF
A POLYLACTIC ACID ELECTRET
TO STIMULATE BONE GROWTH**

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Robbye NyCole Grier

December 2001

DEDICATION

This thesis is dedicated to my parents and siblings,

Rev. and Mrs. Robert and Brenda, Ashelea, Robert II, and Brendt Grier

for their love, support, patience, and understanding,

and

Dr. Roberto S. Benson

for his motivation, support, and commitment.

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ABSTRACT

Bone remodeling and regeneration has perplexed the scientific community for ages. This project explores the possibility of stimulating bone regeneration at the sight of a circular femoral defect in rabbits through the implantation of a polylactic acid electret. Polylactic acid (PLA), a biodegradable polymer that resorbs into the body in the form of naturally occurring substances, is ideal for biomedical applications. An electret is a dielectric material that holds a semi-permanent static electrical charge. The electret is prepared via production of a polylactic acid thin film through compression molding, and then electrostatically charged using the process of corona charging. Upon completion of preparation, the electret was surgically implanted between the femur and periosteum of twelve New Zealand White rabbits. The animals were divided into four categories: Group A - four rabbits that had negatively charged films placed in contact with the defect; Group B - four animals in which a positive electret was applied to the defect; Group C - another four rabbits that served as a control group with an uncharged implant; and Group D - two animals that served as the non-surgical baseline control group to provide a guideline for comparison of the effects of surgery on the daily behavior of the animals. The animals were monitored over a period of 30 days, the time for necessary for a complete osteon, or bone cycle. Each rabbit was administered doses of tetracycline and calcein on days 21 and 27, respectively in order to calculate bone apposition rates. Final analysis of the harvested bone demonstrated that negative electrets contributed to osteoblastic activity, whereas positive electrets contributed minimally or not at all. This pilot study showed promise of future use in biomedical applications.

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Chapter 1

Introduction

Bone is the primary structural element of the human body. Its function is to protect the vital internal organs and provide a framework necessary for skeletal locomotion. A unique property of bone is its ability to repair itself and alter its properties in response to changes in mechanical demand. One of the modern techniques of regenerating bone accounts for its bioelectric properties. The study of the piezoelectric properties of bone originated in 1957 through the work of Fukada and Yasuda. Their experimental and theoretical work demonstrated an example of Wolff's law, which states that bone adapts during growth to the forces that are applied to it (Wolff, 1870).

Researchers have extensively used such polymers as poly-lactic acid (PLA), poly-glycolic acid (PGA), or a PGA/PLA co-polymer in biomedical applications, including temporary fixation devices and drug delivery devices (Slivka, 1997). These polymers possess the unique property of biodegradability, an essential characteristic in projects pertaining to live tissue and organs. Use of biodegradable polymers for clinical applications of this nature is ideal because they eliminate the need for an additional surgery to remove the implant. Additionally, they allow regeneration to occur at the site of injury, and effectively transfer external stress to the healing bone resulting in a stronger bone structure (Shikinami and Okuno, 1999). PLA is the chosen biodegradable polymeric material for use in this research. Its by-product, lactic acid, is a naturally occurring substance in the body, and therefore metabolizes through a normal pathway in water and carbon dioxide.

An electret is a dielectric material capable of maintaining a semi-permanent static electrical charge. Electrets have modern uses in filtration systems and domestic products. Charging of electrets is achieved in four major ways, including triboelectric charging, induction charging, electrostatic spinning, and corona charging. The biomedical application of electrets is a recent area of interest. Researchers have used the knowledge of the piezoelectric properties of bone to attempt bone regeneration in animals including rabbits (Laspos, 1999), dogs, and rats. Recently, Dr. Laspos (1999) showed that bone healing is increased when a negative charge was placed near a bone defect. Some strides are also being made on human subjects in the area of back surgeries in which charged materials were used as sutures. Additionally, electrets have been used as antithrombogenic surfaces and to understand membranes, neural signals, biological memory in regeneration, and electrical mediation in tissue growth (Sessler, 1980).

The focus of this research model is the regeneration of bone tissue via electrical stimulation. A poly-lactic acid electret in the form of a bi-axially stretched thin film charged using corona-charging techniques will serve as the mode of administering the charge to the bone. Corona charging involves embedding the electrical field onto the surface of the material to be charged. Due to the semi-permanent nature of the charge, factors causing charge decay must be considered. These factors include time, environmental changes (i.e. temperature and humidity), surface changes (touch), and chemical reactions during sterilization. Studies show that the higher the surface resistivity of a polymer, the better its ability to be charged and retain that charge (Tsai and Wadsworth, 1995). The present problem with PLA is its inability to retain charge over long periods of time. However, preliminary experiments show that the bi-axially

stretched nature of the PLA is a good contributor to charge retention. The initial measures of charge decay show that charge remains fairly constant over a period of 4 weeks (28 days), which is the length of the experimental study. Preliminary charging and measurements of decay indicate a fairly constant relationship with time when environmental factors are constant.

The purpose of this experimental study is to determine what effect the use of a biodegradable polymer electret will have on bone healing in New Zealand white rabbits. Electrets have a dual charge; one side is positive and the other side is negative. Over a period of 30 days, the animals were monitored closely after the initial surgery. The animals are divided into four groups: positive, negative, control, and baseline. Two doses of labeling drugs (tetracycline and calcein) were administered and used to identify the areas of bone formation and rate of regeneration. Finally, an analysis of the harvested bone reveals the effect of positive and negative charges placed at the site of the defect.

Chapter 2

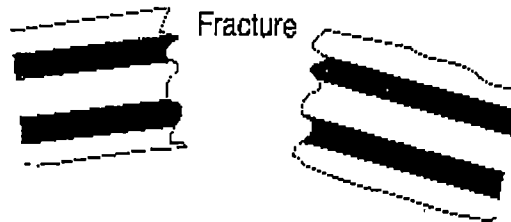
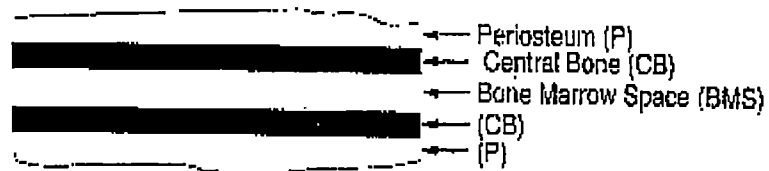
Background and Literature Review

2.1 Bone

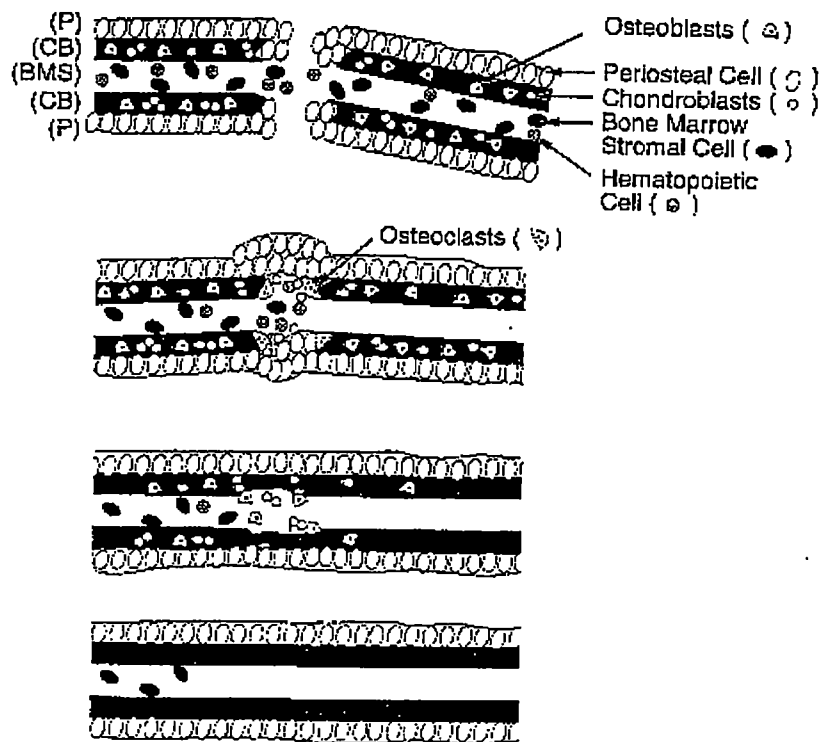
2.1.1 Bone Healing

Bone possesses the unique ability to repair and remodel itself. Understanding this phenomenon begins at the cellular level with the interaction of osteoblasts and osteoclasts. Osteoblasts are cells responsible for creating and laying down the bone matrix during osteogenesis. Osteoclasts, by contrast, are the cellular components that play a significant role in bone resorption. The delicate balance of activity among the aforementioned constituents determines the rate at which bone can be formed.

In its most basic form, Wolff's law simply states that form follows function. The skeletal system's function is to provide a sufficient framework for locomotion, and protect vital body organs. Therefore, upon injury, the system begins to compensate in order for the body to retain its normal functions. An example of such an injury is a bone fracture, which results from trauma causing discontinuity between any two parts of a bone. The cellular events occurring during the healing of a fracture can be explained in **Figures 1a and 1b** (Rosen, 1995). After the initial injury, the damaged ends bleed and form a clot that temporarily fills the space between the two surfaces. Acute inflammatory response occurs as a result of clot formation. This process leads to formation of granulation tissue containing undifferentiated cells. Bone repair actually begins with generation of cells in the periosteum that serve to bridge the gap between the two bone ends. Mesenchymal cells then create a fracture callus, which is eventually replaced by new bone. The osteoclasts tunnel through and create a space for osteoblasts to lie down



a: Creation of Fracture



b: Cellular events

Figure 1: Fracture healing (Rosen, 1995)

thin woven bone. This is the preliminary bone type that undergoes remodeling and develops into dense cortical bone as in the original bone before fracture (Rosen, 1995).

2.1.2 Fracture Fixation

Internal fracture fixation devices affect bone remodeling. These implants are designed to assist in correcting skeletal deformity, and can also serve as joint replacements (Buckwalter, 1996). Though these implants are useful, they alter the loading of the skeleton, thus interfering with the normal remodeling process of bone healing. The ideal fracture-healing device will allow for the skeleton to absorb more load with increasing strength. It is necessary for the device to provide stability initially, in order to relieve the stresses of the surrounding tissues. However, as healing progresses, the amount of stability provided by the implant device should decrease, allowing transfer of load to the original tissue (Slivka, 1997).

The cellular response to such internal fixation devices as pins, screws, and plates, contribute to their limitations. Often these devices are metallic in nature and do not transfer load throughout the healing process, resulting in unused tissue and consequently, stalled tissue growth (Slivka, 1997). Polymeric fixation devices show some advantages over metallic devices in terms of their degradation rates and biocompatibility. However, the debris from these devices can cause adverse tissue response (Hull, 1981). Additionally, the polymeric strength is significantly less than that of metals.

There are a number of factors that add to the complexity of bone remodeling associated with implants. These factors include: "variables related to the design of the

implant, materials used, and method of fixation" (Buckwalter, 1996). Additionally, characteristics of the bone itself, including shape and density, affect the remodeling process. Finally, the overall health, age, sex, and hormonal balance of the patient may contribute to complications of bone remodeling after implantation (Buckwalter, 1996). Gaining better insight into these variables will contribute to greater long-term success of internal fixation devices.

2.1.3 Electrical Properties of Bone

Previous research has demonstrated the electronegative property of bone during osteogenesis in two main categories: surgically invasive and non-invasive (Eriksson, 1976). Surgically invasive techniques involve implantation of electrodes into the bone surface, whereas non-invasive techniques apply an electric field without applied current to the tissue. The overwhelming majority of experiments attempting to electrically induce osteogenesis involve the former of the two techniques. DC current is allowed to flow between two electrodes (positive and negative) resulting in bone formation occurring around the cathode for currents ranging from 1-100 μ A. The anode, by contrast was shown to participate in as well as inhibit osteogenesis (Eriksson, 1976).

In a recent study conducted in 1999 by Dr. Laspos at the University of Tennessee, Knoxville, the electrode implantation technique was utilized to study the effects of electric current on the condylectomized mandibles of growing rabbits. Twenty-four animals were divided into four categories including an anode group, a cathode group, a non-stimulation group, and a control group, each containing six animals. A condylectomy was performed on the right temporomandibular joint (TMJ) of three

experimental groups. The anode electrode was placed in the lateral side of the joint with the cathode electrode in the ear cartilage of the animals in the anode group. The reverse orientation was performed on the subjects of the cathode group. A constant current of 25 μ A was administered to these two groups. No electrical stimulation was given to the non-stimulation group, however, an identical placement of an electrode in the lateral side of the right TMJ was performed. The control group also received a condylectomy, however, no electrode was placed on the joint (Laspos, 1999).

During the experimental period of 30 days, vital dyes (calcein and tetracycline) were administered on days 22 and 28 to quantify the bone apposition rates. Histomorphometric analysis demonstrated that animals receiving negative stimulation displayed increased *osteoblastic* activity, whereas those receiving positive stimulation experienced increased *osteoclastic* activity. Additionally, the anode group showed an increased percentage of bone volume on the condylar capitulum surrounding the condylectomy. The opposite was true of the cathode group. This characteristic suggests that negative stimulation is associated with osteogenesis; contrarily, positive stimulation appears to be associated with bone destruction (Laspos, 1999).

Surgically noninvasive techniques have also been performed to induce osteogenesis. Bassett *et al.* (1974) demonstrated this technique through the study of externally applied static and pulsed electric fields upon fracture healing. With no current passing through the tissue, an electric field (static) of 100V/cm was created by connecting insulated brass plates, across the fractured limb of an animal, to a battery pack of 100V. The pulsed electric field was created through production of time-varying magnetic fields at the fracture site, which in turn, created a corresponding field within the tissue.

Mechanical analysis illustrated a 25% stronger fibula when the lateral plate was negative. A positive plate resulted in a weaker fibula as well as a histologically larger mass of cartilage (Eriksson, 1976).

A third area of experimentation to induce osteogenesis involves electrically polarized implants. Recently in Japan, a study was conducted using negative surface charges of electrically polarized hydroxyapatite (HAp) to enhance osteobonding in canines. Kobayashi *et al.* (2000) demonstrated that bones integrate better with HAp when its surface is negatively charged. Six male beagle dogs were the subjects of this experiment. Each animal's femur was exposed by lateral luxation. Rectangular through-holes were bored through the lateral face of the bone (**Figure 2**). Similarly in the tibia, rectangular holes were bored on the medial face of the bone. The implants were HAp powder, calcined at 850°C and compressed in a mold at 200 MPa. The ceramic samples were rectangular cubes (5.0 x 8.0 x 1.0 mm³). The samples were electrically polarized in a DC field of 1kV/cm using a pair of platinum electrodes at 300°C for one hour (**Figure 3**). The charge on the negative surface was maintained for more than sixty days.

Bones were extracted at 3, 7, 14, and 28 days for analysis. The negative surface of the HAp promoted direct bonding and bone formation. By day 7, the negative surface of the HAp had formed a unidirectional oriented bone layer in direct contact with its surface. From the analysis, it was deduced that negative surface charges induced by polarization lead to highly oriented bone layers formed by activated osteoblastic cells. This is a significant improvement over conventional hydroxyapatite, which can take a minimum of 28 days to establish bone-to-bone contact (Kobayashi, 2001).

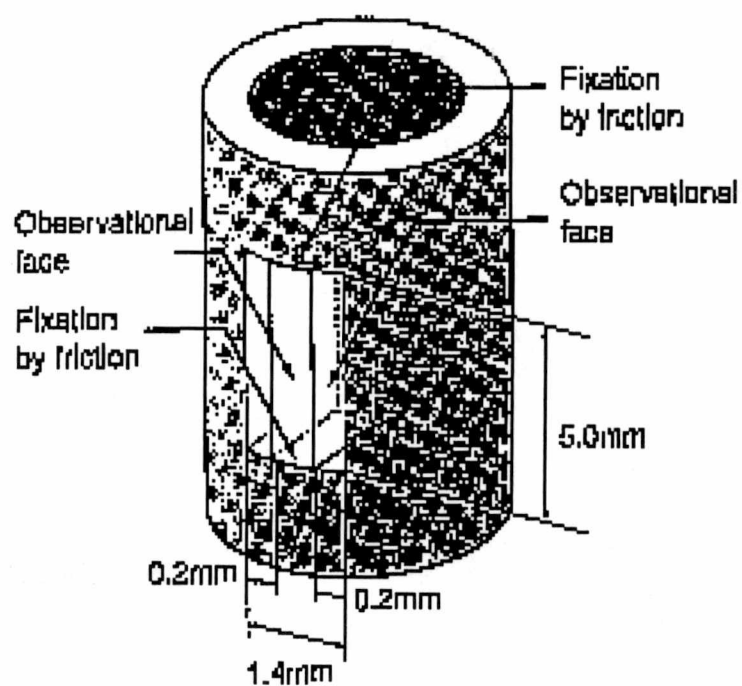


Figure 2: Schematic of implantation of HAp implants (Kobayashi, 2001)

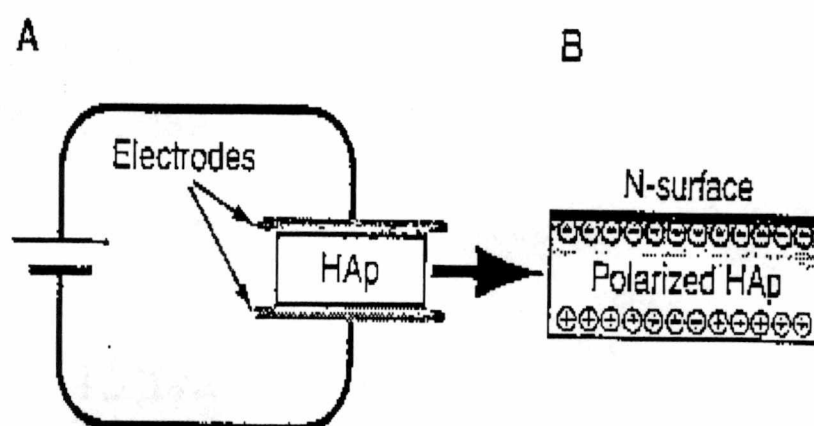


Figure 3: Polarization of HAp samples (Kobayashi, 2001)

Friedenberg and Brighton (1966) have established a characteristic curve of the surface potential of fractured bone in both human beings and rabbits (Figures 4 and 5). It has been established that bone exhibits negative potential at the site of fracture and remains negative until healing is complete. The negative nature of the fracture site is consistent with observations showing electronegative dc potentials in areas of active growth and active membrane surfaces (Eriksson, 1976).

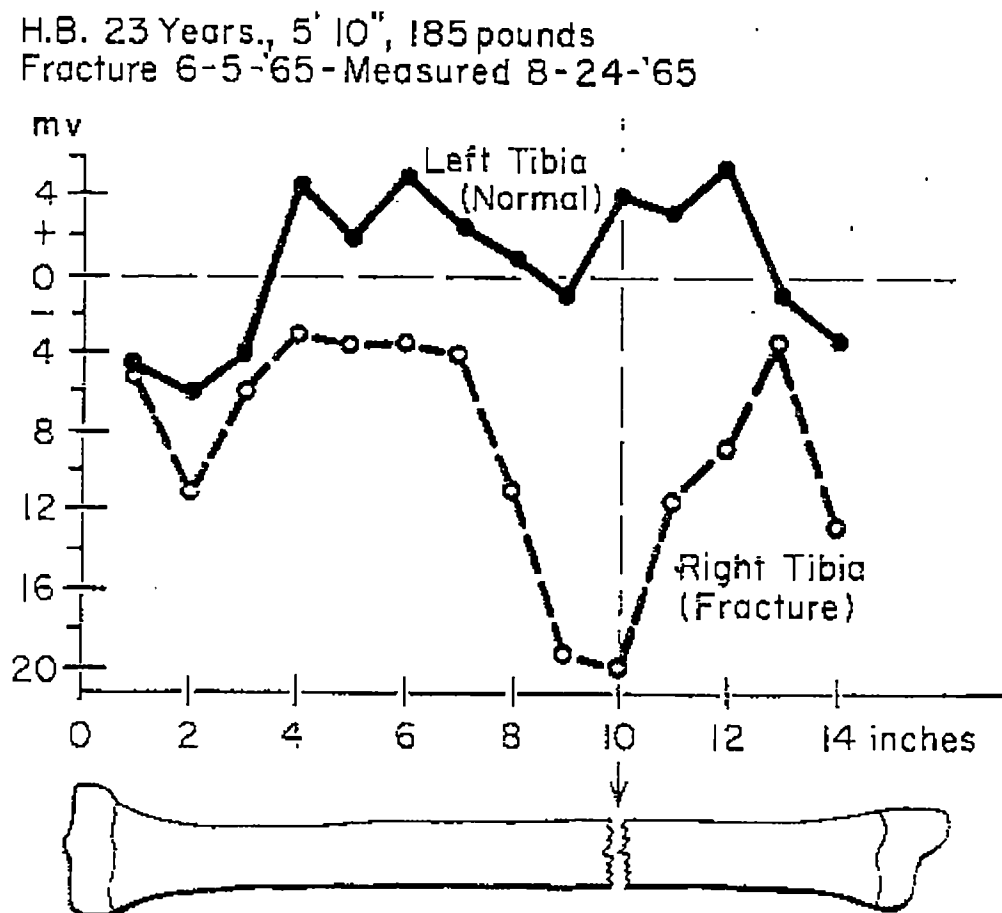


Figure 4: Bone surface potentials from human tibia before/after fracture
(Friedenberg and Brighton, 1966)

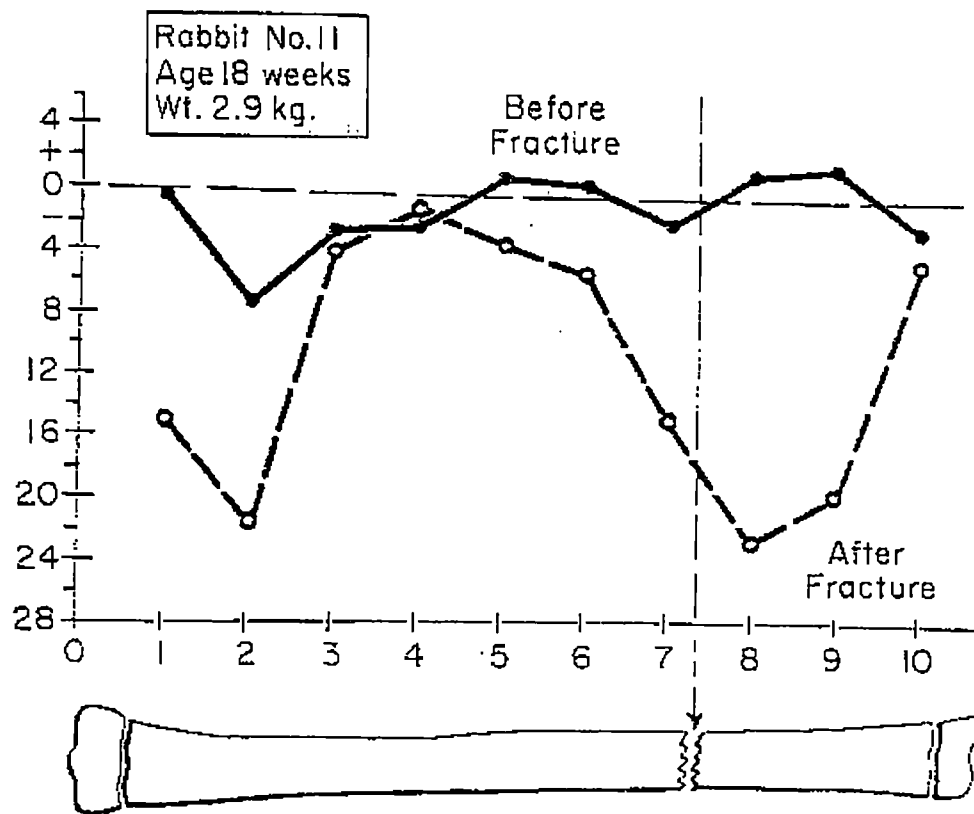


**Figure 5: Bone surface potentials from rabbit limb before/after fracture
(Friedenberg and Brighton, 1966)**

The mechanism by which bone is formed through induction of electric fields is still unclear. One hypothesis proposes that collagen has a large dipole moment with a positively charged head and negatively charged tail (Athenstaedt, 1970). This property of collagen leads Eriksson (1976) to believe that perhaps collagen migrates toward both electrodes (positive and negative) due to dielectrophoresis. Whether bone is regenerated or resorbed is contingent upon which electrode has the greater charge presence.

Our proposed mechanism in this study is embedded within the electronegative nature of fracture healing itself. It is proposed that using negative electrical stimulation

attracts positively charged particles, such as bone morphogenic proteins, that stimulate osteoblasts to form and lay down a new bone matrix. Past research has confirmed that anodes enhance osteogenesis and cathodes inhibit bone growth (Laspos, 1999). Additionally, it is also known that proteins usually have some charge associated with them, leading to their attraction to negative stimulation.

2.2 Biodegradable Polymers

Biodegradable polymers used as biomedical implants have significant advantages. Their ability to degrade via natural bodily mechanisms eliminates the necessity of a second surgery to remove the implant, a shortcoming of modern biomedical implants. Such polymers also have a controllable degradation rate that is affected by a number of factors including crystallinity, chain branching, chain mobility, molecular weight, and impurities (Gita, 1992). Biodegradable polymers also perform well in hydrolytic environments, which is important given that the body is more than 80% water. Additionally, these polymers can be mixed to form a copolymer with ideal properties suitable for the specific biomedical application (Kimura, 1993). **Table 1** gives common applications of biodegradable polymers.

2.2.1 Poly-lactic acid

Poly-lactic acid is a chiral molecule that exists in the form of four distinct polymers including two stereoregular polymers poly (D-lactic acid), or PDLA, and poly (L-lactic acid), or PLLA. A third form of poly-lactic acid exists in an optically inactive form called

Table 1: Common Applications of Biodegradable Polymers (Kimura, 1993)

Application	Shape	Example
Binding Materials	Suture, clip	PLA, PGA
Bone-setting materials	Plate, screw, rod, pin, splint	PLLA
Matrix for tissue culture	Sponge, mesh, nonwoven fabric, tubes	PLLA
Artificial blood vessel	Fiber, porous material	PLLA
Artificial tendon and ligament	Fiber	PLLA, polyglactin
Synthetic Skin	Fiber	PLA, polyurethane
Drug delivery system	Microcapsule, microsphere, hollow fiber	Polycaprolactone, glycolide/lactide polymers

poly(DL-lactic acid), or PLA. The fourth distinct polymer, poly(meso lactic acid), or meso-PLA is not generally used in practice (Kimura, 1993 and Kohm, 1996). PLLA distinguishes itself as an exceptional implant material because its by-products are non-toxic, easily excreted, naturally occurring substances in the body. Upon hydrolysis, it breaks down into lactic acid, which is metabolized through natural pathways into carbon dioxide and water. The basic chemical structure of PLA is given in Figure 6 (Kohm, 1996).

The differences in crystallinity between PDLA and PLLA affect the practical uses of each polymer. A common problem with PDLA is its amorphous nature. Thus, it is often preferred for drug delivery applications, where a homogenous environment is necessary. PLLA, on the other hand, because of its high molecular weight, is used in biomedical applications requiring high mechanical strength as is the case for sutures and orthopedic devices (Kohm, 1996).

2.2.2 Polyglycolic Acid

Polyglycolic acid (PGA), similar to PLA, is used widely in biomedical applications

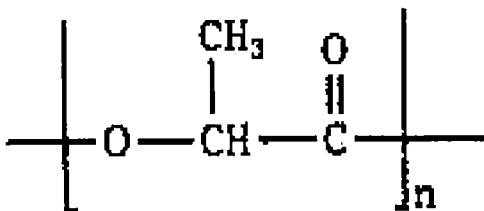


Figure 6: Chemical structure of polylactic acid (PLA) (Kohm, 1996)

as a biodegradable polymer. Because of its simplistic nature, PGA was used in the first totally absorbable suture as well as in internal fixation implants. PGA is highly crystalline, and is characterized by a high melting point and low solubility in organic solvents. Though PGA has been used for medical purposes, its limitation is its rapid loss of mechanical strength due to the linearity of its chemical structure (**Figure 7**). Consequently, it is often mixed with PLA to form a copolymer that increases its hydrophobicity in the biological environment. Though one aspect of PGA's morphology is improved, its crystallinity degrades even more rapidly when mixed half and half with PLA due to increased rates of hydrolysis. As a result, a copolymer of PGA/PLA is actually weaker than either polymer alone (Kohm, 1996).

2.2.3 Polydioxanone

Polydioxanone, or PDS (**Figure 8**), is one of the three synthetic, biodegradable polymers in addition to PLA and PGA that is used systematically in human medicine. PDS is crystalline and also degradable via hydrolysis. Its major medical contributions include

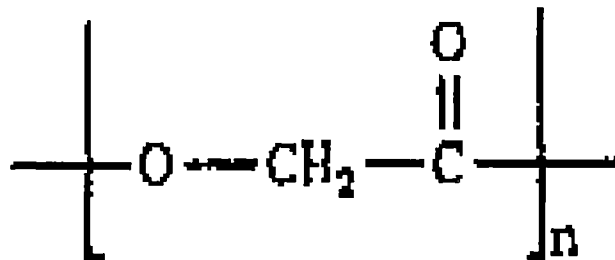


Figure 7: Chemical structure of polyglycolic acid (PGA) (Kohm, 1996)

sutures, orthopedics, and surgical applications (Ray, 1981). Though it has been used consistently for biomedical purposes, it is not as widely recognized as PGA and PLA for implantable materials.

2.2.4 Polycaprolactone

Polycaprolactone is a semicrystalline polymer characterized by high solubility, low melting point, and degradation by microorganisms. It has a much slower rate of degradation than PLA, which establishes its validity for use as long-term drug delivery systems. At present, this polymer is considered non-toxic and biocompatible. In fact, it is currently used as a wound closure device in European countries (Kohm, 1996). Research is still being conducted to determine its candidacy as a biomaterial. However, its use is not as widespread as that of PGA and PLA. Its chemical structure is given in **Figure 9** (Kohm, 1996).

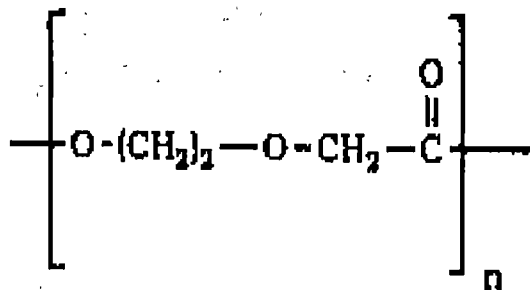


Figure 8: Chemical structure of polydioxanone (PDS) (Kohm, 1996)

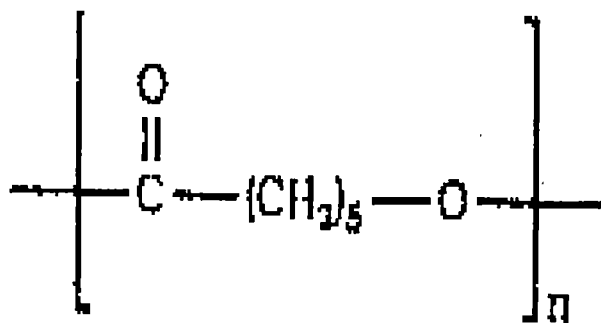


Figure 9: Chemical structure of polycaprolactone (Kohm, 1996)

2.3 Electrets

An electret is a dielectric material that maintains a semi-permanent electrical charge without externally applied voltage. Its quasi-permanent nature is attributed to the time constants that are characteristic of the decay rate. The time necessary to truly determine the rate of decay is often longer than experimental studies involving electrets (Sessler, 1980). Electrets are often compared with magnets, however, one important distinction is their production. Magnets generally result from dipole charges, whereas, electrets may

be the product of monopole or dipole charges (**Figure 10**). Another significant difference in magnets and electrets is the type of electric field they possess. Electrets have a static field; magnets, on the other hand, have a magnetic field. They have many practical applications including bioelectrets, transducers, generators, air filters, electrostatic recording, piezoelectric polymer transducers, and pyroelectric polymer devices (Sessler, 1980).

2.3.1 Charging Techniques (Huang, 2001)

In the formation of electrets, there are four major charging techniques: triboelectric charging, charging by induction, electrostatic spinning, and corona charging. Triboelectric charging is the simplest process because there is no necessity for an external power source. Electric charge is exchanged when two electrically dissimilar polymers

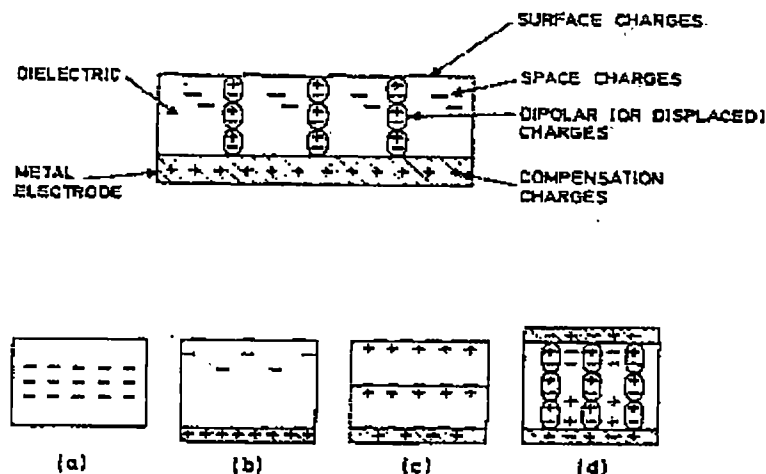


Figure 10: Electret configurations (Sessler, 1980)

are rubbed together, resulting in one polymer with a positive charge and other polymer with a negative charge. A major disadvantage to triboelectric charging is the instability of the charge; it decays quickly. A second method of charging electrets is charging by induction. This process involves placing a material in a strong electric field, and allowing charges to develop on its surface, creating a dipole material. The material will have one side with positive charges and the other side with an equal amount of negative charges. The third type of charging is electrostatic spinning in which electric forces are used to produce polymer fibers. A polymer solution is allowed to flow through a high potentially charged pipette. The melting polymer carries electric charges with it as it is ejected from the pipette into the air. When the polymer cools and solidifies, the charges are trapped within the fibers of the polymer, resulting in an electret material.

The fourth type of charging, and choice for this experimental investigation, is called corona charging. High voltage and low current generally characterize Corona charging. **Figure 11** illustrates the theory behind corona charging. When air molecules are ionized by cosmic rays or radiation, the electric field strength is powerful enough to reach air breakdown potential. In the case of corona charging, the electric field strength necessary for air breakdown depends on the diameter of the wire electrodes used for charging. **Equation 1** illustrates the aforementioned relationship.

$$E_b = 3000 + \frac{127}{\sqrt{d_w}} \quad (1)$$

where E_b is the electric field strength (kV/m) and d_w is the wire diameter (m)

From this equation, it can be concluded that if an electrode possesses a small radius of curvature, then high electric field strength is possible in the small regions near the electrode, creating a discharge. Corona charging generates a discharge by applying varying voltage between a sharp-edged electrode and an electrode behind the sample to be charged. **Figure 12** illustrates the four possible electrode configurations for corona charging. Their corresponding equations are given in **Equations 2 – 5**.

Configuration 1 (Tsai, 1998):

$$E_s = \frac{V_o}{a} \cdot \left(\frac{1}{1 - \frac{a}{b}} \right) \quad (2)$$

where E_s is the surface electric field, V_o is the applied voltage, a is the radius of the sharp point or wire, and b is the distance from the sharp point or wire to the plate.

Configuration 2 (Tsai, 1998):

$$E_s = \frac{V_o}{a \ln(b/a)} \quad (3)$$

where E_s is the surface electric field, V_o is the applied voltage, a is the radius of the wire, and b is the radius of the shell.

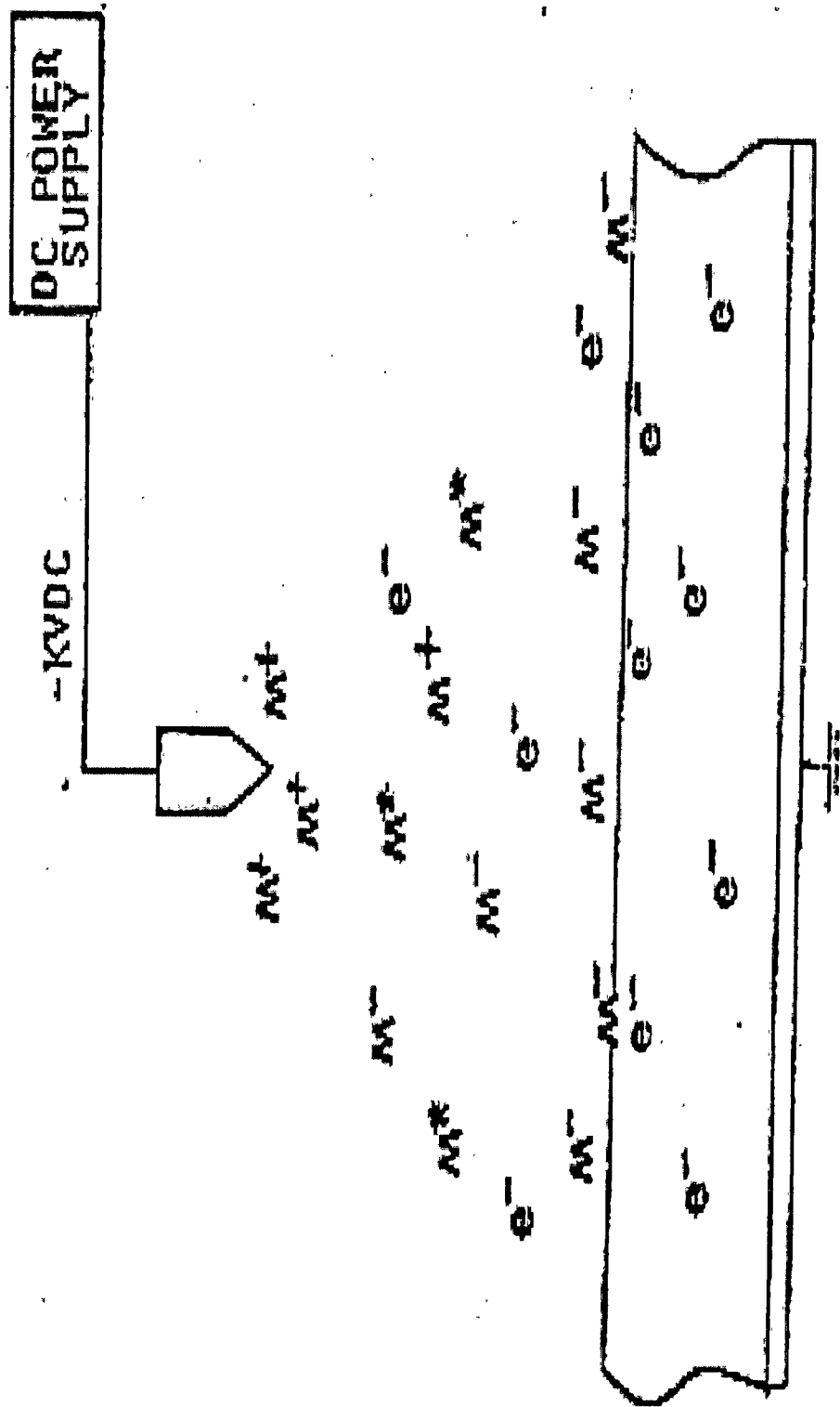
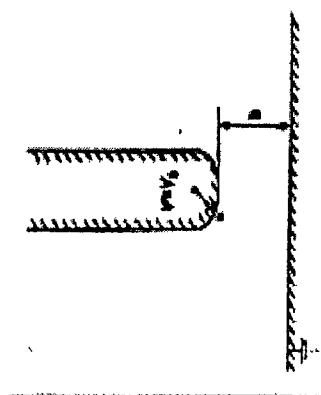
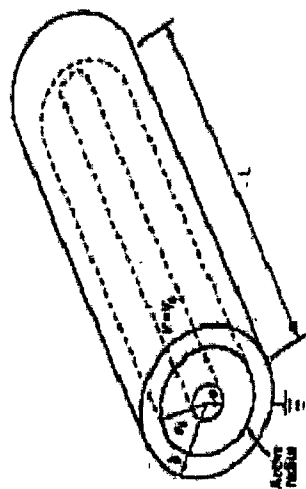


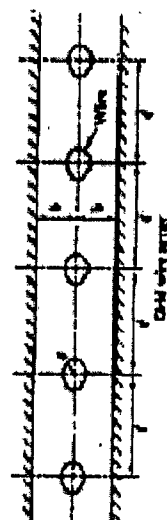
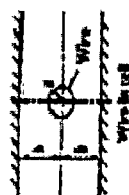
Figure 11: Corona charging theory (Huang, 2001)



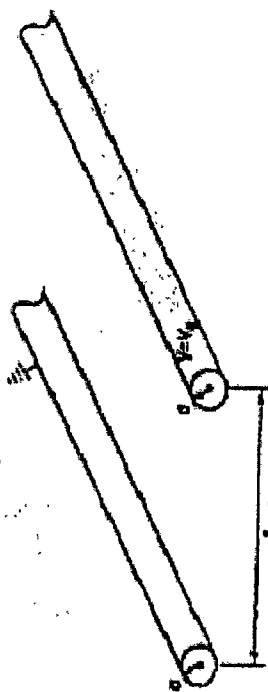
Configuration 1



Configuration 2



Configuration 3



Configuration 4

Figure 12: Electrode configurations for corona charging (Tsai, 1998)

Configuration 3 (Tsai, 1998):

$$E_s = \frac{V_o}{a \ln(4b/sa)} \quad (4)$$

where E_s is the surface electric field, V_o is the applied voltage, a is the radius of the wire, and b is the distance from the wire center to the plate

Configuration 4 (Tsai, 1998):

$$E_s = \frac{V_o}{a \ln(s/a)} \quad (5)$$

where E_s is the surface electric field, V_o is the applied voltage, a is the radius of the wire, and s is the distance between the two wires (in this case $s > a$)

There are two types of corona charging, positive and negative. Positive corona charging generates a stable bluish-green colored discharge, whereas negative corona charging produces a dancing motion-type discharge. Negative corona charging has the advantage of being able to operate at higher voltage and efficiency; thus, it is more often used in industry than positive corona charging.

The films used in this investigation were charged on a melt-blowing line at 40,000V and 0.8mA. **Figure 13** illustrates the five charging locations on the line. This investigation utilized a variation of this design.

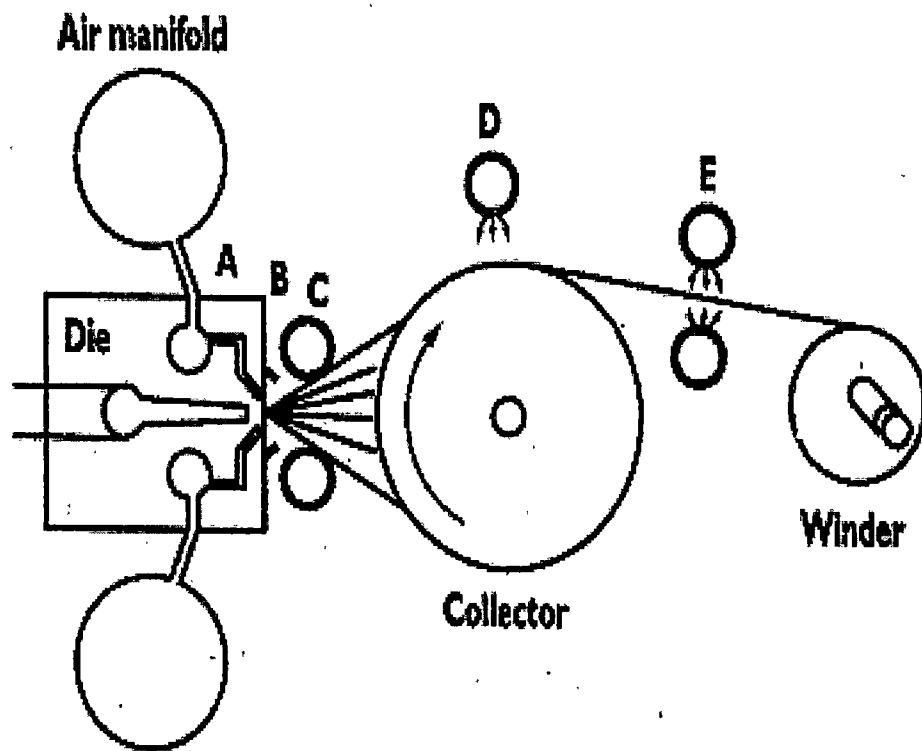


Figure 13: Charging locations on the melt-blowing line (Tsai, 1998)

2.3.2 Dielectric Constant

One of the most important applications of electrets is their practicality in biomedical science. Recent and current research has suggested and provided substantial evidence that negative electrical stimulation enhances osteogenesis in the bones of animals (Laspos, 1999 and Eriksson, 1976). The ability of a polymer electret to retain the specified charge for an allotted time is paramount to the success of the implant. This capacity to store electrical energy is referred to as the dielectric constant, and is a dimensionless quantity (Richardson, 1983).

2.3.3 Rate of Charge Decay

Related to the dielectric constant is the charge decay rate of the polymer. Previous research has demonstrated that charge decay occurs most rapidly when a structural group of a polymer serves as a side chain substituent as opposed to being present on the main chain (**Figure 14**). Thus, it can be hypothesized that polymers with higher dielectric constants may be better suited for use as bioelectrets. Calculations of dielectric constants (ϵ) were made for all of the biodegradable polymers described earlier (PLA, PGA, PDA, and polycaprolactone) using the group contribution theory. Group contribution theory takes into account the individual contributions of each functional group within a polymer to the overall dielectric constant, or capacity to retain charge. **Table 2** gives the values of common functional groups in polymers (Van Krevelen, 1976).

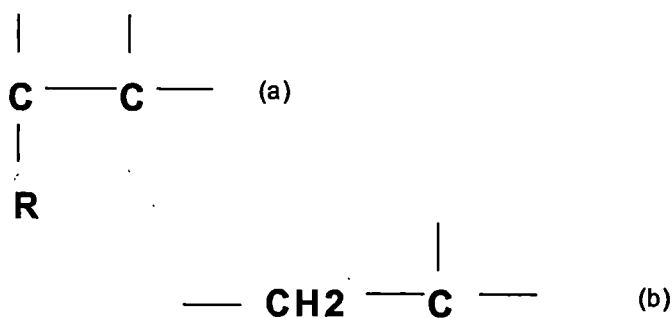


Figure 14: Side chain (a) vs main chain (b)

Table 2: Group contributions to molar dielectric polarization

(Van Krevelen, 1976)

Group	For $P_{LL} = \frac{\epsilon - 1}{\epsilon + 2} V$	For $P_V = \epsilon^{1/2} M$
-CH ₃	5.64	17.66
-CH ₂ -	4.65	20.64
>CH-	3.62	23.5
	2.58	26.4
	25.5	123.5
	25.0	128.6
-O-	5.2	(30)
	(10)	(65)
-COO-	15	95
-CONH-	30	125
-O-COO-	22	125
-F	(1.8)	(20)
-Cl	(9.5)	(60)
-C≡N	1.1	(50)
-CF ₂ -	6.25	70
-CCl ₂ -	17.7	145
-CHCl-	13.7	90
-S-	8	(60)
-OH (alcohol)	(6)	(30)
-OH (phenol)	~20	~100

Equation 6 was used to calculate the dielectric constant (ϵ).

$$P_V = \epsilon^{1/2} M \quad (6)$$

where P_V is the group contribution to molar dielectric polarization and M is the molecular weight. Table 3 gives the calculated dielectric constants of each of the aforementioned polymers as well as values of other polymers. Detailed calculations for polylactic acid, polyglycolic acid, polydioxanone, and polycarbonone may be found in Appendix 1. Comparison of the two most widely used polymers in biomedical applications, PGA and

PLA; it appears that PGA may be another possible candidate for use as an electret implant for stimulating osteogenesis.

Table 3: Calculated values of dielectric constants for polymers

Polymer	Dielectric constant (ϵ)
Polyglycolic Acid	3.97
Polylactic Acid	3.58
Polycaprolactone	3.02
Polydioxanone	1.54
Polyethylene	2.30
Polypropylene	2.40

Chapter 3

Experimental Methods

3.1 Sample Preparation

The sample used is a bi-axially stretched thin film of polylactic acid (PLA). 2" x 2" plaques of PLA were prepared by compression molding at 170°C using a Carver Press. After slow cooling, thin films were prepared from the PLA plaques via biaxial 4" x 4" stretching using a T.M. long stretcher.

3.2 Formation of the Electret

Corona charging, which is characterized by high voltage and low current, was used to prepare the electrets. In this case, the voltage was set to 40,000V and the current to 0.8mA. Upon completion of charging, the films were handled carefully, so as not to disturb the charge on the films. Using a surface charge potential tester, the charge was verified to be 30,000V on the positive surface and -45,000V on the negative surface.

3.3 Charge Determination of Experimental Samples

A surface charge potential tester was utilized to determine the average charge potential of each film. Thirty-six random points along the film were measured to give a general charge distribution curve.

3.4 Sterilization

The films were sterilized using the Novascan® technique for a period of 13.5 hours. Of the three large films, the one with the greatest charge was cut into smaller

squares of approximately 1in. x 1in. Each small film was placed in a sterilized beaker in Novascan® solution for 12 consecutive hours. The films were then removed and placed in a saline bath for one hour. A second saline bath was given for 15 minutes, and repeated for an additional 15 minutes. The films were removed and allowed to air dry in a sterile container. The total charge loss due to sterilization was 50V, which is insignificant for our purposes.

Techniques to preserve a sterile atmosphere for the surgeries were utilized. All instruments were autoclaved and soaked overnight in Novascan®. During surgery, used instruments were re-sterilized in Novascan®. Sterile gloves, caps, masks, and gowns were worn while in the operating room. Any portion of unused sutures was discarded. Used needles were discarded in a biohazard bin. A new drill burr was also installed for each animal.

3.5 Animal Study

The animal study was conducted over a period of 30 days, the time necessary for a complete osteon, or bone cycle to occur in New Zealand White rabbits. The rabbits were allowed a minimum of 7 days to recover from the stress induced by transportation and environmental change. They were housed individually in stainless steel cages (62 cm x 38.5 cm) with slotted floors (**Figure 15**) at the University of Tennessee Medical Center Animal Facility. The animals were fed and provided water by trained Animal Facility Staff persons. Fourteen rabbits were categorized into four groups according to the type of electret applied to the defect site. Group A consisted of four rabbits that had negatively charged films placed in contact with the defect. Group B included four

animals in which a positive electret was applied to the defect. Group C contained another four rabbits that served as a control group with an uncharged implant. Finally, Group D had only two animals that served as the non-surgical baseline control group. This group provided a guideline for comparison of the effects of surgery on the daily behavior of the animals.

Rabbits were chosen as the appropriate subject of this study for numerous reasons. They are large enough, so as to allow the placement of the electret over the femoral defect. This animal model is familiar due to its use in previous studies.

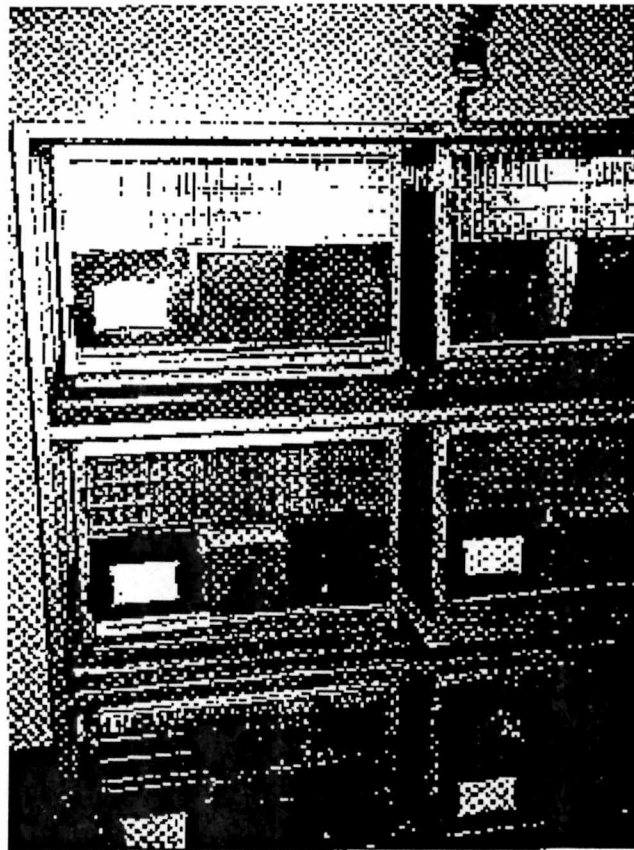


Figure 15: Cages used to house rabbits for study

Additionally, its compatibility with intramuscular labeling of the bone is excellent. Intramuscular labeling minimizes the discomfort of the animals and eliminates the need to sedate the animals during drug delivery as is necessary when administering intravenously.

Safety and minimal discomfort of the animals was paramount. As stated earlier, the two rabbits in the baseline category served as examples of the eating habits and activity levels of the animals in their normal state. Pain, distress, or discomfort were assessed using the following criteria: loss of appetite; weight loss; restlessness; abnormal resting postures in which the animal appears to be sleeping or is hunched up; licking, biting, or scratching the area of surgery; loss of mobility; unresponsiveness; and self mutilation. Any animal that did not fully recover from the effects of the surgical procedure by day 3, or experienced weight loss up to 20 % was euthanized immediately so as not prolong the discomfort. Fortunately, we did not experience this situation.

3.6 Surgical Protocol

Pre-surgery treatments included food restriction, anesthesia, and preparation of the surgery site. Food intake was restricted for 12 hours to prevent possible complications from aspiration. The anesthesia was administered intramuscularly in two parts half an hour prior to surgery. First, the animals were given a single pre-anesthetic dose of Ancef® (35mg/kg/IM). Next, each animal was given individual doses of ketamine® (3.5mg/kg/BW) and xylazine® (5mg/kg/BW). The level of the anesthesia was determined by observing respiration rate, heart rate, corneal reflex, color of mucus membranes, and muscular relaxation. Finally, the surgery site was prepared by shaving

the area surrounding the femur, and then sterilizing the area with a skin sterilant called betadine (**Figure 16**).

With the animals under appropriate anesthesia, the duration of each surgery was approximately 30 minutes. A 3-4cm vertical incision was made along the lateral side of the right thigh of each animal (**Figure 17**). Blunt dissection directly to the femur (**Figure 18**) was employed with special care to avoid damaging the surrounding tissues. The defect was created using a #3 fissured tapered burr under continuous saline irrigation (**Figure 19**). The resulting hole was 4mm in diameter and depth. An appropriate biodegradable polymer electret (positive, negative, or control) was superimposed on the defect, and held in place with the periosteum; thus, eliminating the need for an additional suture. Skin closure was achieved using running sutures of Vicryl® (**Figures 20 and 21**).

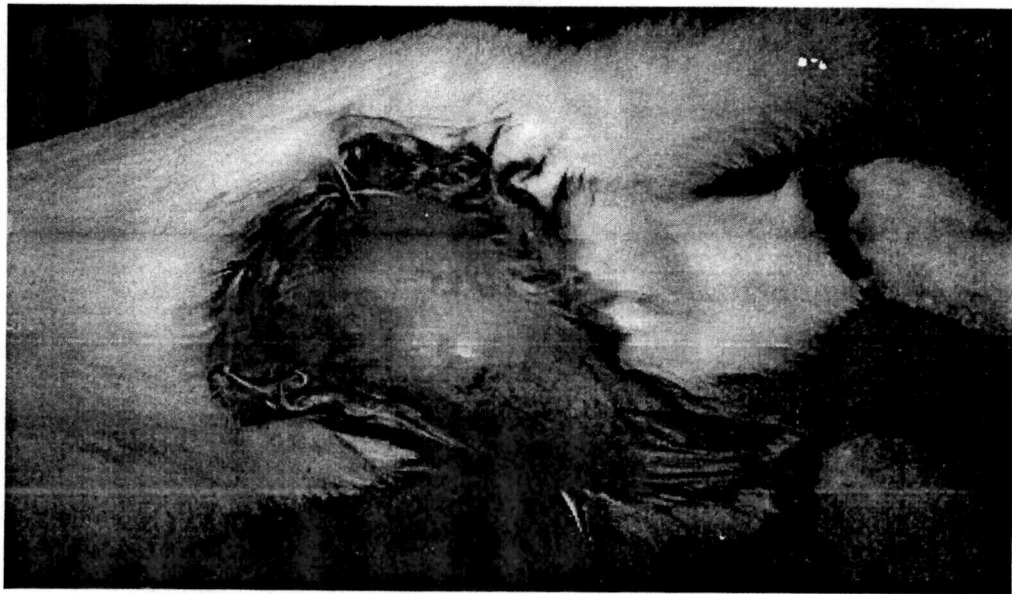


Figure 16: Sterilization of surgery area with betadine



Figure 17: 3-4cm vertical incision along lateral surface of femur

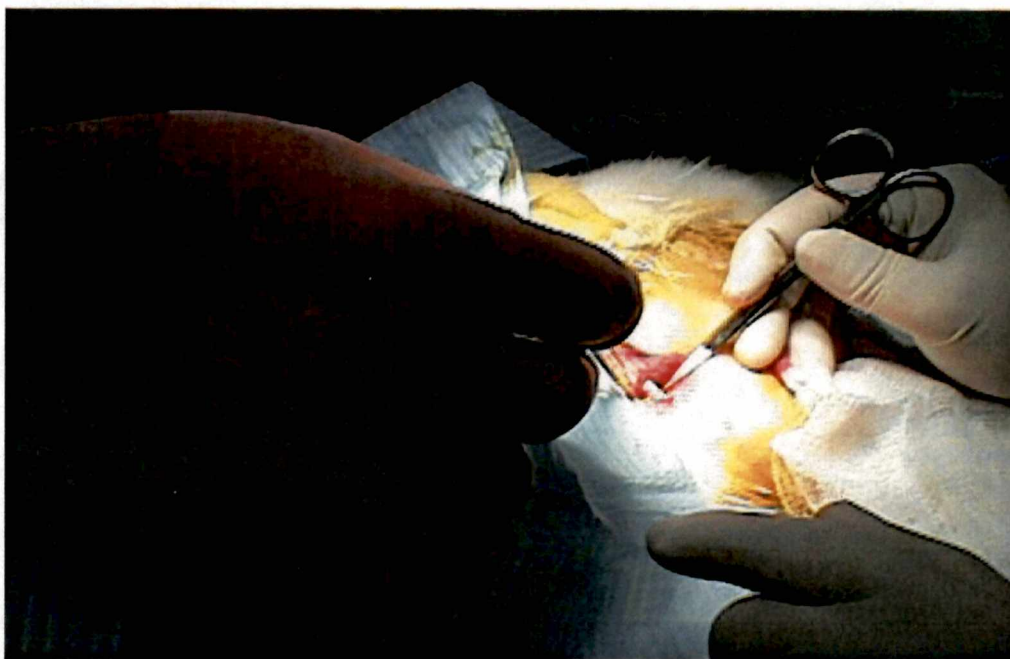


Figure 18: Exposed femur of rabbit with periosteum pulled back

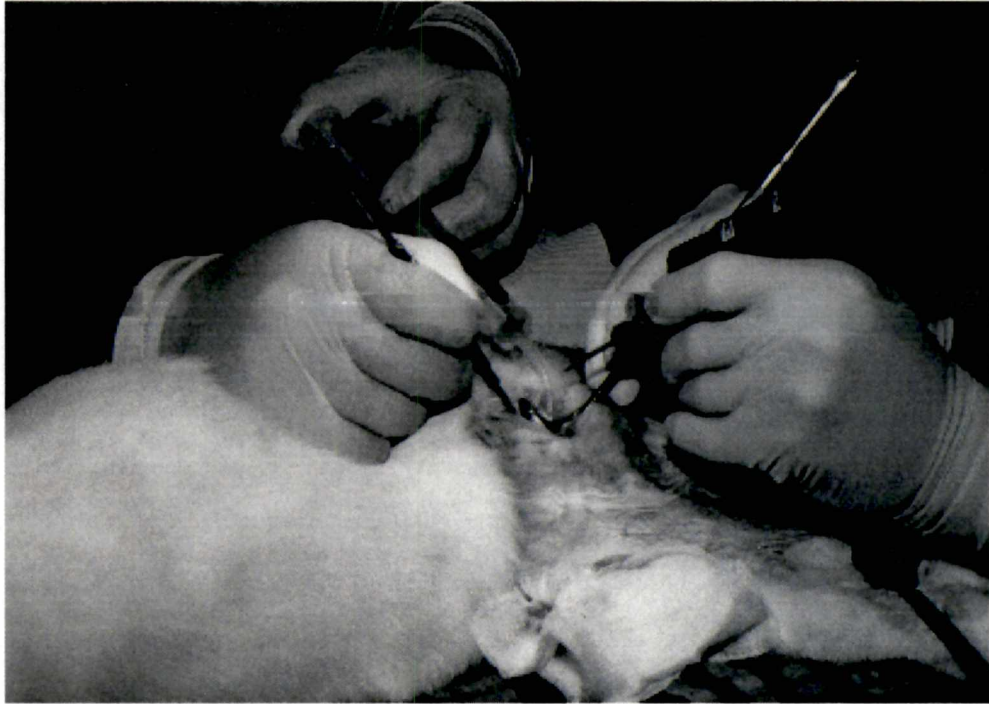


Figure 19: Creation of defect under continuous saline irrigation

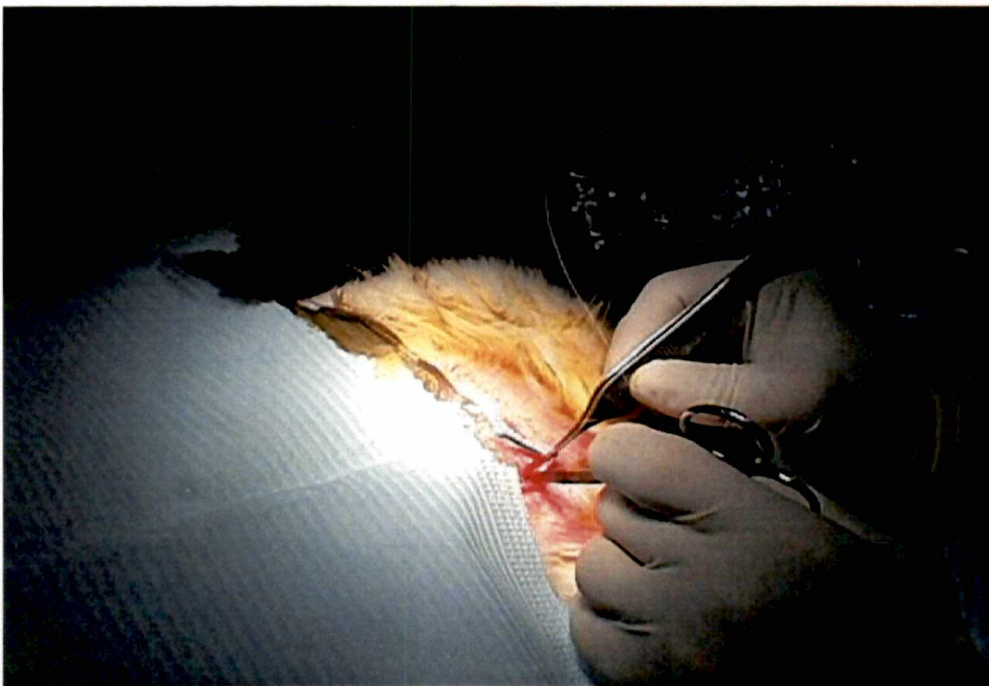


Figure 20: Suturing of wound site

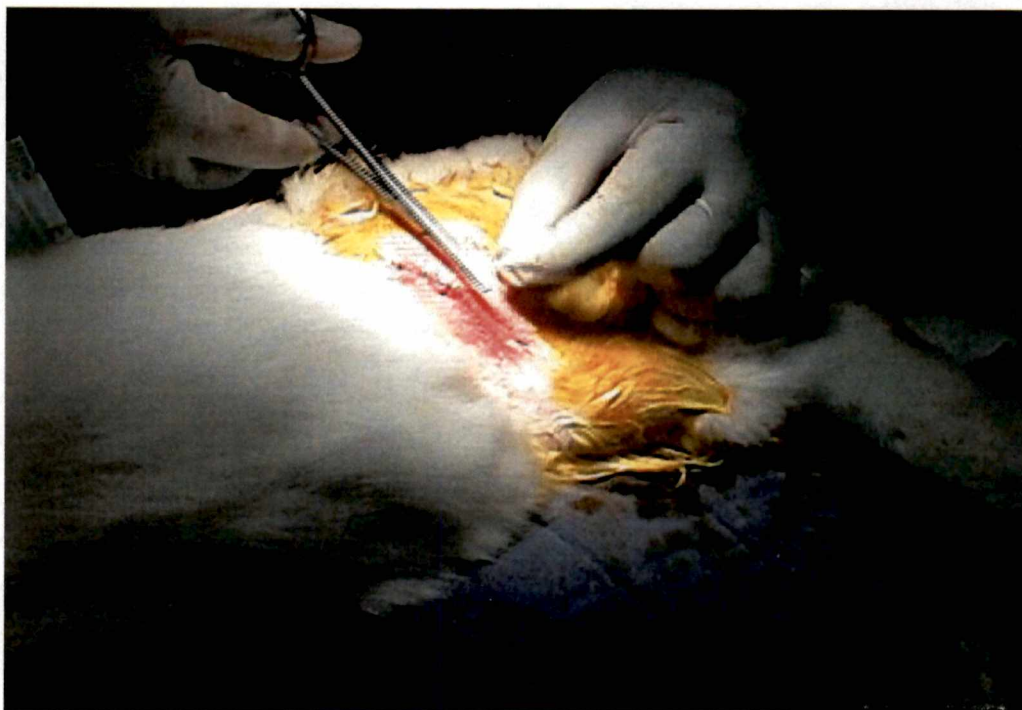


Figure 21: Final wound closure with Vicryl® suture

Immediately post-surgery, each animal was administered a dose of Buprenorphine® (0.05mg/kg/sc) as an analgesic once, and then as needed for pain. The recovery period lasted approximately one hour during which the animals were monitored continuously. All animals were expected to return to normal eating and activity patterns. Throughout the 30 days that followed, the rabbits were monitored at least twice daily by observation, and weighed once a week. On day 21, each animal was administered an injection of tetracycline (10mg/kg/BW) mixed with 1 cc of sterile saline intramuscularly in the hip. On day 27, another labeling drug, calcein (10mg/kg/BW) was mixed with 1 cc of 2% NaHCO₃ solution and administered intramuscularly. The injections were given slowly since both dyes bind calcium and may cause muscle, cardiac, and neural complications if given too rapidly. Both drugs have an affinity for bone, and are

fluorescent for easy viewing under a microscope. Use of these labels allowed us to measure where bone is being formed and, how fast it is being generated. As a result, we were able to establish the effects of the positive, negative, and control films on bone formation.

On day 30, the animals were euthanized via an anesthetic overdose of Beuthanasia® intravenously (1mg/10lb/BW). The animal carcasses were disposed of appropriately through incineration. All methods of euthanasia and disposal are in compliance with the AVMA panel report of 1993.

3.7 Vital Labeling Dyes (Laspos, 1999)

To quantify apposition rates of bone, two vital dyes were administered at different periods during the experiment (Days 21 and 27). Double labeling allows calculation of average bone formation over a specified interval. **Figure 22** illustrates the process of double labeling with tetracycline. These intravital markers, calcein green and tetracycline are only incorporated in areas of active bone formation. They produce secondary fluorescence when exposed to light in the blue spectrum (450-490nm) during microscopic analysis. The dye color (fluorescent color) differs from the initial color of the dye upon mixing preparation. Tetracycline is yellow upon preparation and gives an orange fluorescent color. Calcein green is orange upon preparation and gives a yellow to green fluorescent color. Each dye was prepared fresh prior to each injection.

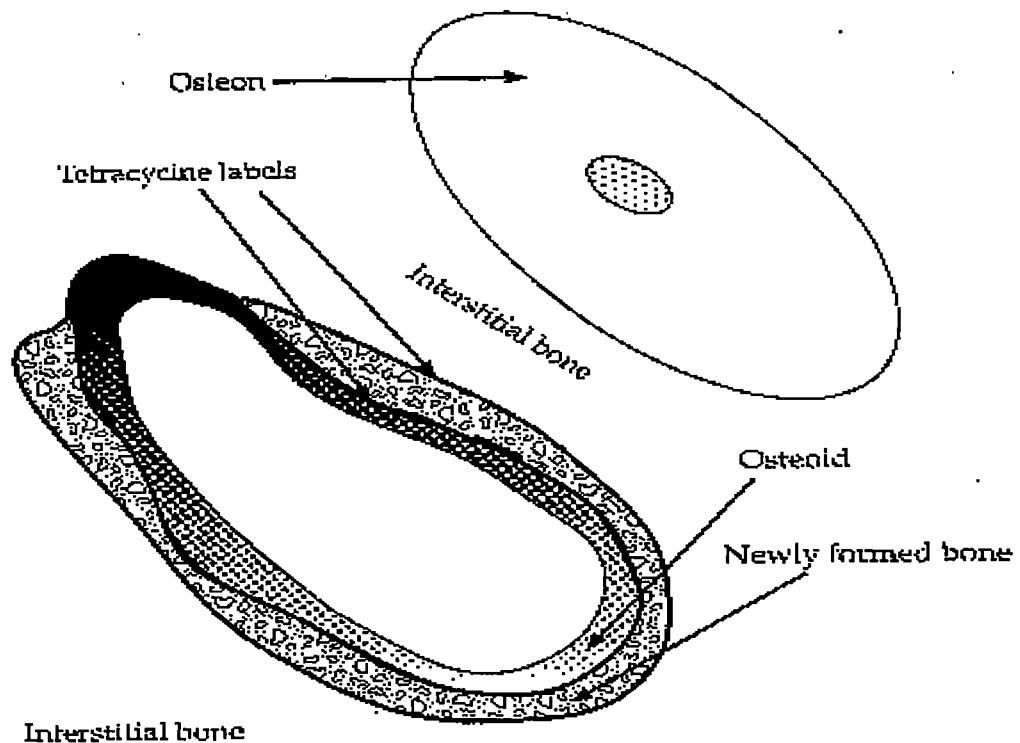


Figure 22: Schematic of tetracycline double labeling (Laspos, 1999)

Calcein, (disodium dihydrogen ethylenediamine tetraacetate), was described by Diehl and Ellingboe (1956) and Wallach et al. (1959). It was prepared in an attempt to measure calcium in microscopic sections of normal and malignant tissues. Since then, it has been used as an intravital marker in several studies with success. Parr et al. (1997) used calcein as a flouochrome in their experiment for sutural expansion using rigidly integrated endosseous implants in rabbits. For these reasons, calcein was chosen for use in the present investigation.

Tetracycline bone labeling properties have been described in previous research techniques. It is a temporarily stable tissue marker which provides true bone formation rates and cell population dynamic parameters. It is deposited *in vivo* under natural

conditions so that subsequent study of the labels produced cannot disturb the cells whose behavior have been recorded previously. Tetracyclines positively identify centers of new bone formation by labeling the first stage of mineralization of new organic bone matrix. Since the first stage of mineralization of an organic matrix parallels the rate of synthesis of new matrix, these markers allow measurement of rates of protein synthesis by all cells. After deposition in new bone, tetracyclines in undecalcified sections fluoresce under ultraviolet or blue light. Tetracycline marker records cell behavior regardless of composition and form, intracellular morphological, energetic, and chemical mechanisms. Tetracycline labeling is a chelation and absorption with calcium at the calcification front, where the first histologically visible mineral deposition occurs in new bone matrix. New bone mineralizes in two functionally distinct stages. The initial stage is the one which is consistently labeled with tetracyclines. It deposits in one day about 20% of the entire mineral the organic can accept. This requires the presence of viable osteoblasts. The second stage, which deposits the remainder of the mineral over many weeks, does not require viable osteoblasts and does not usually label permanently with tetracyclines.

3.8 Histology and Histomorphometry (Laspos, 1999)

The rabbits were euthanized on Day 30 of the animal study. The femur of each animal was dissected from the leg, cleaned, and placed immediately in a 10% formalin solution for fixation. The femurs remained in the formalin for about 48 hours. Leaving the bone in the formalin solution for more than 48 hours would result in the destruction of the vital dyes as well as the permeability and staining characteristics of the fresh bone. The femurs were then placed in 70% ethanol for the remainder of the time. Ethanol was

changed twice a week into a fresh solution until the end of the end of the experiment and preparation for the histologic and histomorphometric study. Ethanol has the advantage of fixing the vital dyes. Once the specimens are in 70% EtOH, the labels are stable and can stay in solution for over one year.

The femurs were then cut and embedded in an embedding mixture, Spurr's plastic®. The following dehydration and embedding procedure was used:

1. 50% EtOH for 24 hours, 2 to 3 changes, continuous agitation if possible.
2. 70% EtOH for 48 hours, 3 to 4 changes, continuous agitation if possible.
3. 90% EtOH for 48 hours, 3 to 4 changes, continuous agitation if possible.
4. 95% EtOH for 48 hours, 3 to 4 changes, continuous agitation if possible.
5. 100% EtOH for 48 hours, 3 to 4 changes, continuous agitation if possible.
6. 100% acetone for 24 to 48 hours, 3 to 4 changes, continuous agitation if possible.
7. 2/3 acetone, 1/3 Spurr's plastic® without accelerator for 24 hours.
8. 1/3 acetone, 2/3 Spurr's plastic® without accelerator for 24 hours.
9. 100% Spurr's plastic® with accelerator under vacuum for 24 hours.
10. 100% Spurr's plastic® with accelerator under vacuum in 60°C oven for 24 hours.

The Spurr's plastic® low viscosity embedding mixture is recommended because of its excellent penetration qualities, which provide good and rapid infiltration of tissues. It is easy to prepare and mixes rapidly via shaking and swirling. The hardness is adjusted by altering the amount of the flexibilizer, DER 736. The blocks have good trimming and sectioning qualities. The following ingredients are present in the Spurr's plastic®:

1. ERL 4206: vinyl cyclohexene dioxide (VCHD) is a cycloaliphatic diepoxide, with a MW of 140.18 and an epoxide equivalent of 74 to 78.
2. DER 736: diglycidyl ether of polypropylene glycol, a flexibilizer to control the hardness of the polymerized block. It was selected because of its low viscosity: 30 to 60 cp at 25°C. Its molecular weight is 380, and it has an epoxy equivalent of 175 to 205.
3. NSA: nonenyl succinic anhydride, a hardener with a relatively low viscosity of 102.8 cp at 25°C and MW of 227. A minimum exposure to air is recommended to avoid hydrolysis.
4. DMAE: dimethylaminoethanol (S-1), an accelerator, used because of its low viscosity and ability to result in blocks with less color. In addition, it induces rapid cure when the temperature is elevated to 70°C. It is effective in a very low concentration, 0.7% to 0.75%.

The following quantities were used in preparation of Spurr's plastic® in this experimental study:

ERL 420.....610.0g,
DER 736.....6.0g,
NSA2.....6.0g, and
DMAE.....0.33g.

The specimens were then cut with a Delta Bench Band Saw® and cut with a Delta trimmer Model 31-080®. Cutting and trimming was important so that the specimens would be parallel with the diamond saw when mounted on the blocks for sectioning.

A diamond saw was used for the sectioning of the specimens (Leitz 1600). The Leitz 1600 saw microtome is specifically designed for cutting hard and brittle material such as bone, ceramics, and reinforced plastics. To make a section, the object is guided extremely slowly against the saw rotating at a speed of approximately 600rpm. The built-in water-cooling device prevents heating of the object and removes sawdust from the cutting edge. The amount of water is regulated with a valve. The section thickness is set manually with a knurled screw on the object arm.

The sections were then polished at one side in a Mark V lab series grinder/polisher machine, which has two rotating wheels. The first wheel holds a disk of sand paper with 1500 grit for the initial grinding of the sections. The second wheel holds a cloth that was used with an alumina suspension, 1 micron lubricant, in continuous water supply. This finishing gave the sections a glossy surface on one side. The sections were then allowed to dry and glued to plastic slides with cyanoacrylate. Care was taken to ensure that no air bubbles were left under the section. To eliminate air bubbles, sets of 12 slides were pressed together in a vice for 24 hours. The sliders were then polished and cleaned from the cyanocacrylate. A clean surface of the slide at the specimen area was critical for proper microscopic examination.

Two sections of each femur were used. One of the sections was used for the fluorescent study, and the other for staining with Sanderson's Rapid Bone Stain®. This staining technique can be used for differential staining of mineralized sections of approximately 100µm of bone. The slides were immersed in the stain for approximately five minutes. Finally, counter staining with Alizarin Red® was performed next.

Chapter 4

Results and Discussion

4.1 Charging Results

Initially, three 4" x 4" films were charged to 3500V on the positive side and -2500V on the negative side. The films were kept rolled between the polypropylene melt blown webs, and were tested for surface potential at 30-day intervals over a period of three months. Care was taken to avoid exposure of the films to extreme (hot or cold) temperatures, touch, and other chemicals. Below are the charge decay curves for the positive and negative films.

The uncharged control film, of course, experienced no decay. On the positive curve (**Figure 23**), film #3 had the most drastic decay within the first sixty-day interval, and then adapted a more stable decline. Film #2 experienced an initial rapid decline followed by steadier during the last thirty days. Because of their rapid drops in charge at various times, the positive sides of films #2 and #3 were not selected. Film #1 had the steadiest decline, losing only 300V in the first month, 610V in the second, and 600V in the third month. This was the film chosen for use as the positive electrets.

The negative charge decay of the electret is presented in **Figure 24**. Films #1 and #2 exhibit almost no decay (about 60V) during the first thirty days. During the second month, films #1 and #2 exhibit sharper decline. Overall, film #1 showed the least decline again, thus its negative side was used for the negative electrets. Film #3 experienced an initial rapid decay followed by a more stable decrease in the charge.

The final charge on the films, immediately prior to sterilization was 3500V on the positive side and -3800V on the negative side. Immediately after trial sterilization, the

charge potential of the electrets was measured resulting in an average charge decay due to sterilization of only 50V. So, it is estimated that the charge on the films preceding surgery was about 3450V and -3750V on the positive and negative interfaces, respectively.

4.2 Animal Data

Table 4 gives data on each animal for comparative analysis. As the table indicates, all of the animals recovered fully from the anesthesia and were behaving normally within two hours post-surgery. Observations in the days post-surgery were made to note any additional analgesic that may have been given, and any unusual observations including lethargy, loss of appetite, increased respiration, and weight loss (**Table 5**). None of the animals needed additional drugs for pain, and all returned to normal eating habits and activities. Animal #3 experienced some bleeding during surgery due to a possible hematoma. However, as stated above, the swelling had subsided by day 4 post-surgery.

Each animal was weighed once a week over the entire four-week duration. The rabbits were not expected to lose weight, as this would be an implication of a complication in the surgery. The majority of the animals experienced no weight loss; instead, most of them remained the same or slightly gained weight (**Figure 25**). Animal #10 had an initial weight of 3.05kg, and gained 0.25kg by week two. However, when weighed in week three, the animal had lost 0.20kg, all of which were regained by week four. Possible explanations include loss of appetite during the third week, lethargy, or human error.

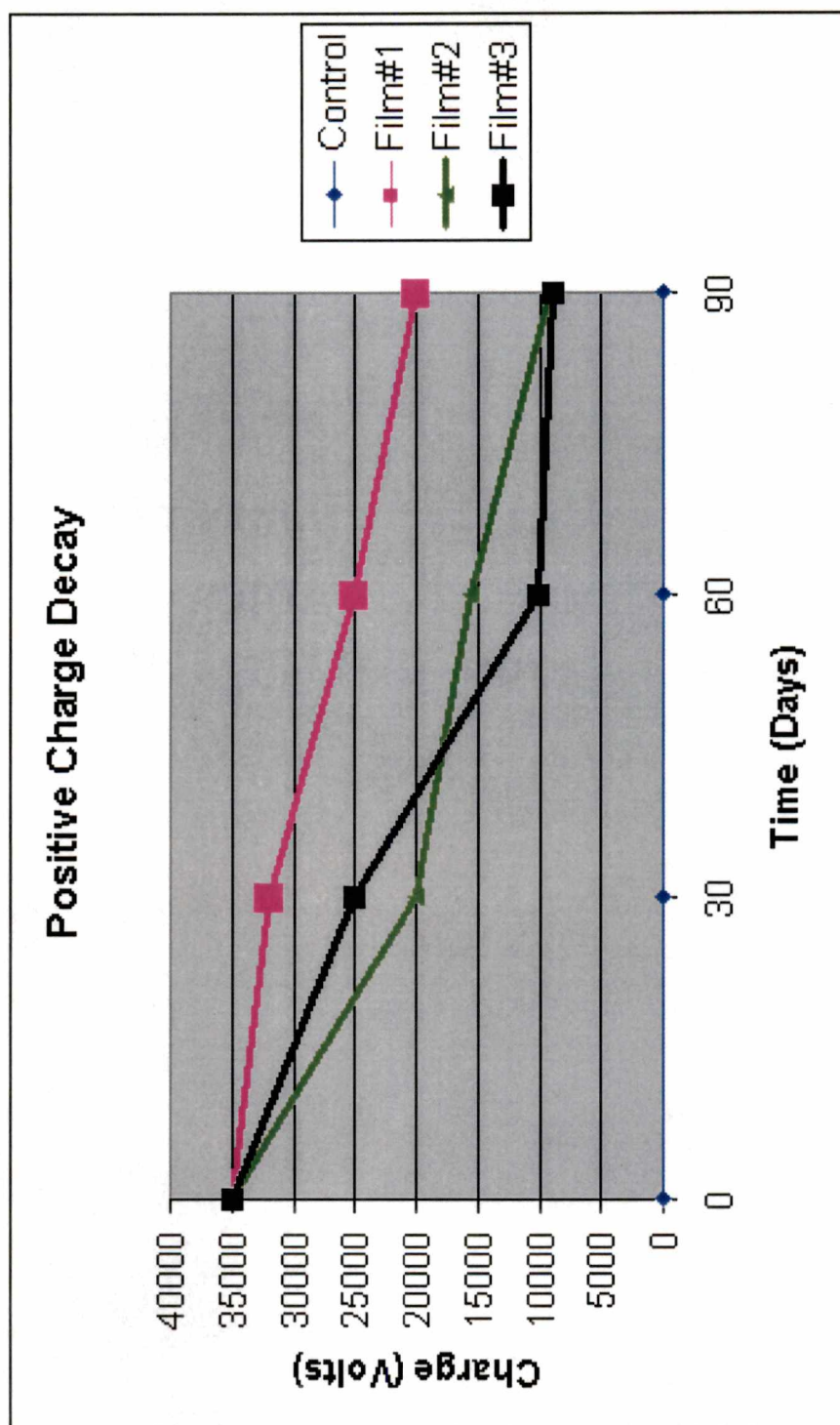


Figure 23: Charge decay for positive electrets

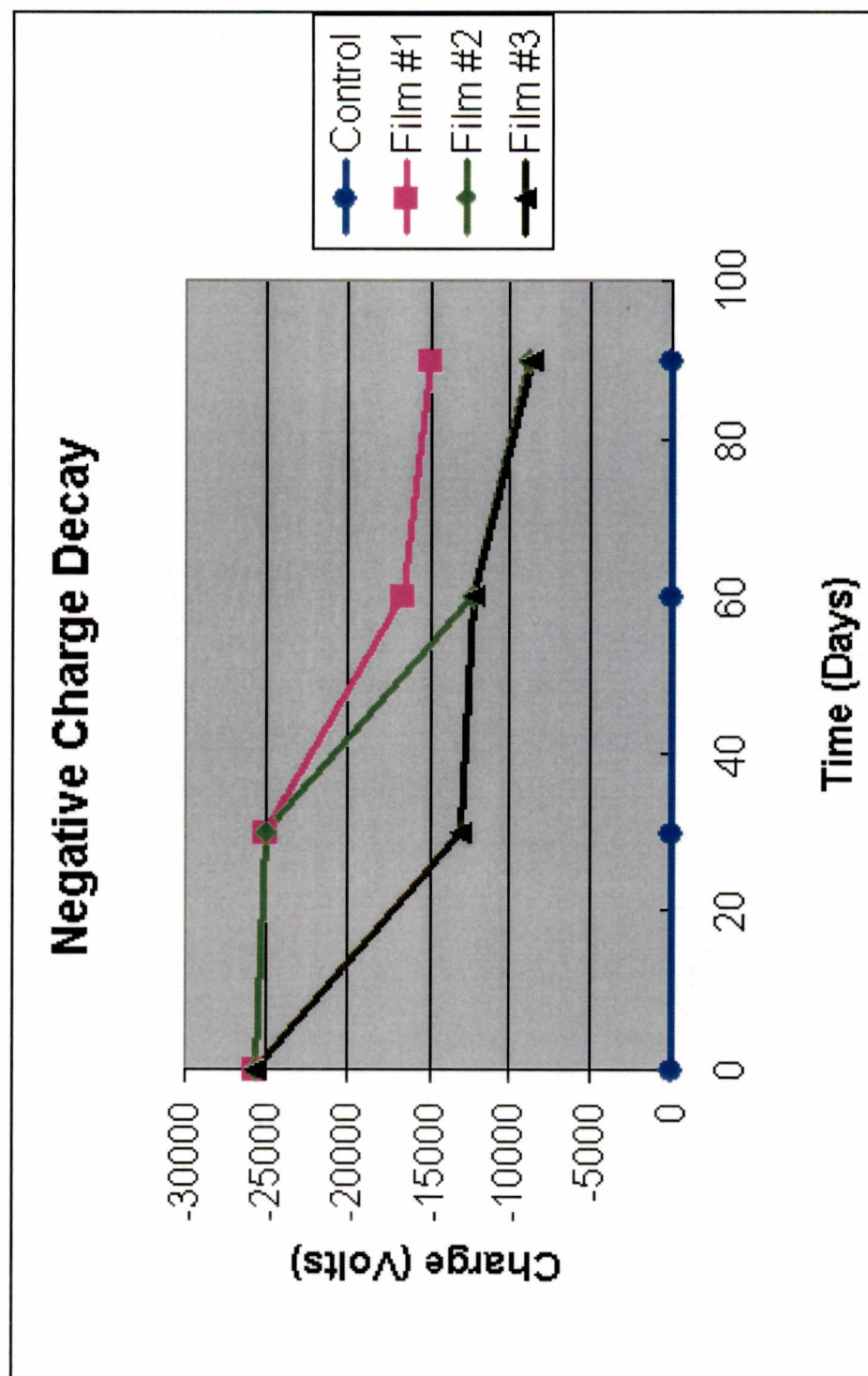


Figure 24: Charge decay for negative electrets

Table 4: Observations immediately post-surgery

Animal	Initial Weight (kg)	Date of Surgery	Duration on Surgery (min.)	Electret Type	Post-surgery Comments
1	2.50	6/28/01	30	Control	Recovered from anesthesia without incident; alert; moving normally
2	2.30	6/28/01	24	Control	Alert; moving normally
3	2.60	6/28/01	20	Control	More bleeding after creation of defect – possible hematoma; some swelling at surgery site; functioning normally
4	2.60	6/28/01	16	Control	Alert; moving normally
5	2.95	8/9/01	20	Positive	Alert; coordinated movement
6	2.93	8/9/01	18	Negative	Alert; moving; coordinated
7	2.82	8/9/01	28	Positive	Alert; successful recovery from anesthesia
8	2.92	8/9/01	25	Negative	Recovered from anesthesia; alert
9	2.93	8/9/01	20	Negative	Good recovery from anesthesia; alert
10	3.05	8/9/01	15	Positive	Alert; recovered from anesthesia
11	2.96	8/9/01	20	Positive	Eating and drinking normally; alert
12	2.95	8/9/01	20	Negative	Good recovery from anesthesia; alert

Table 5: Observations in days post-surgery

Animal	Observations
1	No additional drugs needed; animal eating and drinking normally; active
2	No additional drugs needed; animal eating and drinking normally; active
3	No additional drugs needed; animal eating and drinking normally; active; swelling at surgery site subsiding by day 4
4	No additional drugs needed; animal eating and drinking normally; active
5	No additional drugs needed; animal eating and drinking normally; active
6	No additional drugs needed; animal eating and drinking normally; active
7	No additional drugs needed; animal eating and drinking normally; active
8	No additional drugs needed; animal eating and drinking normally; active
9	No additional drugs needed; animal eating and drinking normally; active
10	No additional drugs needed; animal eating and drinking normally; active
11	No additional drugs needed; animal eating and drinking normally; active
12	No additional drugs needed; animal eating and drinking normally; active

Weekly Weight Gain of Rabbits

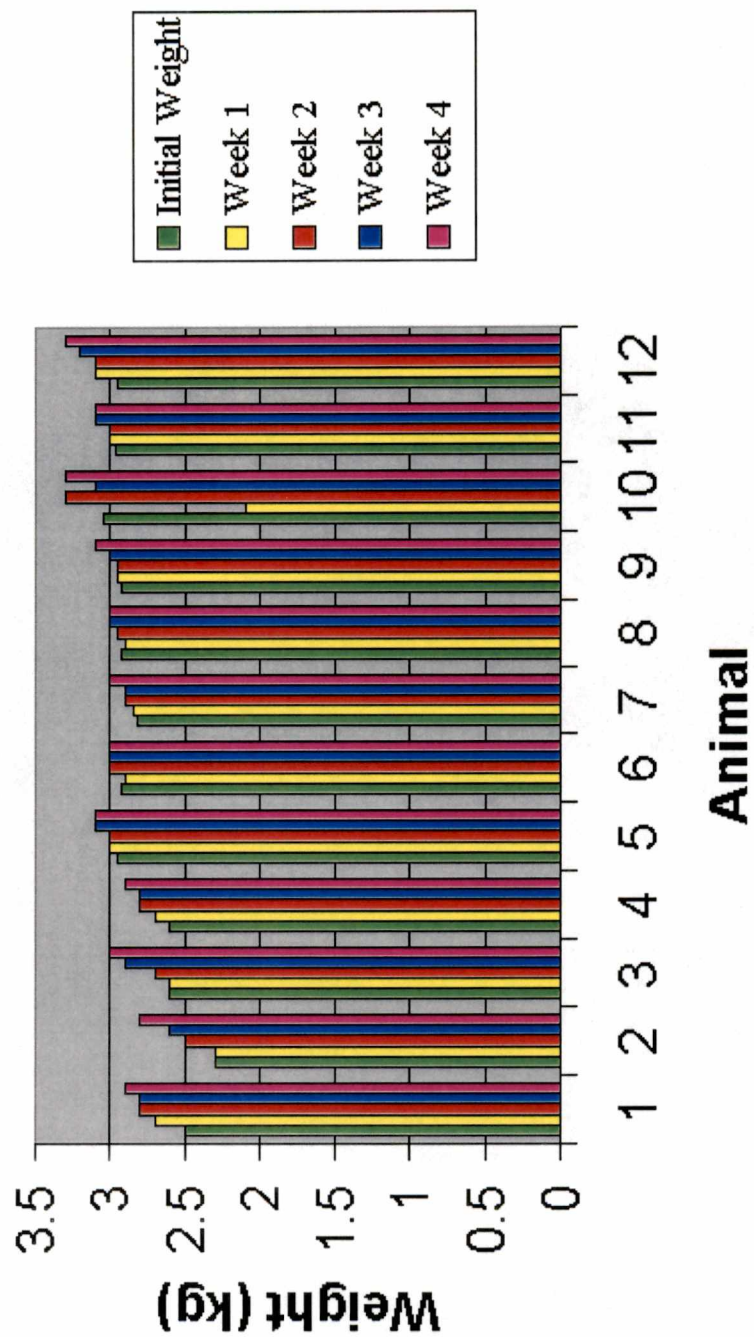


Figure 25: Weekly weight gain comparison

4.3 Observations at Sacrifice

Several observations were noted about each animal at the time of sacrifice. Some displayed large (L), medium (M), and small (S) quantities of heterotopic bone formation. The heterotopic bone appears to be a result of the surgery itself, as it grows up and away from the defect itself. In some cases, it conceals the defect to the naked eye.

Animals 2, 5, 7, and 9 exhibit some presence of the electret film by visual inspection. The fact that only remnants of the film remain validates the biodegradability of PLA. PLA degrades in the body at a controllable rate that can be controlled by mixing with polyglycolic acid. Its degradation products are nontoxic, biocompatible, and easily excreted in the form of CO_2 and H_2O . More importantly, PLA possesses the ability to hold the necessary charge for the specified time frame. All of these factors aid in eliminating the necessity of a second surgery for removal, the major limitation of many biomedical implants.

Additionally, in animals 1, 2, 4, 6, and 8, the defect was still visible. It was not expected that the defect would be completely filled within a thirty-day period, as cortical and trabecular bone require an extended period of time to develop. Woven bone, the initial type of bone matrix, is much less dense than cortical and trabecular, and forms at a more rapid rate. This experiment focused on the remodeling progress made during one osteon, which for a rabbit is 30 days. Animal #3 showed discoloration and erosion of the bone resulting from the hemorrhaging that took place during the surgery itself. **Table 6** illustrates a summary of all observations made at the time of sacrifice.

Table 6: Observations at time of sacrifice

Event	Totally or Partially Visible by Inspection	Not Visible by Inspection
Defect	5	7
Film Attachment	4	8
Heterotopic Bone Formation	8 total (L - 3, M - 4, S - 1)	4

4.4 Apposition

The rate at which woven bone is laid down is called the apposition rate. It is calculated by taking the difference between the labeling fluorescents of calcein and tetracycline in osteons (**Figure 26**) and the periosteum (**Figure 27**). Labeling of secondary osteons follows the opposite pattern of tree ring growth, with the oldest ring outside. Labeling on the periosteum, on the other hand, is identical to tree ring growth, with the oldest ring on the inside. Each of these drugs expresses a natural affinity for cellular bone activity, consequently, adhering to cells involved in osteogenesis. The drugs were administered six days apart, thus dividing by six gives the average apposition rate in microns per day. A comparison of apposition rates at the defect site and in a control area was made to determine the effect of the films. The control area, again, was approximately 1mm away from the site of the injury. A graphical representation of the aforementioned comparison is given in **Figure 28**. The relationship demonstrates that the electrets did not interfere with the normal apposition rate of bone. Any change in apposition rate would then be attributed to the surgery itself.

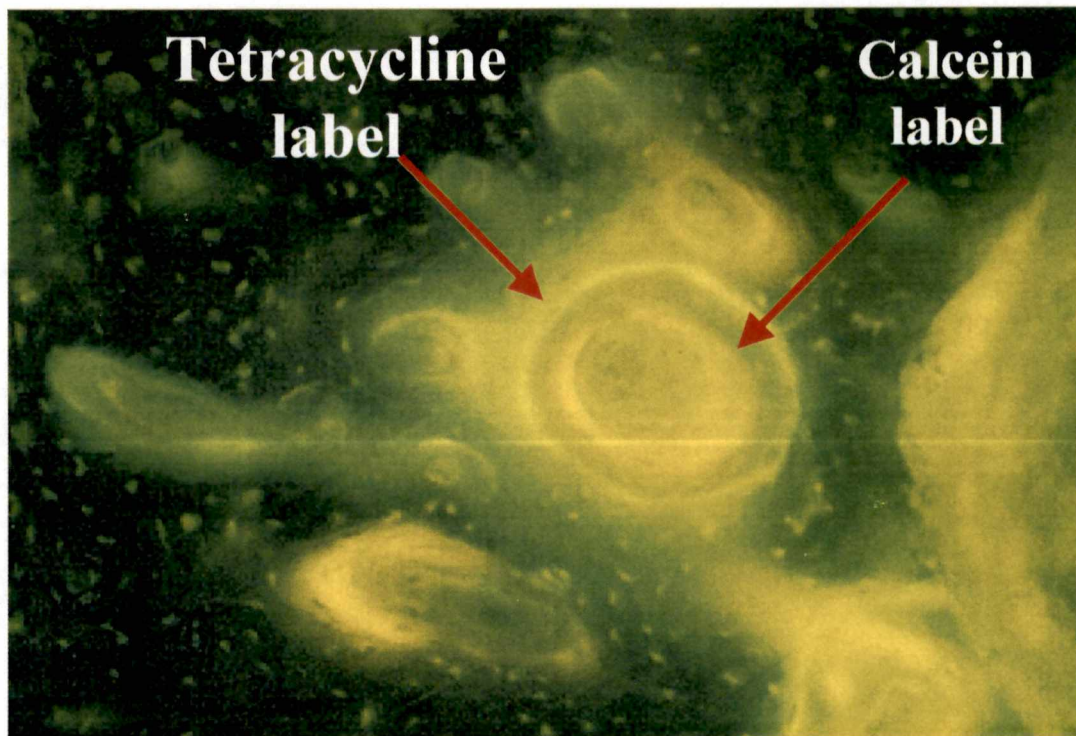


Figure 26: Double labeled osteon with tetracycline and calcein

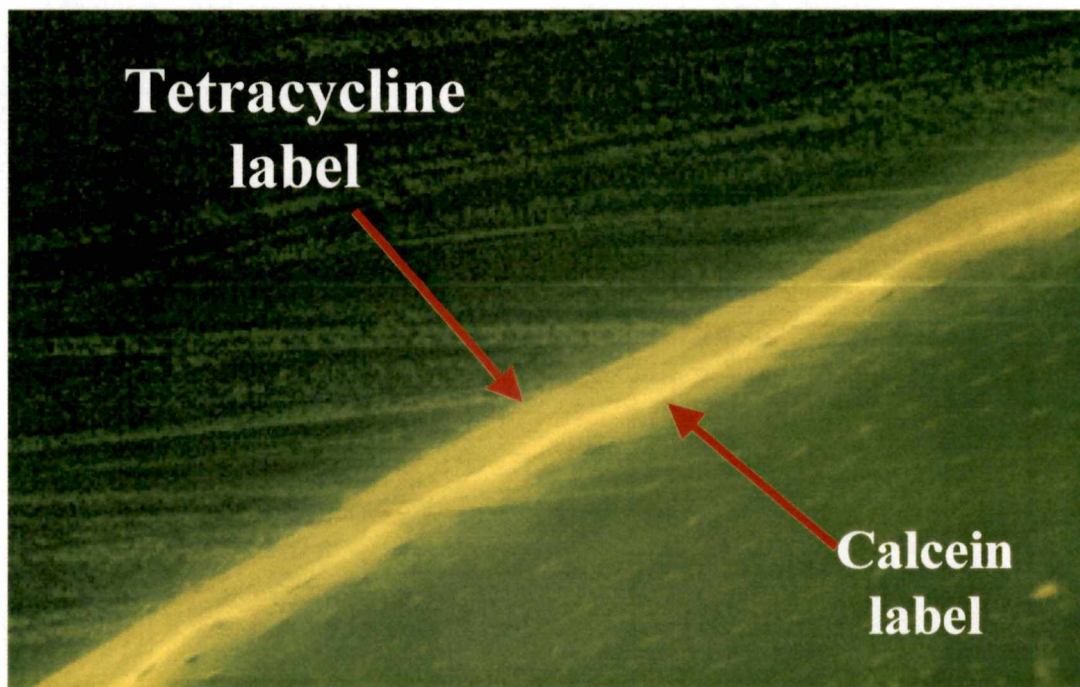


Figure 27: Double labeled periosteum

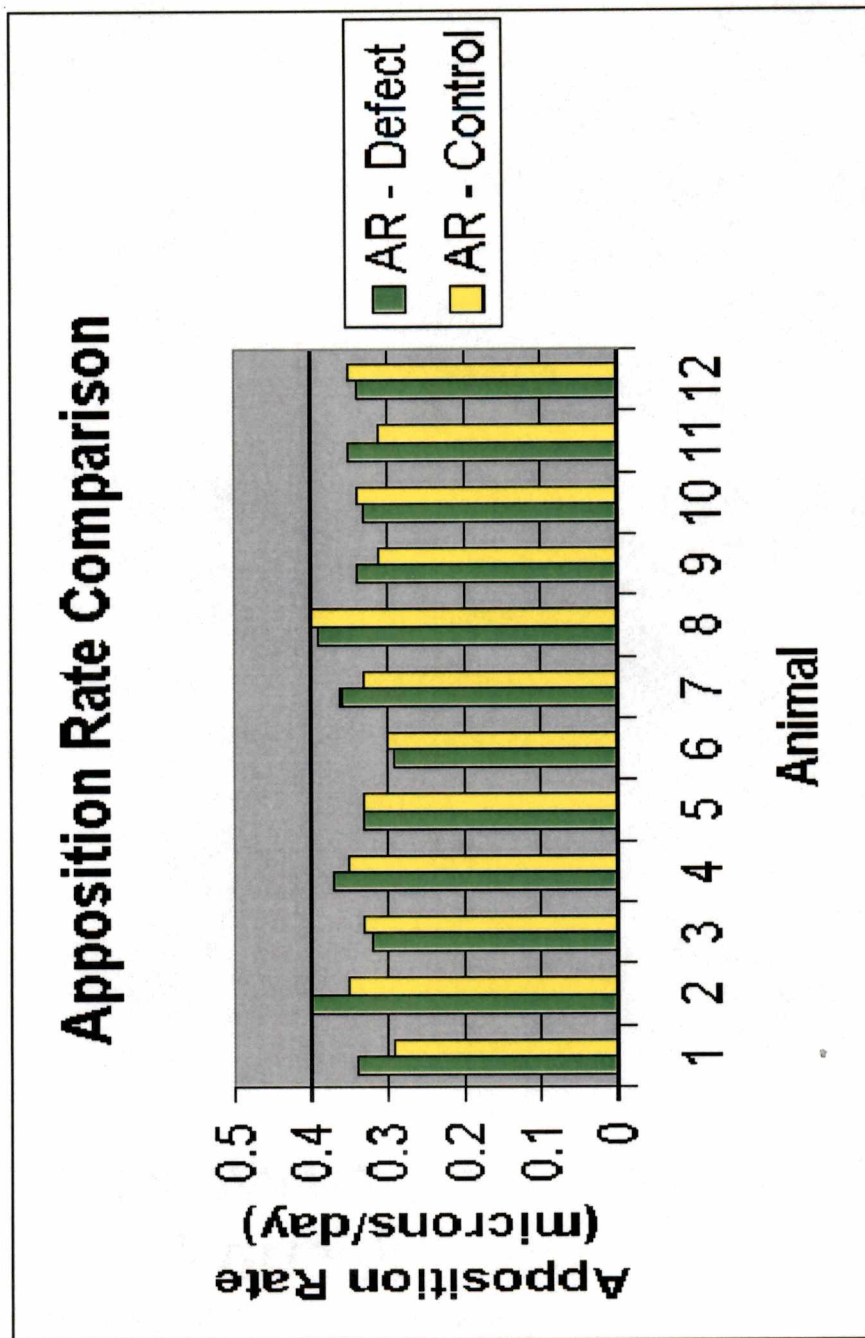


Figure 28: Apposition rate comparison of defect and control site

4.5 Bone Volume Comparison

A comparison of bone volume in the defect and in a nearby control area was made for further explanation of the regeneration activity. Bone volume was calculated as a percentage by measuring the area of the defect site and multiplying a third time to give volumetric units. This calculation was performed on the sections of the bone stained with Sanderson's Rapid Bone Stain® and Alizarin Red®. The control area was a site on the femur located approximately 1mm away from the defect (**Figure 29**).

The bone within the defect is woven bone, the initial structure of bone laid down by osteoblasts during osteogenesis. Woven bone resembles a mesh-like structure of fibers, lightweight, and less dense than cancellous or trabecular bone. It is expected that the volume of the bone in the defect will be less than that in the control area.

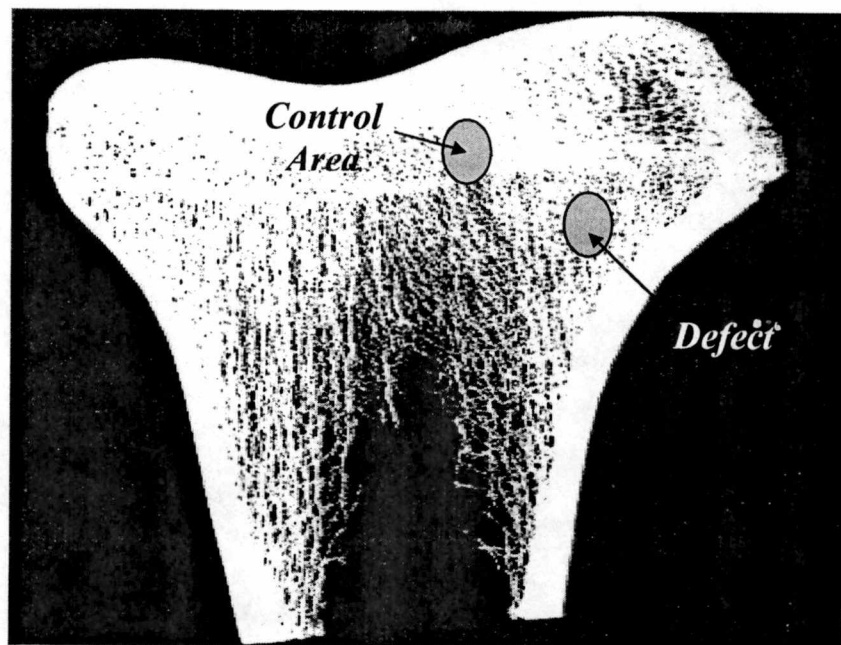


Figure 29: Control area and defect separated by 1mm (Hastings, 1984)

Figure 30 provides a comparison between the bone volumes in the control and defect areas. There are no measurements of a bone volume comparison for animals #3 and #5 because their bone fill percentage was negative; thus, the bone volume of the fill could not be calculated.

4.6 Bone Regeneration

The measurement of bone regeneration was calculated as a percentage of "fill" seen within the defect compared to the original size of the injury site, with 0% being no regeneration at all, and 100% being totally filled. The results from bone regeneration due to placement of the positive and negative electrets are compared to the controls in **Table 7** and illustrated graphically in **Figure 31**. In the control animals, there were two positive bone fill percentages and two negative. The negative percentages indicate that bone was actually resorbed and lost. Though there was no electrical charge applied to the control films, the electronegative properties of bone itself contributed to the percentages of bone activity in those animals. Additionally, the natural reaction to trauma to the body results in heightened cellular activity at the site of the wound. Animal #3 (**Figure 32**) had a -14% bone fill, which is believed to be caused by the complications incurred during surgery with excessive bleeding and swelling at the injury site. Only a few secondary osteons can be seen.

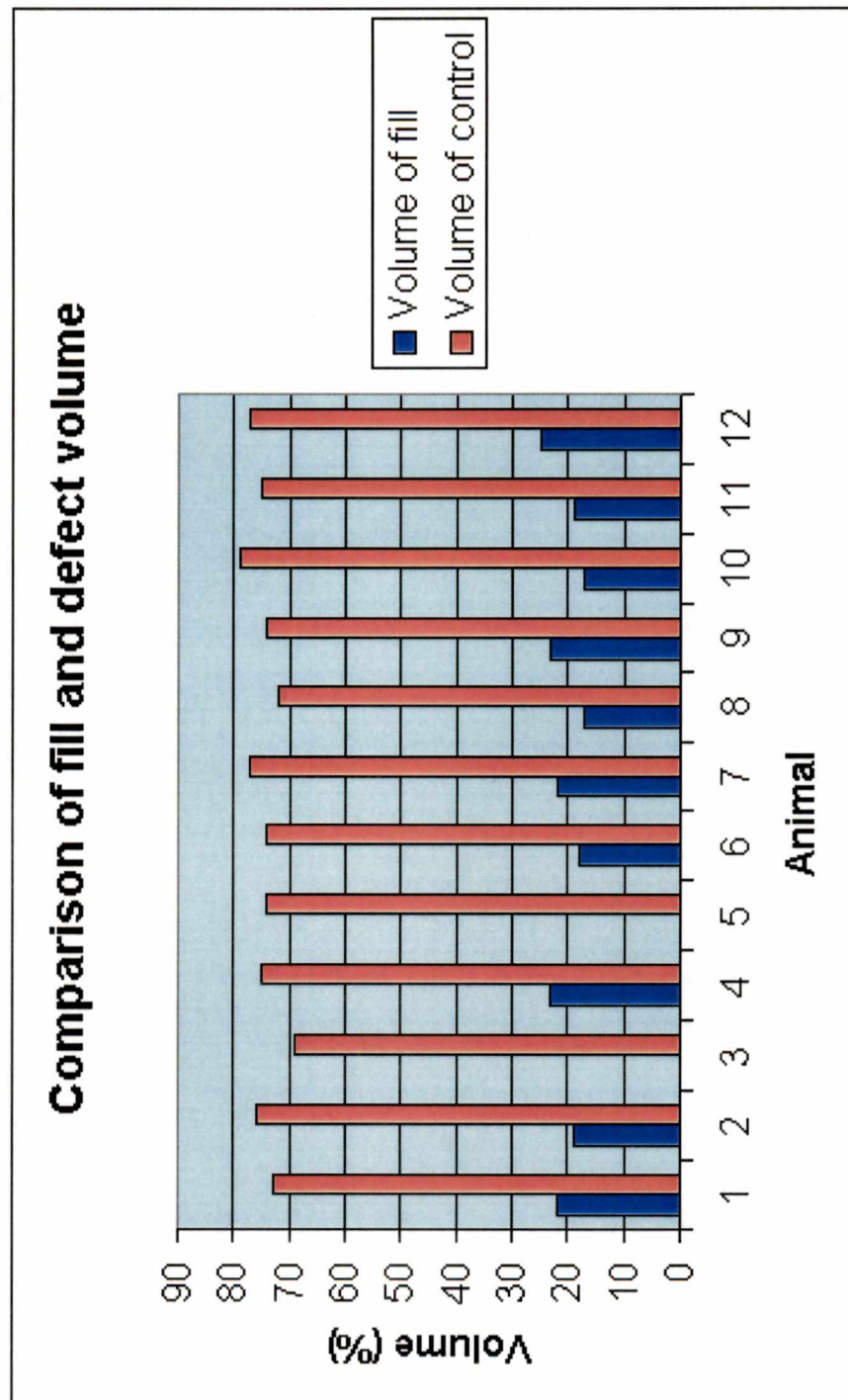


Figure 30: Volume percentage comparison between defect and control

Table 7: Comparison of bone fill percentages of electret groups

Animal	Electret Type	Bone Fill (%)
1	Control	12
2	Control	-5*
3	Control	-14*
4	Control	14
5	Positive	-8
6	Negative	62
7	Positive	14
8	Negative	55
9	Negative	53
10	Positive	16
11	Positive	11
12	Negative	60

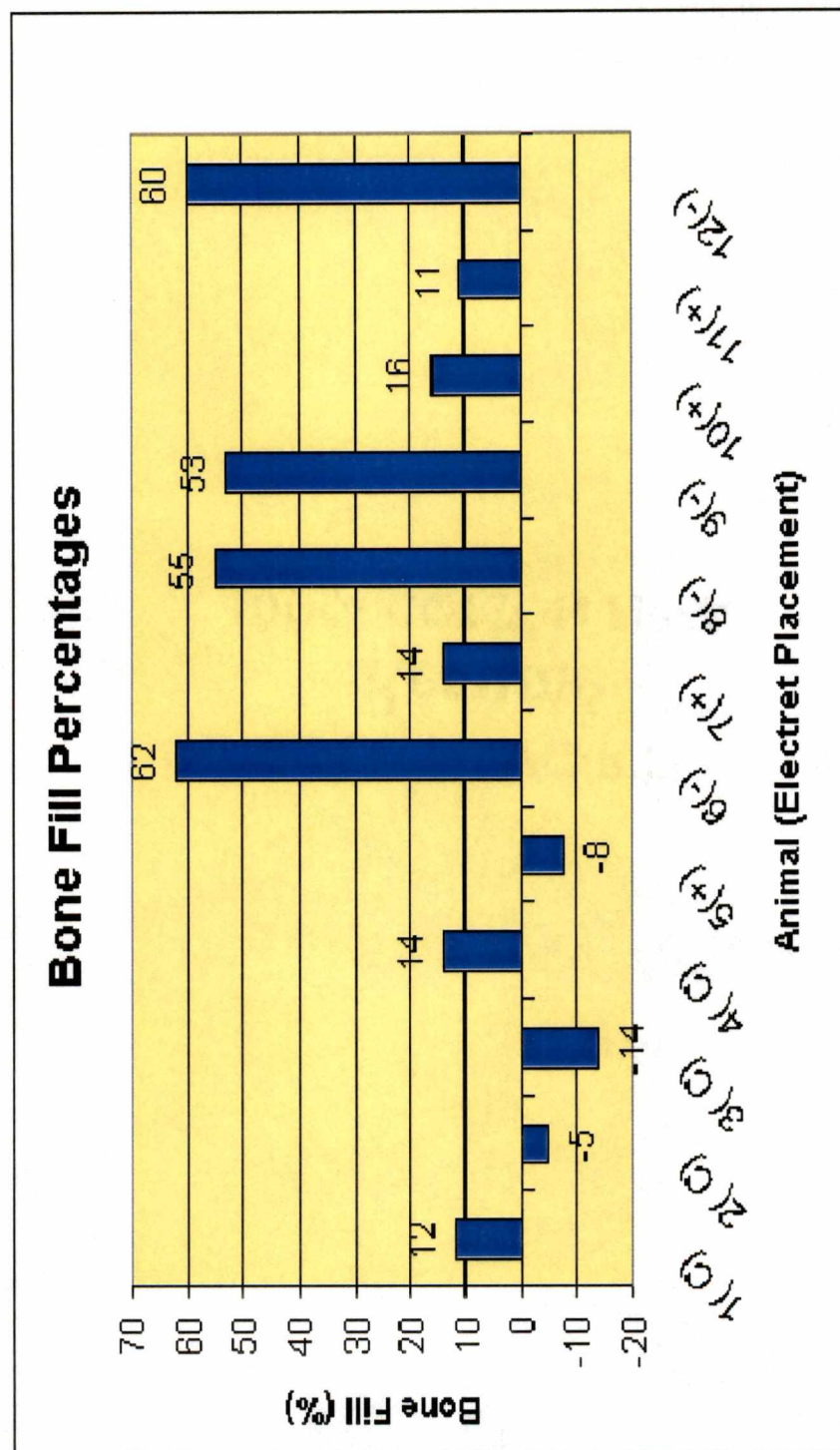


Figure 31: Bone fill percentages of defect site

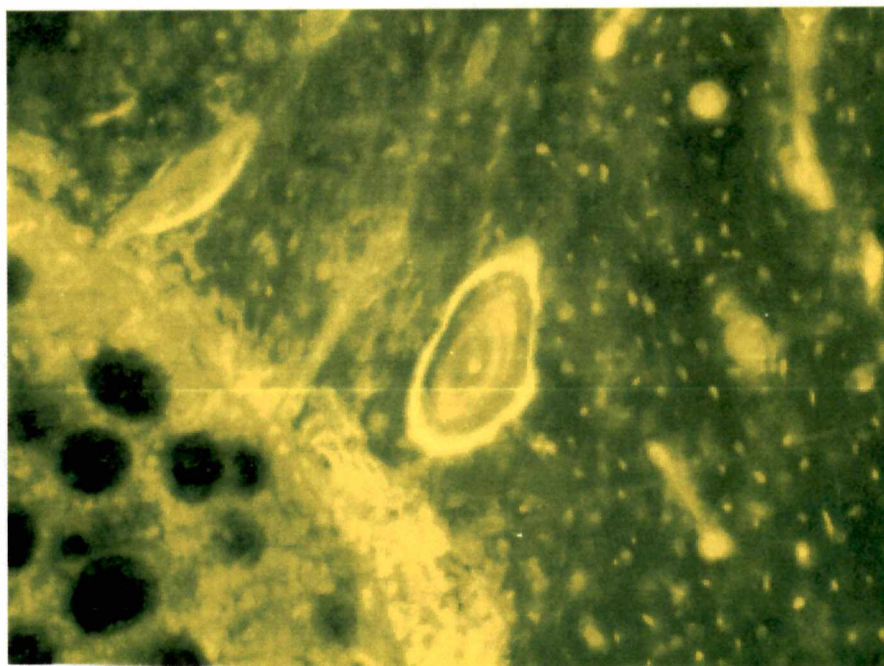


Figure 32: Animal 3 - minimal secondary osteons

Animals 5, 7, 10, and 11, which were given a positive electret implant, generated some bone (**Figure 33**). However, the percentage is significantly less than that regenerated due to negative electret implants, as can be seen by the remaining unfilled portion of the defect (**Figure 34**). Future studies using a positive electret may be useful in determining its effect on bone resorption in cases of osteopetrosis.

Animals 6, 8, 9, and 12, which received the negative interface of the electret applied to the defect, showed expected results of increased bone generation. The range of bone fill in these animals reached a minimum of 53% and maximum of 62%. Animal #8 (**Figure 35 and 36**) clearly illustrates the bone growth that occurred over the experimental duration of 30 days (55%). Additionally, the higher magnification of the cross sectional view of animal #8 shows partial remnants of the PLA electret film. This further validates PLA's biodegradable properties with time.

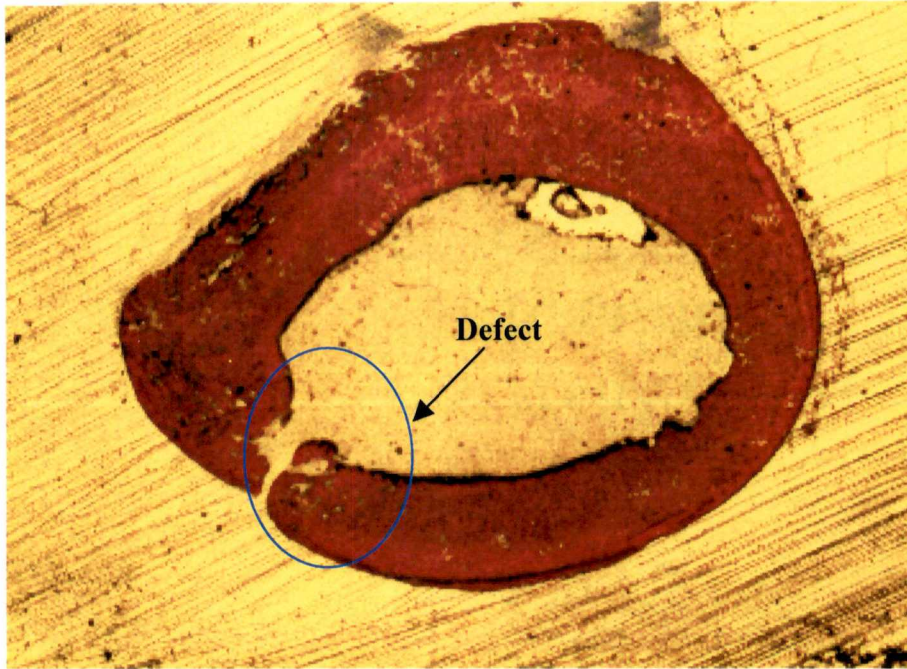


Figure 33: Animal 7 - cross-section of bone with partially filled defect (14%)

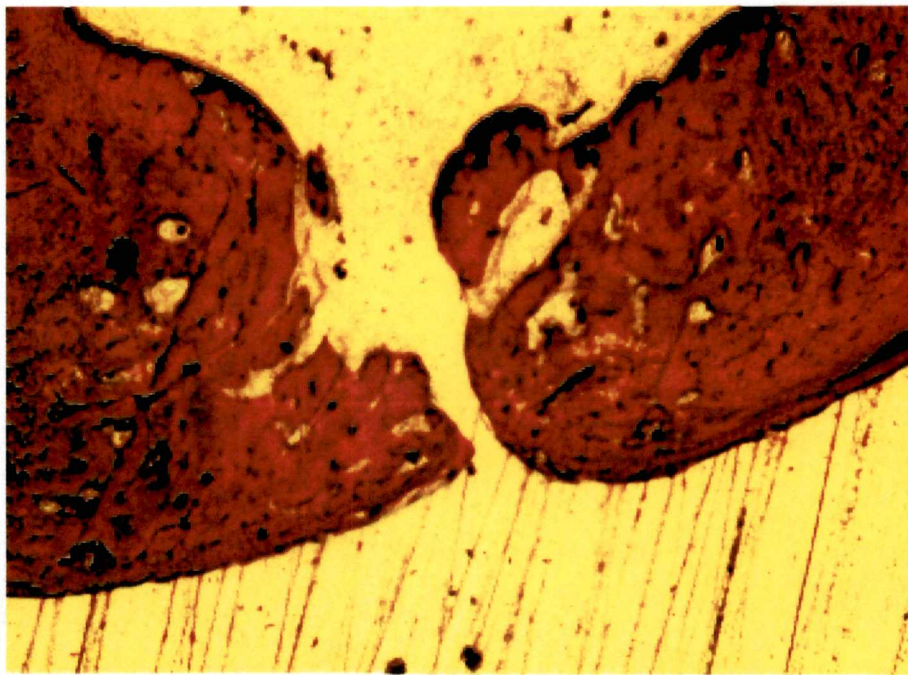


Figure 34: Animal #7 – higher magnification of partially filled defect

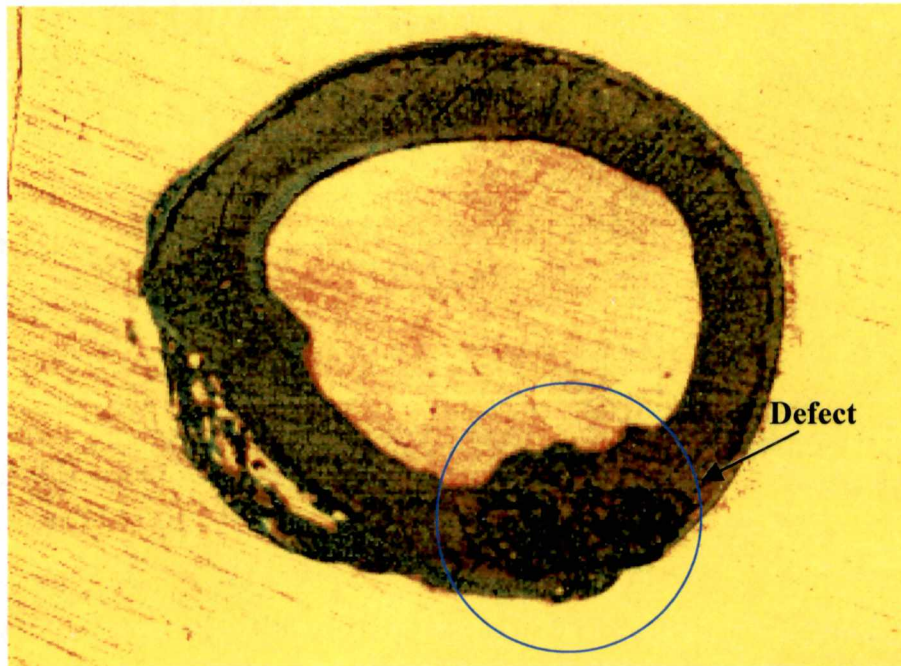


Figure 35: Animal 8 – cross-section of significantly filled defect (55%)

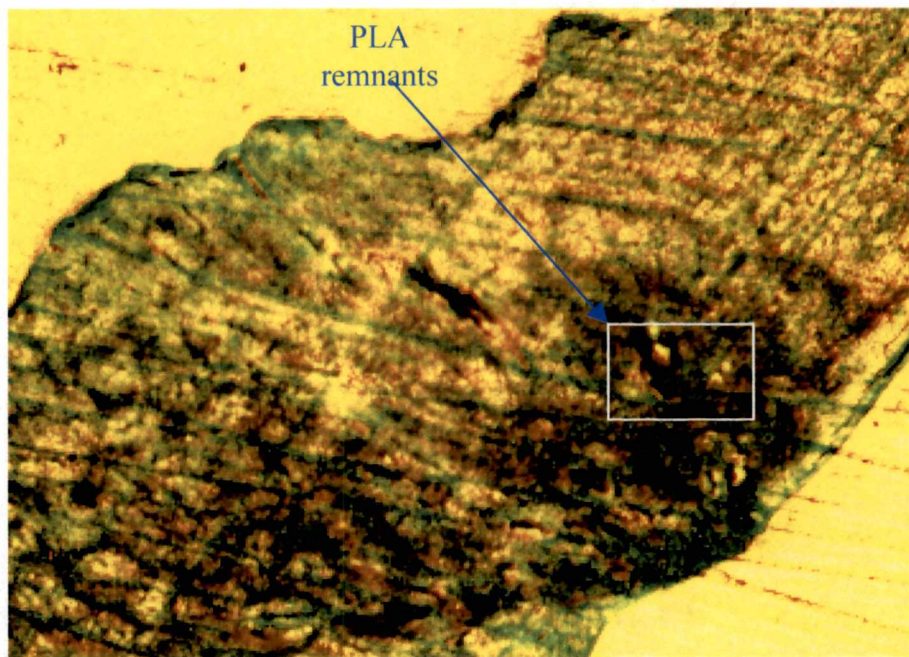


Figure 36: Animal 8 – higher magnification of filled defect

Table 8: Comparative Analysis of Bone Fill

Electret Placement	Average Bone Fill
Control (total)	1.75%
Control (2 positive %)	13%
Control (2 negative %)	-9.5%
Positive (total)	8.25%
Positive (w/o negative %)	13.7%
Negative	57.5%

Table 8 gives average bone fills for the control, negative, and positive electret placements. The total control average was 1.75%, a significantly low percentage that is expected given that the films had no charge. The total average of the positive electrets was 8.25%, greater than the control percentage, but considerably less than the exceptional average of 57.5% among the negative electrets.

Figures of bone fill for each animal may be found in **Appendix 4**.

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

The purpose of this investigation was to study the effects of a biodegradable PLA electret on the femur of growing New Zealand White rabbits. It was hoped that the application of electrostatically charged biomaterials to a defect would assist in regeneration of the bone. This property of electrets may be used long term in fracture healing in patients needing bone repair in various part of the body.

Fourteen rabbits were categorized into four groups. The baseline group, control group, positive group, and negative group. The baseline group, consisting of two animals, served as a guide for comparative analysis of eating, drinking, and behavioral habits. The control group of four animals received identical surgeries, however, their implant was uncharged. The positive electret group, comprised of four rabbits, had a positive electret applied to the site of defect. The negative electret group, including four animals, received a negative electret implant that was applied directly to the defect. At the end of one complete osteon of 30 days, the animals were euthanized and analyzed for bone growth.

The influence of PLA electrets was significant in bone regeneration and remodeling of the femoral defect. Based on the final analysis of the harvested bone, the following conclusions could be drawn:

1. Negative electrets increased osteoblastic activity, leading to bone regeneration within the defect. The results showed a higher percentage of bone fill and volume in those animals that received a negative electret.

2. Positive electrets increase osteoclastic activity, contributing to bone resorption, and in some cases, bone loss within the defect. A second possibility: positive electrets decrease or inhibit osteoblastic activity. Results consistent with these conclusions depicted lower percentages of bone fill and volume in those animals receiving positively charged implants. Some of the animals even showed a negative percentage of bone fill, resulting in actual erosion of the bone.
3. Animals that received a control implant containing no charge, showed some reaction to the trauma of the surgery itself with some positive and some negative percentages of bone fill and volume.
4. Heterotopic bone formation up and away from the defect site resulted in response to the trauma of the surgery in the majority of the animals indicating that the electrets are not responsible for its formation.
5. The biodegradable PLA electret implant eliminates the need for a second surgery for removal or replacement of the device, a major shortcoming of current fracture fixation procedures.

This study depicts the role of negative electrical stimulation in bone regeneration in the femurs of growing New Zealand white rabbits. Its results show promise in using negative electrical stimulation to enhance fracture healing in biomedical applications involving long bones.

5.2 Recommendations

Further study of the effects of negative electrets is necessary. My recommendations for supplementary analysis include:

1. Conducting the same experiment over a longer period of time.
2. Using another animal species for comparison of effects (i.e. dogs, pigs).
3. Utilizing a different biodegradable polymer such as polyglycolic acid (PGA).
4. Testing the effects of positive electrical stimulation on bone resorption for application in diseases involving osteopetrosis.

All of the above applications can be tested using the same mechanisms of this study. The future direction of this study is has many possibilities. These preliminary results add to the establishment of greater confidence in the manipulation of the electrical properties of bone to achieve beneficial medical results.

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APPENDICES

APPENDIX 1: DIELECTRIC CONSTANT CALCULATIONS

Dielectric Constant Equation:

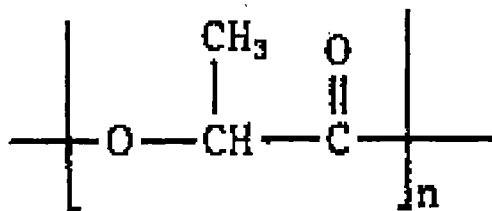
$$P_v = \epsilon^{1/2} M \quad (1)$$

$$\epsilon = \left(\frac{P_v}{M} \right)^2 \quad (2)$$

where P_v is the group contribution to molar dielectric polarization and M is the molecular weight. Utilizing information from the table below, the following values were calculated.

Group	For $P_{LL} = \frac{\epsilon - 1}{\epsilon + 2} V$	For $P_v = \epsilon^{1/2} M$
$-\text{CH}_3$	5.64	17.66
$-\text{CH}_2-$	4.63	20.64
>CH-	3.62	23.5
<C<	2.58	26.4
$-\text{C}_6\text{H}_5$	25.5	123.5
$-\text{C}_6\text{H}_4-$	25.0	128.6
$-\text{O}-$	5.2	(30)
>C=O	(10)	(65)
$-\text{COO}-$	15	95
$-\text{CONH}-$	30	125
$-\text{O}-\text{COO}-$	22	125
$-\text{F}$	(1.8)	(20)
$-\text{Cl}$	(9.5)	(60)
$-\text{C}\equiv\text{N}$	11	(50)
$-\text{CF}_2-$	6.25	70
$-\text{CCl}_2-$	17.7	145
$-\text{CHCl}-$	13.7	90
$-\text{S}-$	8	(60)
$-\text{OH}$ (alcohol)	(6)	(30)
$-\text{OH}$ (phenol)	~20	~100

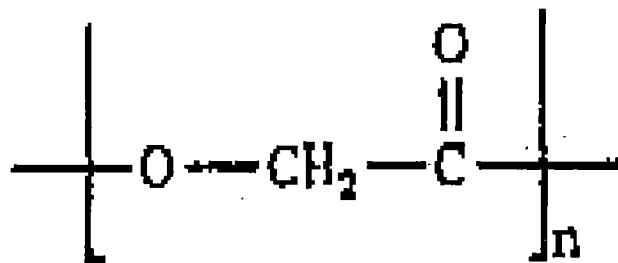
Polylactic Acid:



Group	# of Groups	Contribution	Molecular Weight
$\begin{array}{c} \text{O} \\ \\ -\text{C}-\text{O}- \end{array}$	1	95	44
CH ₃	1	17.66	15
CH	1	23.5	13

$$\varepsilon = \left(\frac{136.16}{72} \right)^2 = (1.891)^2 = 3.58$$

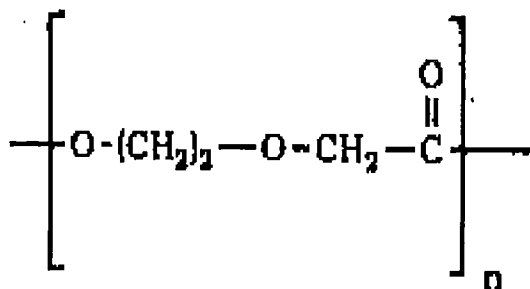
Polyglycolic Acid:



Group	# of Groups	Contribution	Molecular Weight
$\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{O}- \end{array}$	1	95	44
CH_2	1	20.64	14

$$\varepsilon = \left(\frac{115.64}{58} \right)^2 = (1.99)^2 = 3.97$$

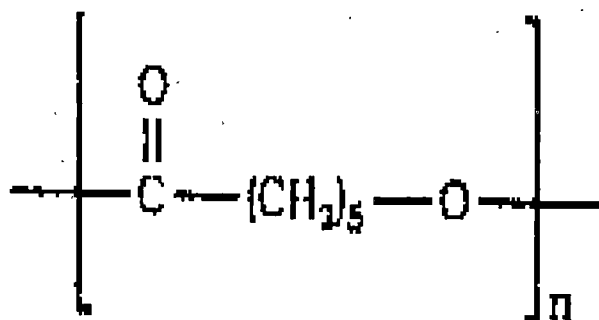
Polydioxanone:



Group	# of Groups	Contribution	Molecular Weight
$\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{O}- \end{array}$	1	95	44
CH_2	3	61.92	42
$-\text{O}-$	1	-30	16

$$\varepsilon = \left(\frac{126.92}{102} \right)^2 = (1.24)^2 = 1.54$$

Polycaprolactone:



Group	# of Groups	Contribution	Molecular Weight
$\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{O}- \end{array}$	1	95	44
CH ₂	5	103	70

$$\varepsilon = \left(\frac{198}{114} \right)^2 = (1.74)^2 = 3.02$$

APPENDIX 2: PROTOCOL FOR LIVE VERTEBRATES

**PROTOCOL FOR USE OF LIVE VERTEBRATES
THE UNIVERSITY OF TENNESSEE-KNOXVILLE**

PROTOCOL WILL BE RETURNED IF INCOMPLETE
PLEASE DO NOT ALTER THE PROTOCOL FORM

Submit 3 TYPED copies to:

Office of Laboratory Animal Care (OLAC)
A205 Veterinary Teaching Hospital
Phone # 974-5634

Effect of an Electret Polymer on Bone Healing in New Zealand White Rabbits

Title of Project/Course

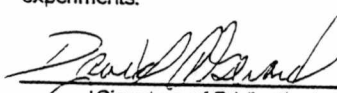
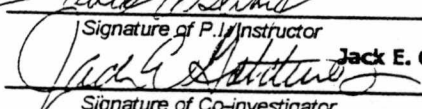
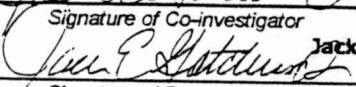
David A. Gerard

Principal Investigator/Instructor

544-9461 dpilling@mc.utmck.edu UT Graduate School of Medicine Oral&Maxillofacial Surgery

Wk. Phone E-Mail College Department

Your signature as P.I., Co-investigator, or Department Head on this application verifies that: (1) the information herein is true and correct and that you are familiar with and will comply with the legal standards of animal care and use established under federal and state laws and policies as well as university policies; (2) the proposal has received approval for scientific and/or educational merit by peer review; and (3) the activities do not unnecessarily duplicate previous experiments.

	David A. Gerard	3/30/01
<i>Signature of P.I./Instructor</i>		<i>Date</i>
	Jack E. Gotcher	3/30/01
<i>Signature of Co-investigator</i>		<i>Date</i>
	Jack E. Gotcher	3/30/01
<i>Signature of Department Head</i>		<i>Date</i>

FOR OLAC USE ONLY		Revised 6/00
IACUC Number _____	Ethical Concern Category A B C D E	
Assigned to: Full IACUC Review	Expedited _____	

General Information

1. This is a ☒ New ☐ 3-year rewrite
☒ Research ☐ Teaching Courses/CE Seminars covered by this protocol:

2. ☐ Yes ☒ No This protocol includes the use of farm animals used or intended for use as food or fiber, or livestock or poultry used or for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber.
3. Veterinarian responsible for clinical care
☒ OLAC Staff Veterinarians
☐ Dr. Dallas Goble
☐ LACS Ambulatory Service for the Department of Animal Science
☐ Other, list _____ If other, clinical veterinarian must follow the IACUC "Attending Veterinarian/Researcher Veterinarian" policy.
4. Funding Source:
UT College of Engineering-Graduate Research Fund
5. Non-Surgical Procedures (Pertains to any experimental procedure - including non-surgical, pre-surgical and post-surgical procedures using animals)
☒ NO ☐ YES:
6. Surgical Procedures (Pertains to any surgical procedure, including non-survival surgery. If other procedures are done on animals prior to or after surgery, complete applicable sections.)
☐ NO ☒ YES:
7. Field Studies Involving Wild Animals
☒ NO ☐ YES: (this section may be obtained from the OLAC office 974-5634)
8. Hazardous Agents
☒ NO ☐ YES:
9. Prescription Drugs/Controlled Substances
☒ NO ☐ YES:
10. Euthanasia
☒ NO ☐ YES:

A. Non-Technical Summary

Summarize, in lay person language (8th grade level), the rationale and purpose for the study, and the proposed use of the animals. Include time sequence of all procedures. For complex projects sequence of events can be depicted in a flow sheet (i.e., day one, group one, treatment; day two, etc.). For resubmissions, state what has been accomplished under this protocol so far. Please define acronyms and abbreviations.

Please see Addendum A

B. Animal Housing Facilities

In order to provide assurance of humane care and use of laboratory animals, all animal housing facilities on the UTK campus will be operated according to federal laws, AAALAC and NIH Guidelines, and the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching as appropriate. Housing of animals, including those actively on experiment, for periods longer than 12 hours, is restricted to facilities meeting such guidelines and laws.

1. Please check your preferred housing location.

- ☐ College of Arts and Sciences Facility in the Walters Life Sciences Building (WLS).
- ☐ College of Human Ecology Animal Facility in the Jessie Harris Building.
- ☐ College of Veterinary Medicine (CVM) Laboratory Animal Facility VTH/Cherokee
- ☐ CVM Large Animal Clinical Sciences in VTH/Cherokee Farm
- ☐ CVM Small Animal Clinical Sciences Research Units
- ☒ UT Medical Center Lab Animal Facility
- ☐ Joe E. Johnson Animal Research and Teaching Unit
- ☐ IACUC Approved Satellite Facility List bldg/room #
- ☐ *Other List:

*Any newly established facility must be approved before housing animals.

2. Please list all special housing or husbandry requirements.

N/A

3. Will wire bottom cages be used for rodents? If so, please provide scientific justification for use of wire bottom cages.

N/A

4. Environmental enrichment is routinely provided for animals. Please indicate if environmental enrichment is NOT to be provided and justification why it would interfere with research.

N/A

In accordance with NIH Guidelines and Federal Law, the IACUC formally reviews all animal housing facilities and satellites semi-annually and files a report to the Office for Protection from Research Risks (OLAW) in Washington, DC and the United States Department of Agriculture (USDA).

C. DESCRIPTION OF ANIMALS

Common Name	Strain/Breed	Sex	Weight/Age	Source	# per Day	Total # per project (3 years)
Rabbit	NewZealand	M/F	4-5 lbs	Myrtles	14	14

1. Animals will be removed from the animal housing facility:

☒ No

☐ Yes. Please answer the following:

- Animals will be taken to (bldg/room number): _____
- Animal manipulations that will be performed in the laboratory include: _____

- Estimated total time period live animals will be kept in the laboratory: _____ hours
- Animals will be returned to the facility YES NO

2. Disposal of animals after completion of activity:

- ☐ Return to production/breeding unit/facility inventory
☐ Slaughter, (must conform to the Humane Slaughter of Livestock, 9 CFR, part 313)
☐ Sold
☐ Transfer to another research project - please list protocol # and Investigator _____
☒ Euthanatized
☐ Returned to owner
☐ Other (Please describe) _____

D. QUALIFICATIONS OF PERSONNEL

List all individuals who will be working with the animals on this project. Include all investigators, student employees, post-doctoral researchers, staff research associates and laboratory assistants who will actually work with the animals. If personnel do not have experience, state how they will be trained.

The Occupational Health Program (OHP) is mandatory for all personnel who work with laboratory animals.

If an individual having animal contact is not currently enrolled in the occupational health program call the OLAC office at 974-5634 for information on enrolling.

Name/Degree(s)	OHP*	Procedure(s)/Description of Relevant Experience
Jack Gotcher, DMD, PhD		Will perform all surgeries and administer bone labels-Dr. Gotcher did all prior menisectomy surgeries on rabbits, also Dr. Gotcher has performed research surgeries on dogs and minipigs (implant placement) for previous studies
David Gerard, MS, PhD		Will assist with all surgeries, will harvest appropriate tissues at time of euthanasia. Dr. Gerard has assisted with all prior rabbit surgeries, as well as dog and minipig surgeries. He has been in charge of all tissue harvesting and processing for prior animal studies done by Oral Surgery
Ron Roberts, Animal care supervisor		Will assist in anesthesia and monitoring of animals during surgery and post-surgery

E. Animal Welfare

1. In addition to procedures which obviously cause pain, distress, or discomfort, USDA (Policy 11 & 12) states that any procedure which requires the use of an anesthetic or analgesic to prevent pain or discomfort is by definition a painful procedure (examples of painful procedures include: survival and non-survival surgery, use of Freund's Adjuvant, monoclonal antibody production, food/water deprivation, and application of noxious stimuli).

According to the above definition does this project involve pain or distress? [☒] yes [☐] no

If yes, please provide a written narrative that at minimum should include:

- a. the databases searched or other sources consulted to confirm that less painful alternative methods are not available,
- b. the date of the search and the years covered by the search.
- c. key words and/or search strategy used,
- d. a narrative written in such a way that the IACUC can readily assess whether the search topics were appropriate and whether the search was sufficiently thorough

This search should consider:

- >Replacement of existing animal methods with nonanimal methods whenever possible.
- >Reduction of the number of animals needed.
- >Refinement of research procedures to minimize pain and discomfort.

Please see Addendum B for narrative on animal welfare.

2. Does the proposed research duplicate any previous work? [☐] yes [☒] no If yes, explain why it is scientifically necessary to replicate the experiment.

3. Explain why the proposed species is/are the most appropriate.
The rabbit is a large enough animal to allow the placement of the electret film over a surgically created defect on the femur of the animal. Making films of a smaller size to be used in a smaller animal would be technically difficult, and these films would be difficult to place. We have worked with this animal model in a number of previous studies including one looking at the effect of electric charge on bone healing, and so are quite familiar with the animal model. Also, we have used bone labels in this animal model in previous studies and have found that IM administration of the labels works quite well. This minimizes the discomfort to the animals and negates the possible need for sedation during administration that is generally necessary with dogs and minipigs where the labels must be given IV.

4. Describe the steps you have taken to reduce the number of animals in your study. (refining experimental design, replacing animals with in vitro procedures, etc.)
Four animals per study group is the minimum that is necessary for adequate statistical analysis of the data, using analysis of variance for repeated measurements to assess any statistical differences within and between groups (ref. L.A. Woodbury, *Biostatistics*, 1971).

5. Provide an explanation of how the numbers of animals to be used were derived. If used in an experiment (test a hypothesis) numbers should be based on scientific and statistical requirements (ex: power tests or previous experience) to achieve objectives. Please see #4 above. Also, previous studies healing using electrical stimulation in this model have used these numbers of animals with good results.

Laspos, Chris. Effect of positive and negative charge on healing of condylectomized rabbits. *Masters Thesis, UT College of Dentistry, 1999*

Laspos, C, DA Gerard, E Harris. Effect of electric current on healing of condylectomized rabbits. J Orthodon, in press.

6. During The Study:

a. How often will the clinical condition of animals be monitored?

Daily-this will include observation of eating habits and activity levels as well as any other signs of distress or discomfort including any sign of lameness

b. Who will monitor the clinical condition of the animals?

Dr. Gerard and the animal facility staff

c. Are animals expected to experience any specific study-induced or related problems (i.e. health problems, pain, distress, complications, etc.) or any health problems as a result of the phenotype of the animal?

No. In 4 previous studies using rabbits, no signs of discomfort or distress were observed at any time post-surgery. Care will be taken at the time of surgery to use blunt dissection moving aside muscles to gain access to the femur. This will insure a minimum of discomfort combined with post-surgery analgesics. In previous studies animals continued to eat, gain weight and have normal activity levels. All animals either maintained or gained weight. Animals should not show any sign of lameness or other discomfort. In this study we will have 2 animals that serve as non-surgical baseline controls. These animals will also serve as indicators of comparative activity levels and eating habits.

d. What criteria will be used to assess pain, distress, or discomfort?

Generally in these animals level of activity and eating and drinking habits are very good indicators of pain, distress, or discomfort. The 2 non-surgery control animals will also serve as indicator of comparative activity levels and eating habits.

Check all that apply:

- ☒ Loss of appetite.
- ☒ Loss of weight.
- ☒ Restlessness.
- ☒ Abnormal resting postures in which the animal appears to be sleeping or is hunched up.
- ☒ Licking, biting, scratching, or shaking a particular area.
- ☐ Failure to show normal patterns of inquisitiveness.
- ☐ Failure to groom, causing unkempt appearance.
- ☐ Guarding (protecting the painful area).
- ☒ Loss of mobility.
- ☐ Red stain around the eyes of rats.
- ☒ Unresponsiveness.
- ☒ Self-mutilation.
- ☐ Labored breathing.
- ☐ Other. (please list)

1. SITES OF STUDY

2. STUDY PROCEDURES :

- [] Polyclonal antibody production*
- [] Blood* or other body fluid withdrawal, tissue collection, injections, tail clip, gavaging
- [] Restraint with mechanical devices
- [] Projects involving food and water deprivation, or dietary manipulation
- [] Tumor and disease models* or toxicity testing
- [] Anesthesia or analgesia* (for non-surgical procedures)
- [] Behavioral studies
- [] Endoscopy, fluoroscopy, radiology, ultrasound, MRI, CT, PET, or other diagnostic procedures
- [] Monoclonal antibody production
- [] Use of tissues, serum, tumor lines, hybridoma, etc.

POLYCLONAL ANTIBODY PRODUCTION

N/A

Describe in detail method(s), needle sizes, volume(s) collected or administered, and frequency of collection or injections:

	Location	Needle Size/ Catheter Size	Biopsy Size	Volume Collected	Volume Administered	Frequency
Body Fluid Withdrawal						
Tissue Collection						
Injection/ Infusion	IM/hip-Bi. <u>femoralis</u>	22 gauge			10mg/kg body weight	Twice 6 days apart
Tail Clip						
Gavaging						
Other						

e. What criteria will be used for removing animals from the study prematurely?
Any animal that is not fully recovered from the effects of the surgical procedure by day 3 (i.e. normal appetite and activity level and showing no other adverse signs) will be euthanized immediately. Also, any animal that loses up to 20% of their body weight will be euthanized.

RESTRAINT WITH MECHANICAL DEVICES

Describe device, duration of restraint, frequency of observation, conditioning procedures and steps to assure comfort and well-being:

N/A

PROJECTS INVOLVING FOOD AND WATER DEPRIVATION, OR DIETARY MANIPULATION

Describe methodology. State objective criteria used to assess physical condition and pain, discomfort, stress, and distress during the course of study. Include clinical signs or manifestations expected from the procedure. What criteria will be used to determine a humane endpoint before severe morbidity and death?

N/A

	Amount Restricted/Added	Duration	Compound Supplemented	Compound Deleted	Frequency
Food Restriction					
Fluid Restriction					
Nutrient Alterations					

TUMOR AND DISEASE MODELS, TOXICITY TESTING

Describe methodology used for tumor/disease induction and/or toxicity testing. State objective criteria used to assess physical condition and pain, discomfort, stress, and distress during the course of study. Include clinical signs or manifestations expected from the procedure. What criteria will be used to determine a humane endpoint before severe morbidity and death?

N/A

ANESTHESIA/ANALGESIA/TRANQUILIZATION (OTHER THAN SURGERY)

Adequate records describing anesthetic monitoring and recovery must be maintained and available to the attending veterinarian and animal care staff.

Agent	Dose (mg/kg)	Route	Frequency	For which procedure
ketamine	3 5mg/kg	IM	1 time	surgeries
xylazine	5mg/kg	IM	1 time	surgeries

Name and qualifications of person administering drugs: _____

RON ROBERTS-SENIOR ANIMAL CARETAKER WHO HAS ADMINISTERED ANESTHESIA FOR ALL OUR PRIOR RABBIT STUDIES

CRITERIA TO ASSESS LEVEL OF ANESTHESIA.

Check all that apply:

☒ Respiration rate

☒ Heart rate

☐ ECG

☐ Toe pinch

☐ Tail pinch

☒ Corneal reflex

☒ Color of mucous membranes

☒ Muscular relaxation

☐ Other (pulse oximeter, respirometer) please list _____

ANESTHESIA RECOVERY MONITORING

a. Will analgesia be provided? ☒ Yes ☐ No
If no, please explain why analgesics are withheld:

b. What is the anticipated duration for recovery from anesthesia?
1 HOUR

c. How often will animal(s) be monitored during recovery?
Continuously

d. What specifically will be monitored?
Heart rate, respiration, gum color, corneal response, muscle coordination

e. Who will be monitoring them?
Dr. Gerard, Animal facility staff

RECOVERY MONITORING

a. Following anesthesia recovery, what parameters will be monitored?
Heart rate, respiration, gum color, corneal response, muscle coordination

b. Who will monitor the animals?
Dr. Gerard, Animal facility staff

c. How frequently will animals be monitored?
AFTER THE INITIAL RECOVERY FROM THE ANESTHESIA, ANIMALS WILL BE CHECKED TWICE DAILY. THIS WILL INCLUDE OBSERVATION OF RESPIRATION, EATING HABITS, AND ACTIVITY LEVELS. ANIMALS WILL BE WEIGHED AT THE BEGINNING OF THE STUDY AND WEEKLY DURING THE COURSE OF THE STUDY.

BEHAVIORAL STUDIES

Describe in detail types of behavioral manipulations, including placement in testing chambers or apparatus, use of aversive stimuli, duration of test periods, and frequency of test periods:

N/A

ENDOSCOPY, FLUOROSCOPY, X-RAY, ULTRASOUND, MRI, CT, PET,
OR OTHER DIAGNOSTIC PROCEDURES

Describe, in detail, methodology and animal manipulations:

N/A

MONOCLONAL ANTIBODY PRODUCTION

Please provide scientific justification explaining why specific *in vitro* monoclonal antibody production methods cannot be used. IACUC monoclonal antibody production guidelines must be followed. Any deviation from these guidelines must be specified and scientifically justified below.

N/A

USE OF TISSUES, SERUM, TUMOR LINES, HYBRIDOMA ETC.

All tissues must be MAP or PCR tested to ensure that tissues are free of infectious agents. Please provide evidence of this testing:

N/A

SURGICAL PROCEDURES

The attending veterinarian must be consulted on anesthetic regimens, surgical procedures and post-surgical care.

Adequate records describing surgical procedures, anesthetic monitoring and postoperative care must be maintained and available to the attending veterinarian and animal care staff.

TYPE OF SURGERY

☒ Nonsurvival surgery: (animals euthanized without regaining consciousness).

☐ Major survival surgery: (major surgery penetrates and exposes a body cavity or produces substantial impairment of physiologic function).

☐ Minor survival surgery.

☐ Multiple survival surgery?

If yes, provide justification for multiple survival surgical procedures:

1. Surgeon's name and experience with species and procedures to be performed:

Dr. Jack Gotcher-Dr. Gotcher has been involved with numerous animal studies here in the past 15 years including surgery on dogs, minipigs and rabbits.

2. Location where surgery will be done:

UT Hospital-Animal Facility

3. Describe the pre-op preparation of the animals:

a. Food restricted for 12 hours

b. Water restricted for 0 hours

4. Minimal sterile techniques will include (check all that apply):

☒ Sterile instruments

☒ Sterile gloves

☒ Cap and mask

☒ Sterile gown

☒ Sterile operating area

☒ Clipping or plucking of hair or feathers

☒ Skin preparation with a sterilant such as betadine

☒ Practices to maintain sterility of instruments during surgery

5. Describe the following surgical procedures:

a. Skin incision size and location:

Surgeries will be performed under aseptic conditions with the animals under general anesthesia. A 3-4cm incision will be made on the lateral aspect of the right thigh of each animal, with blunt dissection down to the femur, taking care to move the muscles aside and do minimal damage to them and the fascia. A defect measuring 4mm in diameter and depth will be created using a #3 fissured tapered burr under continuous saline irrigation. This defect will be covered with a polymer electret sheet sufficient to allow fixation on either side of the defect using nonresorbable suture.

b. Method of skin closure: (type, suture size, suture pattern, etc.)

Any fascia or muscle tissue that may have been cut will be sutured using vicryl. The skin will then be closed using running sutures with vicryl.

sutured, then the fascia, and finally the skin using vicryl for all sutures.

c: Describe surgery in detail:

3. WILL PARALYZING DRUGS BE USED?
☒ No ☐ Yes _____
Drug Dose

- IF YES, PLEASE JUSTIFY THE NEED TO USE PARALYZING DRUGS:
7. Anesthetic Protocol

	Agent	Dose (mg/kg)	Frequency
Pre-emptive analgesic			
Pre-anesthetic	Atropine	0.1mg/kg/IM	once
Anesthetic	Ketamine/xylazine	3.5mg/kg&5mg/kg IM	once
Analgesic	Butorphanol tartrate	0.2mg/kg SQ	Once, then as needed
Other			

CRITERIA TO ASSESS LEVEL OF ANESTHESIA.

Check all that apply:

- ☒ Respiration rate
☒ Heart rate
☐ ECG
☒ Toe pinch
☐ Tail pinch
☒ Corneal reflex
☒ Color of mucous membranes
☒ Muscular relaxation
☐ Other (pulse oximeter, respirometer) please list _____

8. Describe any behavioral or husbandry manipulations that will be used to alleviate pain, distress, and/or discomfort.

9. ANESTHESIA RECOVERY MONITORING

- a. Will analgesia be provided? ☒ Yes ☐ No
If no, please explain why analgesics are withheld:
- b. What is the anticipated duration for recovery from anesthesia?
1 HOUR
- c. How often will animal(s) be monitored during recovery?
animals will be continuously monitored during recovery
- d. What specifically will be monitored?
respiration, heart rate, corneal reflex, gum color, coordinated movement
- e. Who will be monitoring them?
Dr. Gotcher, Dr. Gerard, animal facility staff

10. POSTSURGICAL RECOVERY MONITORING

- a. Following recovery, what parameters will be monitored?
Return to normal eating habits and activity levels, respiration (observed), weight gain or loss

- b. Who will monitor the animals?
Dr. Gerard, animal facility staff
- c. How frequently will animals be monitored?
At least twice daily by observation. Weights will be checked weekly

HAZARDOUS AGENTS

1. WILL ANIMALS BE SUBJECTED TO ANY OF THE FOLLOWING? **NO**

- _____ 1. *Infectious Agents
- _____ 2. *Toxic Chemicals or Carcinogens
- _____ 3. *Recombinant DNA
- _____ 4. *Malignant Cells or Hybridomas
- _____ 5. **Radionuclides
- _____ 6. *Human tissue

*Biosafety Committee approval required Approval Number _____

**Radiation Safety Committee approval required (Please attach approval letter)

2. IF YES, PLEASE COMPLETE THE ATTACHED ANIMAL HAZARD CONTROL FORM.

3. COMPLETE THE FOLLOWING CHART.

Agent	Concentration	Route of Administration	Duration of Exposure	Post Exposure Maintenance of Animals

ANIMAL HAZARD CONTROL FORM			
The Principal Investigator is responsible for informing all personnel handling animals of the hazards involved and precautions to be taken. The Principal Investigator is also responsible for updating the form in the case of changes in agent or experimental procedure. For assistance in completion of this form please contact the appropriate Safety Officer			
HAZARDOUS AGENTS			
Principal Investigator:	Campus Phone:	Home Phone:	
Department:	E-Mail:		
Secondary Contact: Department:	Campus Phone:	Home Phone:	E-Mail:
IACUC Protocol Number:			
Chemical Agent Used:N/A			
Radioisotopes Used:N/A			
Biohazardous Agents Used:N/A			Biosafety Level:
SPECIAL PERSONAL PROTECTIVE EQUIPMENT REQUIRED:			
Route of Excretion:			
Precautions for Handling Live or Dead Animals:			
Animal Disposal:			
Bedding / Waste Disposal:			
Cage Decontamination:			
Additional Precautions to Protect Personnel, Adjacent Research, and Environment:			
Building:			Room #:

LEGEND (PRESCRIPTION) DRUGS AND/OR CONTROLLED SUBSTANCES

* **Legend Drugs and Controlled Substances** In Tennessee, it is not legal to divert drugs from clinical use to teaching and research use. You, or someone in your group acting as an agent for the group, must have a license for each individual legend drug that you might use in teaching or research. A legend drug is one where the label says "Federal Law restricts this drug to use by or on the order of a licensed veterinarian" or "Federal Law prohibits dispensing without a prescription". If the drug is a controlled substance, you also need a license from the Drug Enforcement Administration for the specific drugs that you may use. Obtaining and using such drugs for research needs to be reviewed by the appropriate Committee. The use of legend drugs and controlled substances must be approved by the appropriate committee.

Do you have the appropriate licenses and legend and controlled drug Committee approval for the drugs you will use in your project or class?

☐] NO ☒] YES

College of Veterinary Medicine personnel contact:
Medical Center personnel contact
All others contact:

Bruce McNeil 974-5670
Mark Smith 544-9363
Brenda Lawson 974-3466

EUTHANASIA

METHODS OF EUTHANASIA MUST COMPLY WITH THE 1993 REPORT OF THE AVMA PANEL ON EUTHANASIA OR OTHER IACUC APPROVED METHODS. DEPARTURES MUST BE JUSTIFIED.

METHOD OF EUTHANASIA

- ☒ Anesthetic overdose.
Drug: beuthanasia
Dose: 1mg/10lb BW IV, IC
Route: IV
☐ Decapitation under anesthesia or tranquilization
☐ Decapitation without anesthesia or tranquilization*
☐ Cervical dislocation under anesthesia or tranquilization
☐ Cervical dislocation without anesthesia or tranquilization*
☐ Exsanguination under anesthesia
☐ Carbon dioxide exposure
☐ Slaughter (covered under the Humane Slaughter of Livestock, 9 CFR, part 313)

☐ Other: Specify:

*PLEASE JUSTIFY ANY DEPARTURE FROM AVMA PANEL REPORT: (EX. CERVICAL DISLOCATION WITHOUT ANESTHESIA)

2. NAME AND QUALIFICATIONS OF PERSON(S) PERFORMING EUTHANASIA:
Animal facility staff-Ron Roberts
3. METHOD OF DISPOSAL. EUTHANIZED ANIMAL CARCASSES MUST BE DISPOSED OF APPROPRIATELY. Please check the appropriate method:
☒ incinerate
☐ by Radiation Safety
☐ carcass will contain hazardous agents and will be autoclaved prior to disposal
☐ slaughter (covered under the Humane Slaughter of Livestock, 9 CFR, part 313)
☐ necropsy
☐ other - describe:

Addendum A

The purpose of this study is to see if a flexible film that can be made to hold an electrical charge (these materials are called electrets) can be used to stimulate bone healing. The rationale behind the study is that many previous studies have shown that an electric current can stimulate both bone formation as well as bone destruction. In a study we did in conjunction with Dr. Chris Laspos from UT Memphis in 1999 we showed increased bone healing in the rabbit when a negative charge was placed near the bone defect. On the other hand, when a positive charge was placed near the bone defect there was a slowing of the healing. If electrets could be used to stimulate bone activity it would eliminate the need for an external source of current (i.e. batteries). In this study a defect will be surgically created in the femur (upper hind leg bone) of rabbits, and the defect will be covered by an electret film. Any material that is made into an electret must have a positively charged surface and a negatively charged surface (for example a sheet of paper would have a + charge on one side and a - charge on the other). In this study we will test the effect of both the positively charged and the negatively charged surface when they are in direct contact with the bone defect. The study will use 14 rabbits placed into 4 groups as follows:

Group A (4 animals)-the negatively charged surface in contact with the defect

Group B (4 animals)-the positively charged surface in contact with the defect

Group C (4 animals)-control group with an identical film with no charge in contact with the defect

Group D (2 animals)-non-surgery controls to obtain baseline bone activity

The study will be 30 days in length, with all animals being euthanized on day 30 after surgery. At day 21 the animals will be given a shot containing tetracycline (10mg/kg BW) in the hip, and on day 27 they will be given a shot of calcein (10mg/kg BW) in the other hip. Tetracycline and calcein are bone directly to any bone that is being made at the time that the animals are given the drug. These two drugs also are fluorescent, and can be seen with a microscope. By using these 2 agents 6 days apart it will be possible to measure where bone is being formed, and how fast it is being formed. This will allow us to measure what effect the positive, negative and control films have on bone formation.

Addendum B

Literature searches were done the beginning of January, 2001, to find other models to examine electrets and bone activity. These searches were done through Medline and PubMed for the period from 1980 to 2000. Topics and key words searched included *electret, bone healing, electrical stimulation-healing, magnetic stimulation-healing*. The only study that could be found using an electret material to affect bone activity was a 2000 study in which the electret material was placed on a calvarial (skull) defect in rats. There are no models that use cell or tissue culture techniques or computer modeling to reproduce the possible response of bone *in situ* to these materials.

The number of animals used in this study is the minimum that will be needed to obtain data that can be statistically analysed to see if differences between groups exist. We have done everything possible to minimize any pain or discomfort that these animals will experience.

Animals will be given a pre-anesthetic dose of Ancef (35mg/kg/IM) prophylactically to prevent infection. Post-surgery, animals will receive one dose of Butorphanol tartrate (0.2mg/kg/SQ) as an analgesic prior to recovery from the surgical anesthesia, and as needed thereafter.

Food will be withheld from the animals for 12 hours prior to surgery to prevent any possible complications from aspiration.

APPENDIX 3: BONE/ELECTRET STUDY – ANIMAL FLOW CHARTS

Bone/Electret Study-Animal flow chart

Animal # 1 Weight 2.5 Ks Date 6/28 Time 11:30am
Presurgery treatment - .26ml
Ancef(35mg/kg/IM) ✓ Time 12:09pm
Surgery 07.5mg
Duration 30mins
Film placement (+, -, C) C
Postsurgery treatment
Buprenorphine 0.05mg/kg/sc ✓ Time 12:09pm 0.125mg = .4ml
Postsurgery observation of vital signs Time 3:10 PM
Comments
CAME OUT OF ANESTHESIA IN 10 MINUTES - NOT COORDINATED
IS ALERT AND MOVING NORMALLY

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased respiration, weight loss

- NO ADDITIONAL DRUG TREATMENT REQUIRED
- ANIMAL CONTINUES TO REMAIN ACTIVE, ALERT AND EATING NORMALLY

Weight-

Week 1 2.7

Week 2 2.8

Week 3 2.2

Week 4 2.9

Day 21 DATE 7/12
Tetracycline (10mg/kg/IM) Time 8:40 AM
Day 27 DATE 7/25
Calcein (10mg/kg/IM) Time 9:20 AM
Day 30 Euthanasia-beuthanasia(1mg/10lb bw iv,ic)
Date 7/28 Time 1:20 PM

Bone/Electret Study-Animal flow chart

Animal # 2 Weight 2.3 Date 6/28 Time 12:20pm
Presurgery treatment .24ml
Ancef(35mg/kg/IM) ✓ Time 12:44pm
Surgery 80.5ms
Duration 24 mins
Film placement (+, -, C) C
Postsurgery treatment
Buprenorphine 0.05mg/kg/sc ✓ Time 12:44pm 0.115mg = .38ml
Postsurgery observation of vital signs Time 3:00 PM
Comments AWAKE AND MOVING NORMALLY

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased respiration, weight loss

-NO ADDITIONAL ANTIBIOTIC OR ANALGESIC APPEARS NEEDED
-ANIMAL IS EATING AND DRINKING NORMALLY

Weight-

Week 1 2.3
Week 2 2.5
Week 3 2.6
Week 4 2.8

Day 21 DATE 7/19
Tetracycline (10mg/kg/IM) Time 9:00 AM
Day 27 DATE 7/25
Calcein (10mg/kg/IM) Time 9:25 AM
Day 30 Euthanasia-beuthanasia(1mg/10lbw iv,ic)
Date 7/28 Time 1:40 PM

Bone/Electret Study-Animal flow chart

Animal # 3 Weight 2.6 Date 6/28 Time 12:50pm

Presurgery treatment .27ml
Ancef(35mg/kg/IM) ✓ Time 11:09pm

Surgery 91mg
Duration 20 mins
Film placement (+, -, C) C

Postsurgery treatment
Buprenorphine 0.05mg/kg/sc ✓ Time 1:10pm 0.13mg = .43ml

Postsurgery observation of vital signs Time 3:30 PM

Comments

SOME BLEEDING AFTER DEFECATION - POSSIBLE HEMATOMA

- ANIMAL APPEARS TO BE FUNCTIONING NORMALLY - SOME SWELLING - NOTED AT SURGERY SITE

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased respiration, weight loss

- NO ADDITIONAL DRUGS NEEDED
- ANIMAL EATING AND DRINKING NORMALLY
- SWELLING AT SURGERY SITE SUBSIDING BY DAY 4

Weight-

Week 1 2.6
Week 2 2.7
Week 3 2.9
Week 4 3.0

Day 21 DATE 7/19
Tetracycline (10mg/kg/IM) Time 9:00 AM

Day 27 DATE 7/25
Calcein (10mg/kg/IM) Time 9:30 AM

Day 30 Euthanasia-beuthanasia(1mg/10lb bw iv,ic)
Date 7/28 Time 1:55 PM

Bone/Electret Study-Animal flow chart

Animal # 4 Weight 2.6 Date 6/28 Time 1:17pm
Presurgery treatment .27ml
Ancef(35mg/kg/IM) ✓ Time 1:33pm
Surgery 91mg
Duration 16mins
Film placement (+, -, C) C
Postsurgery treatment
Buprenorphine 0.05mg/kg/sc ✓ Time 1:33pm 0.13mg = .43ml
Postsurgery observation of vital signs Time 4:00pm
Comments
ANIMAL AWAKE AND ALERT

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased respiration, weight loss

- NO ADDITIONAL DRUGS NEEDED
- ANIMAL EATING AND DRINKING ACTIVELY

Weight-

Week 1 2.7
Week 2 2.8
Week 3 2.8
Week 4 2.9

Day 21 DATE 7/19
Tetracycline (10mg/kg/IM) Time 9:00AM
Day 27 DATE 7/25
Calcein (10mg/kg/IM) Time 9:30 AM
Day 30 Euthanasia-beuthanasia(1mg/10lbw iv,ic)
Date 7/28 Time 2:10 PM

Bone/Electret Study-Animal flow chart

Animal # 5 Weight 2.95 kg Date 8/9/01 Time 2:10pm

Presurgery treatment - .3 ml
Ancef(35mg/kg/IM) _____ Time _____

Surgery
Duration 20 mins
Film placement (+, -, C) +

Postsurgery treatment
Buprenorphine 0.05mg/kg/sc ✓ Time 2:30pm 0.5 ml

Postsurgery observation of vital signs Time 5:30pm

Comments

OUT OF ANESTHESIA ALERT, COORDINATED MOVEMENT

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased respiration, weight loss

- NO ADDITIONAL DRUGS NEEDED

- FUNCTIONING NORMALLY, EATING DENKING + POOL

Weight-

Week 1 3.40
Week 2 3.00
Week 3 3.1
Week 4 3.1

Day 21 DATE 8/30
Tetracycline (10mg/kg/IM) Time 8:00 AM

Day 27 DATE 9/5
Calcein (10mg/kg/IM) Time 8:40 AM

Day 30 Euthanasia-beuthanasia(1mg/10lbw iv,ic)
Date 9/8 Time 10:00 AM

Bone/Electret Study-Animal flow chart

Animal # 6 Weight 2.93kg Date 8/8/01 Time 2:42pm

Presurgery treatment

Ancel(35mg/kg/IM) _____ Time _____

Surgery

Duration 18 mins

Film placement (+, -, C) -

Postsurgery treatment

Buprenorphine 0.05mg/kg/sc ☒ Time 3:00p

Postsurgery observation of vital signs Time 5:30 PM

Comments

ALERT, MOVING, COORDINATE.

Note: more medication was
needed, mouth flinching,
eyes blinking

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased
respiration, weight loss

- NO ADDITIONAL DRUGS GIVEN

- EATING DRINKING NORMAL

Weight-

Week 1 2.9

Week 2 3.0

Week 3 3.0

Week 4 3.0

Day 21 DATE 8/30
Tetracycline (10mg/kg/IM)

Time 8:00 AM

Day 27 DATE 9/5
Calcein (10mg/kg/IM)

Time 2:40 AM

Day 30 Euthanasia-beuthanasia(1mg/10lb bw iv,ic)

Date 9/8

Time 10:10 AM

Bone/Electret Study-Animal flow chart

Animal # 7 Weight 2.32kg Date 8/9/01 Time 3:08 p.

Presurgery treatment
Ancef(35mg/kg/IM) ☒ Time 3:08 p.

Surgery
Duration 28 min
Film placement (+, -, C) +

Postsurgery treatment
Buprenorphine 0.05mg/kg/sc ☒ Time 3:15

Postsurgery observation of vital signs Time 5:30 PM

Comments

- OUT OF ANESTHESIA
- ALERT

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased respiration, weight loss

- NO MORE DRUGS
- EATING - DRINKING NORMALLY

Weight-

Week 1 2.85
Week 2 2.9
Week 3 2.9
Week 4 2.0

Day 21 DATE 8/30
Tetracycline (10mg/kg/IM) Time 8:00 AM

Day 27 DATE 9/5
Calcein (10mg/kg/IM) Time 8:40 AM

Day 30 Euthanasia-beuthanasia(1mg/10lbw iv,ic)
Date 9/18 Time 10:20 A.M

Bone/Electret Study-Animal flow chart

Animal # 8 Weight 2.92kg Date 8/9/01 Time 3:35pm

Presurgery treatment

Ancef(35mg/kg/IM) ✓ Time 3:32pm

Surgery

Duration 25min

Film placement (+, -, C) —

Postsurgery treatment

Buprenorphine 0.05mg/kg/sc ✓ Time 3:32p.

Postsurgery observation of vital signs Time 6:00 PM

Comments

- RECOVERED

- ALIVE

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased respiration, weight loss

- NO MORE DRUGS

- EATING + DRINKING NORMALLY

Weight-

Week 1 2.9

Week 2 2.45

Week 3 2.0

Week 4 3.0

Day 21 DATE 8/30
Tetracycline (10mg/kg/IM)

Time 9:00 AM

Day 27 DATE 9/5
Calcein (10mg/kg/IM)

Time 8:40 AM

Day 30 Euthanasia-beuthanasia(1mg/10lbw iv,ic)

Date 11/2

Time 10:30 AM

Bone/Electret Study-Animal flow chart

Animal # 9 Weight 2.93kg Date 8/1/01 Time 4:00p

Presurgery treatment
Ancef(35mg/kg/IM) ✓ Time 4:00p

Surgery
Duration 20 min
Film placement (+, -, C) -

Postsurgery treatment
Buprenorphine 0.05mg/kg/sc ✓ Time 4:30p
Postsurgery observation of vital signs Time 6:30

Comments
GOOD RECOVERY
ALERT

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased respiration, weight loss

- NO MORE DRUGS
- EATING, DRINKING NORMALLY

Weight-

Week 1 2.95
Week 2 2.95
Week 3 3.0
Week 4 3.1

Day 21 DATE 8/30
Tetracycline (10mg/kg/IM) Time 8:00 AM

Day 27 DATE 9/5
Calcein (10mg/kg/IM) Time 8:40 AM

Day 30 Euthanasia-beuthanasia(1mg/10lb bw iv,ic)
Date 9/8 Time 10:40 AM

Bone/Electret Study-Animal flow chart

Animal # 10 Weight 3.05kg Date 8/9/01 Time 4:25p

Presurgery treatment
Ancef(35mg/kg/IM) ☒ Time 4:40p

Surgery
Duration 15min
Film placement (+, -, C) +

Postsurgery treatment
Buprenorphine 0.05mg/kg/sc ☒ Time 4:40p
Postsurgery observation of vital signs Time 7:00 PM

Comments

- OUT OF ANESTHESIA
- ALERT

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased respiration, weight loss

- NO MORE DRUGS
- EATING, DRINKING NORMALLY

Weight-

Week 1 3.1
Week 2 3.2
Week 3 3.1 ?
Week 4 3.3

Day 21 DATE 8/30
Tetracycline (10mg/kg/IM) Time 8:00 AM

Day 27 DATE 9/5
Calcein (10mg/kg/IM) Time 8:40 AM

Day 30 Euthanasia-beuthanasia(1mg/10lbw iv,ic)
Date 9/8 Time 10:50 AM

Bone/Electret Study-Animal flow chart

Animal # 11 Weight 2.96 kg Date 3/9/01 Time 4:45

Presurgery treatment
Ancef(35mg/kg/IM) ✓ Time 5:05p

Surgery
Duration 20 min
Film placement (+, -, C) +

Postsurgery treatment
Buprenorphine 0.05mg/kg/sc ✓ Time 5:05p

Postsurgery observation of vital signs Time 7:20 PM

Comments

- OUT OF ANESTHESIA
- EATING + DRINKING NORMALLY

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased respiration, weight loss

- NO MORE DRUGS
- EATING, DRINKING NORMALLY

Weight-

Week 1 3.00
Week 2 3.0
Week 3 3.1
Week 4 3.1

Day 21 DATE 8/30 Time 8:00 AM
Tetracycline (10mg/kg/IM)

Day 27 DATE 9/5 Time 8:40 AM
Calcitonin (10mg/kg/IM)

Day 30 Euthanasia-beuthanasia(1mg/10lbw iv,ic)
Date 9/8 Time 11:00 AM

Bone/Electret Study-Animal flow chart

Animal # 12 Weight 2.95kg Date 9/9/01 Time 5:08 p

Presurgery treatment

Ancef(35mg/kg/IM) ✓ Time 5:28

Surgery

Duration 20 min

Film placement (+, -, C) —

Postsurgery treatment

Buprenorphine 0.05mg/kg/sc ✓ Time 5:28

Postsurgery observation of vital signs Time 7:30 AM

Comments

- GOOD REFLEXES
- ALERT

Observations for days postsurgery

Note any additional analgesic given and dosage

Note any unusual observations including lethargy, loss of appetite, increased respiration, weight loss

- NO MORE DRUGS
- EATING, DRINKING NORMALLY

Weight-

Week 1 3.1

Week 2 3.1

Week 3 3.2

Week 4 3.2

Day 21 DATE 9/30
Tetracycline (10mg/kg/IM)

Time 9:00 AM

Day 27 DATE 9/5
Calcein (10mg/kg/IM)

Time 9:40 AM

Day 30 Euthanasia-beuthanasia(1mg/10lbw iv,lc)

Date 9/9

Time 11:20 AM

APPENDIX 4: BONE REGENERATION FIGURES

Microscopic illustrations of bone activity in control, positive, and negative animals are given in **Figures 37-50** that follow.

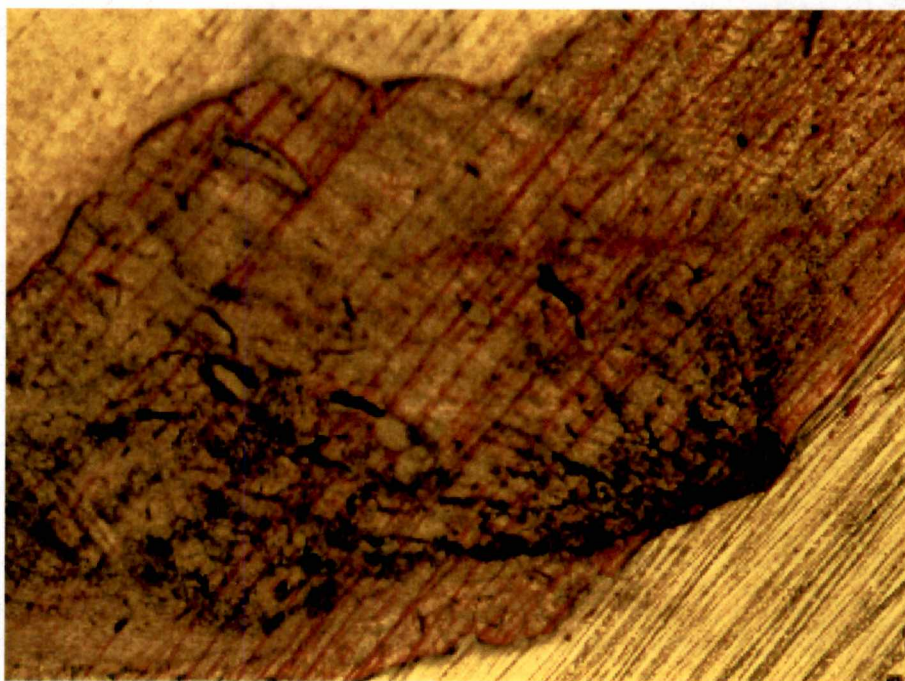


Figure 37: Control Animal 1 - some bone growth within defect (12%)

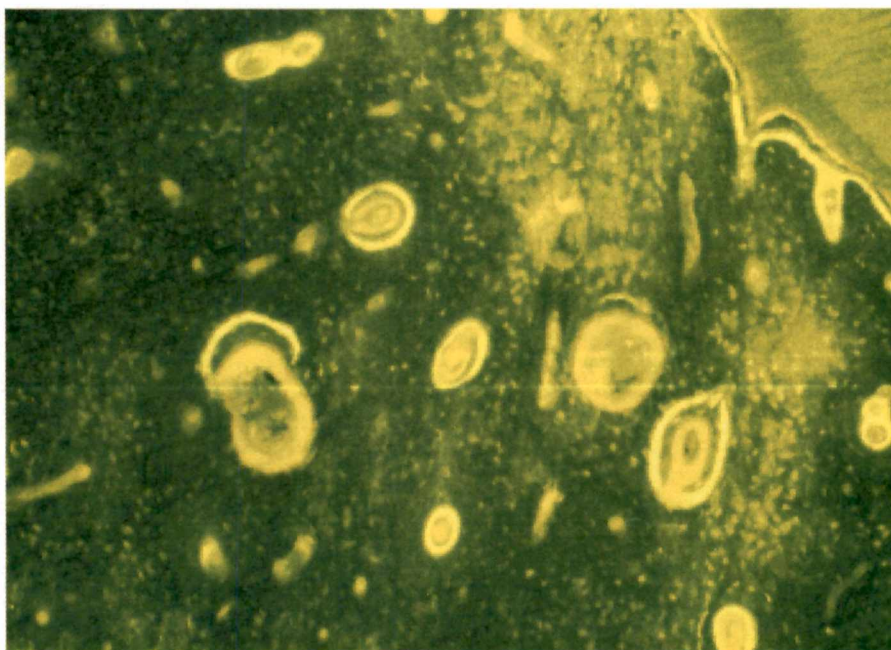


Figure 38: Control Animal 2 – double labeled osteons (-5%)

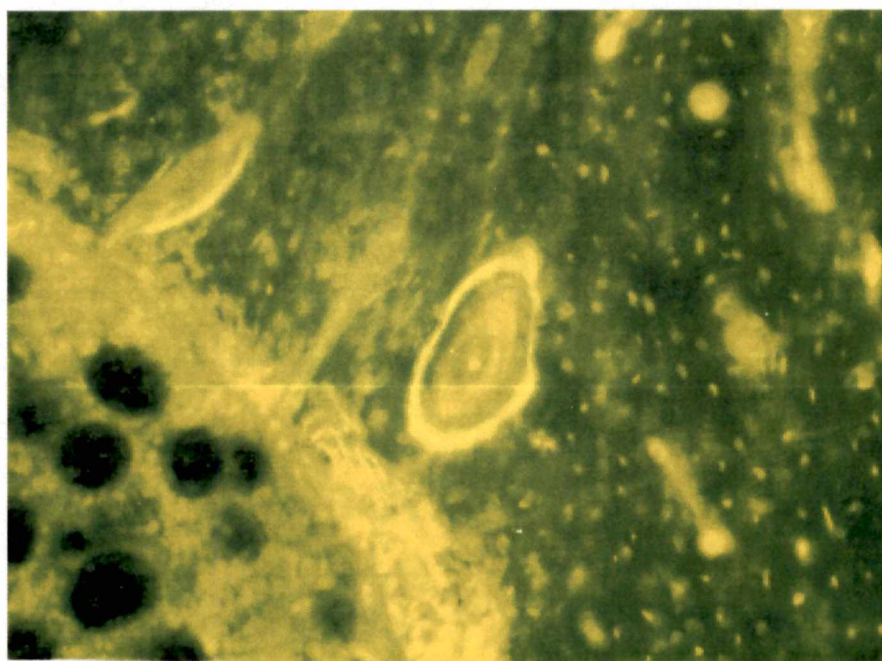


Figure 39: Control Animal 3 – fatty marrow and double labeled osteon (-14%)

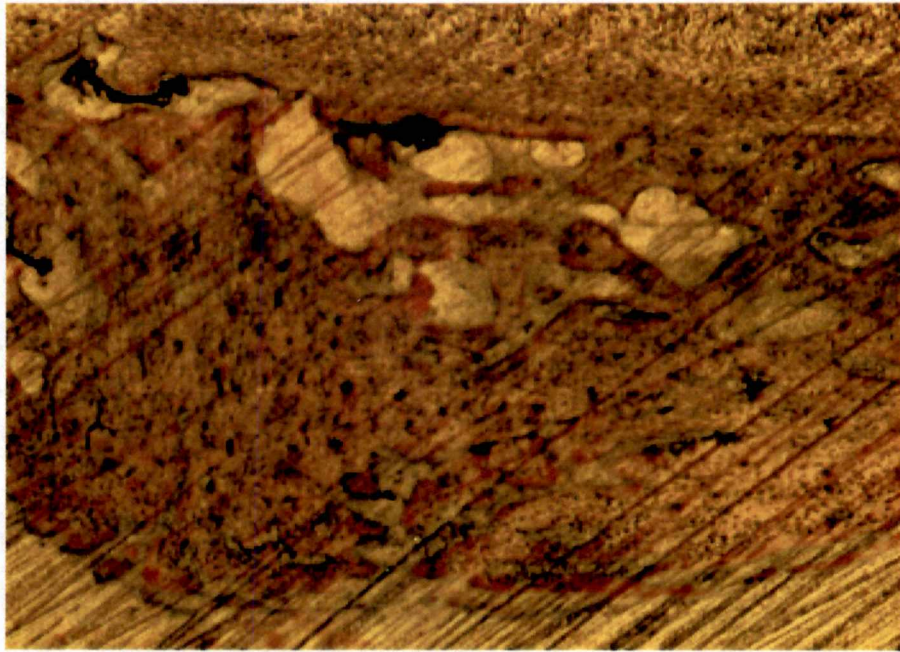


Figure 40: Control Animal 4 – gaps in bone fill within defect (14%)

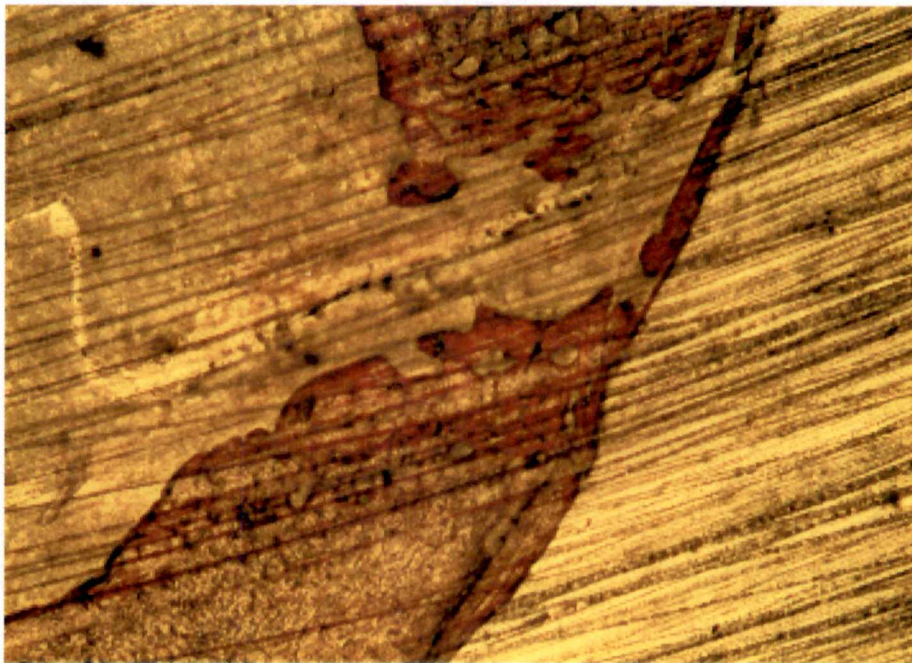


Figure 41: Postive Animal 5 – defect filled only at edge (-8%)

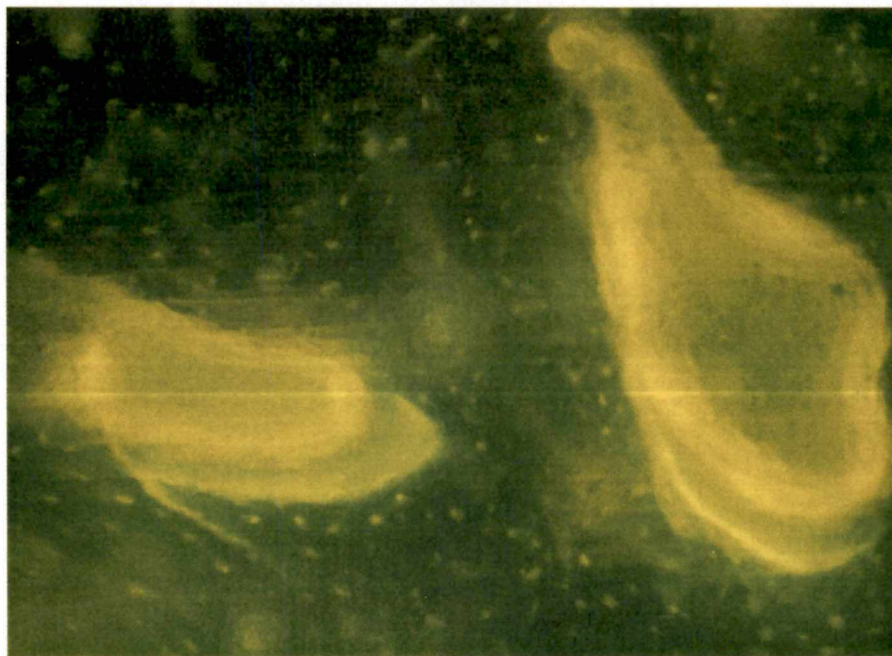


Figure 42: Negative Animal 6 – double labeled osteons (62%)

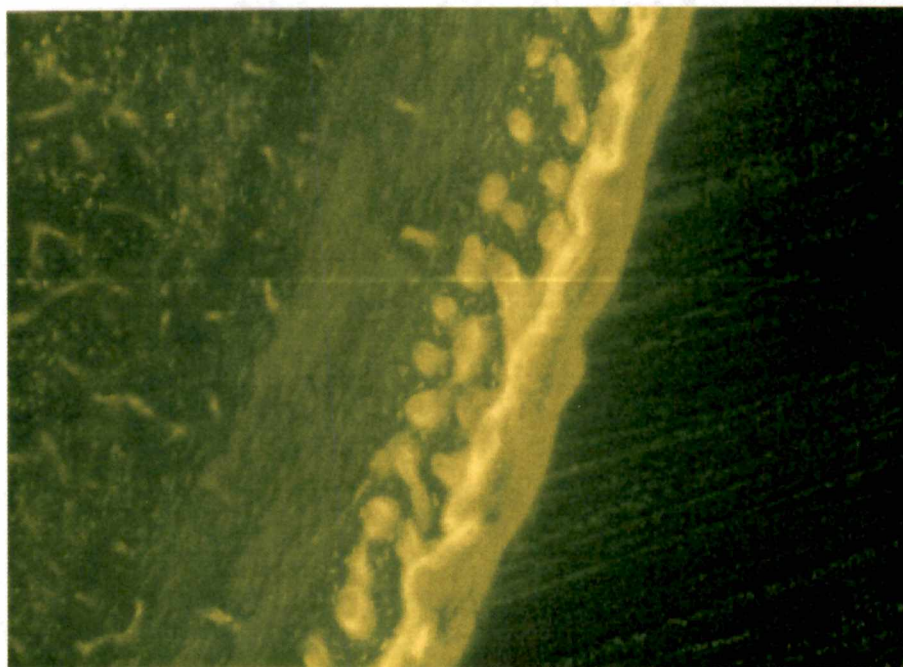


Figure 43: Positive Animal 7 – small amount of subperiosteal growth (14%)

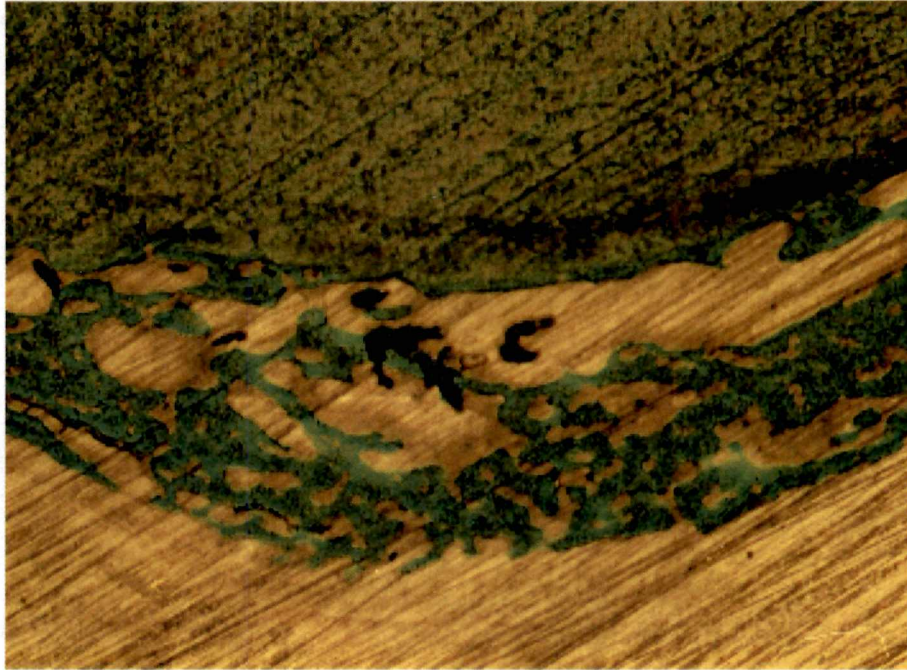


Figure 44: Negative Animal 8 – possible PLA film remnants (55%)

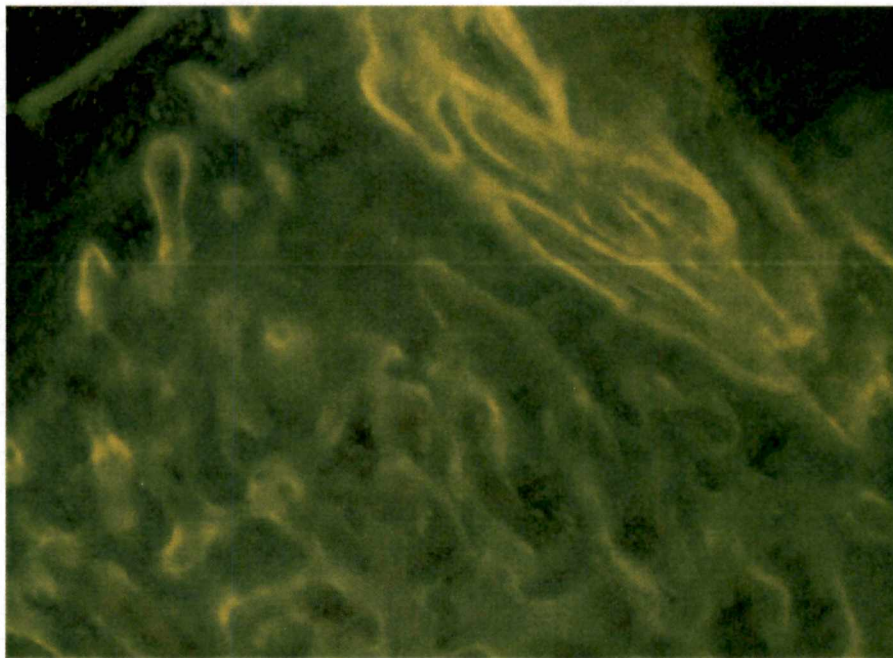


Figure 45: Negative Animal 9 – considerable bone activity within defect (53%)

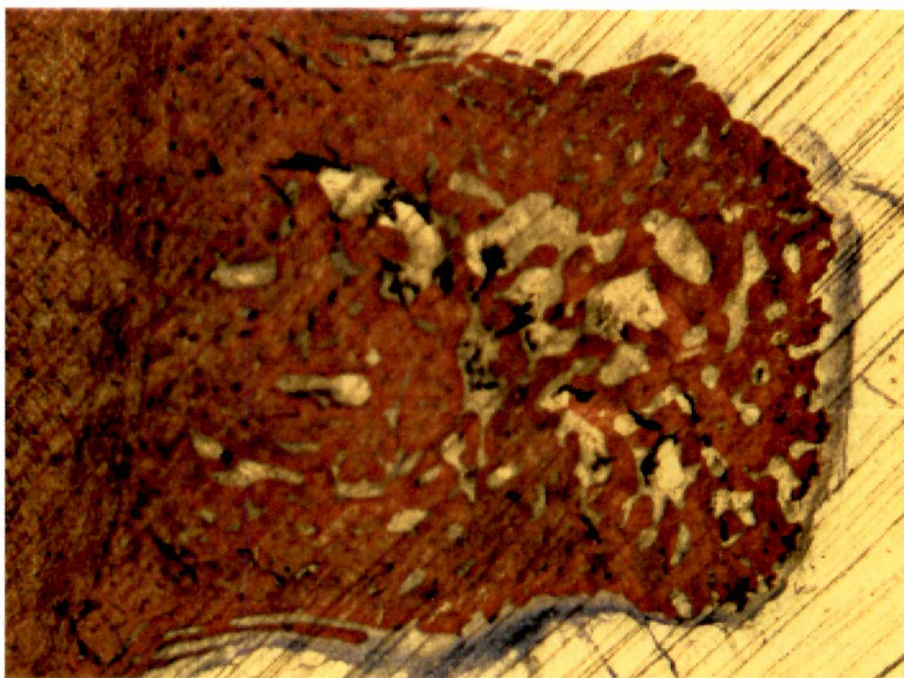


Figure 46: Positive Animal 10 – some woven bone, possible PLA remnants (16%)



Figure 47: Positive Animal 11 – subperiosteal woven bone (11%)

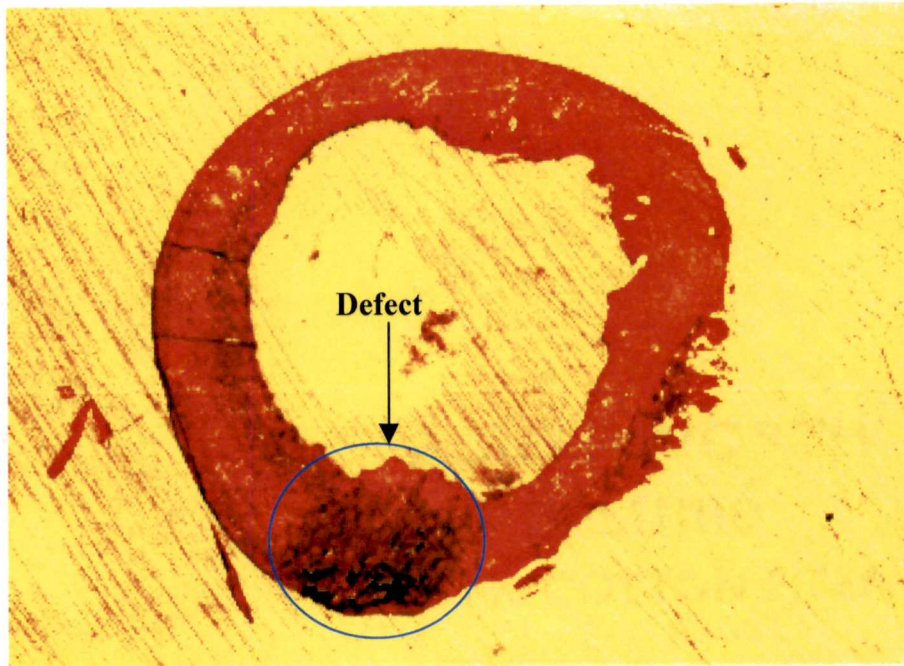


Figure 48: Negative Animal 12 – exceptional woven bone fill in defect (62%)

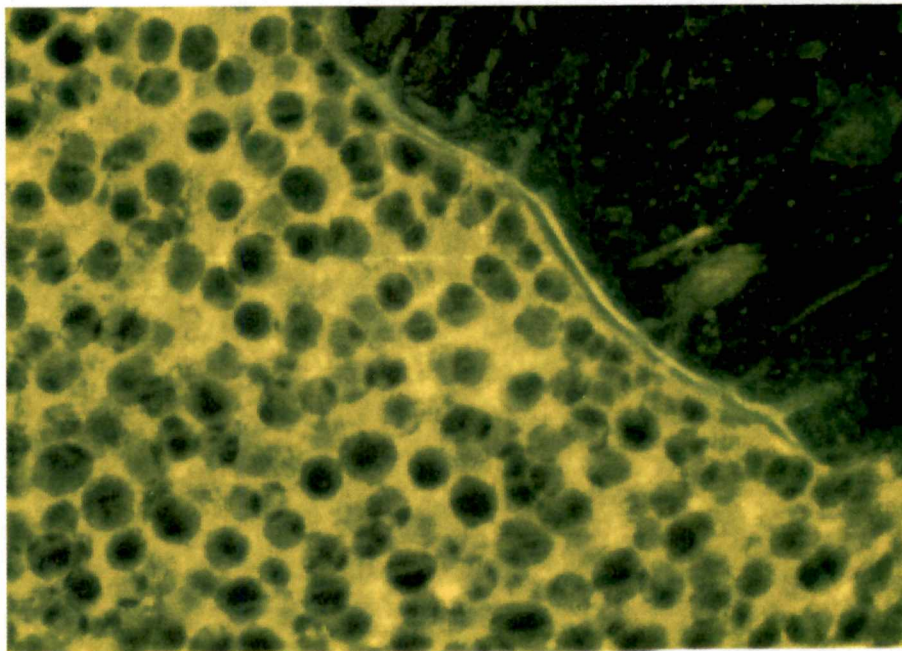


Figure 49: Fatty marrow typical of long bones in rabbits

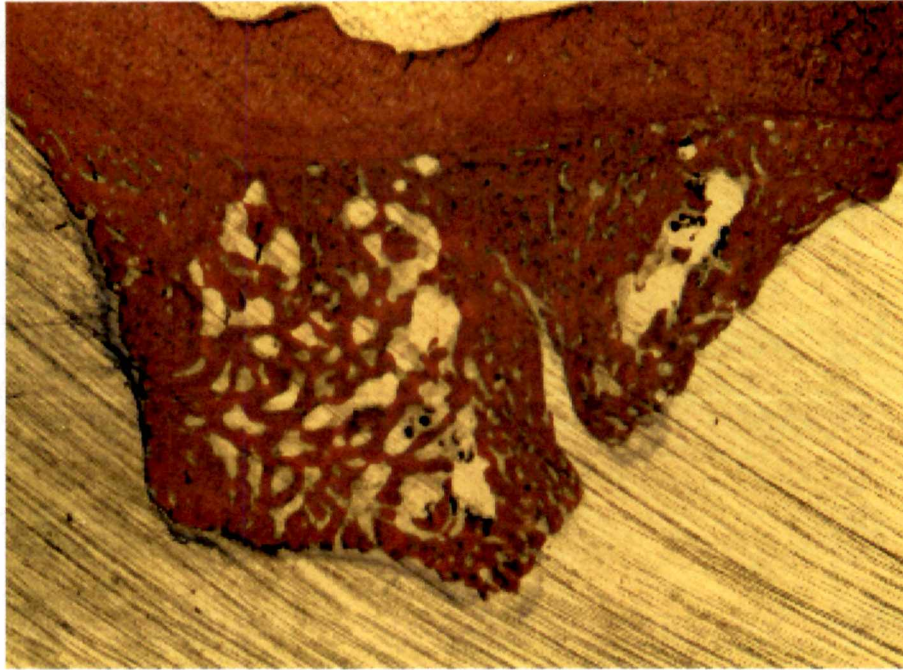


Figure 50: Heterotopic bone formation

VITA

Robbye NyCole Grier was born October 11, 1976 in Atlanta, Georgia. She attended primary and secondary school in the DeKalb County School system, where she graduated from Stone Mountain High School with an Advanced Academic Diploma with Excellence in June, 1995. She matriculated to Vanderbilt University in August of 1995, where she received a Bachelor of Science in Engineering Science on May 14, 1999. Robbye continued her pursuit of higher education at the University of Tennessee, Knoxville in August of 1999. She received her Master of Science Degree in Engineering Science with a concentration in Biomedical Engineering on December 15, 2001.