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ACCELERATED BONE BONDING TO CALCIUM PHOSPHATE CERAMIC COATED STRAIN GAUGES. AN EXPERIMENTAL AND COMPUTATIONAL STUDY

by

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BIOMEDICAL ENGINEERING

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA
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I wish to dedicate this thesis to my parents
for their encouragement and support
throughout my academic career.
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ABSTRACT

Calcium phosphate ceramic (CPC) coated strain gauges have been used for long term *in vivo* bone strain measurements but require 6 to 9 weeks for sufficient bonding. PepTite2000™, OP-1, TGF-β1, Ca₃SO₄·2H₂O, and an endothelial cell layer with and without TGF-β1 were examined as enhancements to accelerate bone to CPC bonding.

Young male Sprague-Dawley rats were implanted with gauges for three weeks and calcein labeled. Following euthanasia, their femurs were explanted and mechanically tested. Histology was completed. Optical Coherence Tomography (OCT) was studied as an alternative to histology. A finite element analysis (FEA) examined bone to gauge strain transfer.

Mechanical testing indicated increased sensing accuracy with TGF-β1 and OP-1 enhancements versus unenhanced gauges. PepTite2000™ and endothelial enhanced gauges displayed lower sensing accuracy and contained vasculature near CPC. TGF-β1 increased bonding with endothelial cells. Ca₃SO₄·2H₂O inhibited bone bonding. OCT unsuccessfully imaged bone to CPC contact. FEA identified geometric and material parameters for improved gauge design.
1 Introduction

The remodeling of bone in response to mechanical stimuli has been a topic of interest and significance in both basic and clinical orthopedic research for over a hundred years. It is well documented that loading characteristics on bone influences bone changes in shape and material properties (Woo et al., 1981 and Rubin and Lanyon, 1984). Although the exact mechanism of mechanotransduction by bone cells still remains unknown, methods for the study of mechanical variables \textit{in vivo} are an important part of the study of bone remodeling and mechanotransduction. The difficulties associated with the \textit{in vivo} measurement of mechanical quantities are quite extensive. One of the most fundamental measurements that must be made is the change in strain patterns that may occur in a bone after a specific treatment or other stimulus.

\textit{In vivo} strain measurements have been previously recorded by bonding strain gauges to bone with a cyanoacrylate adhesive (Lanyon, 1971, Lanyon and Smith, 1970, Keller and Spengler, 1982 and Baggott and Lanyon, 1977). Strain readings have been used to study bone adaptive behavior to various mechanical stimuli (Rubin and Lanyon, 1984 A and B) and to study the effects of implants on this behavior (Lanyon et al., 1981, Szivek et al., 1985 and Szivek et al., 1995). However the cyanoacrylate adhesive is quickly dissolved \textit{in vivo} (Cameron et al., 1965), causing inaccurate strain readings. Cyanoacrylates have difficulty bonding to a surface in an aqueous environment and they
release harmful chemicals during degradation. In addition, acrylics, polyurethanes, and epoxy resins exhibit poor biocompatibility. (Donkerwolcke et al., 1998).

Calcium Phosphate Ceramics (CPC) such as resorbable tricalcium phosphates and bioactive hydroxyapatite ceramics have been used to coat orthopedic implants in order to induce bone bonding (Hench, 1998). Calcium phosphate ceramic (CPC) particles can be used to bond strain gauges to bone. The CPC particles encourage bone growth and attachment, permitting strain transfer from the bone surface to the strain gauge. CPC coated strain gauges have been used for in vivo bone strain measurements in dogs and rats (Szivek and DeYoung, 1997 and Szivek et al., 2000). This technique has allowed gauges to remain bonded for up to 18 weeks (Szivek and DeYoung, 1997). However, previous studies have shown that the time required for sufficient bone bonding to produce an accurate strain reading is at least 6 weeks in rats and 9 weeks in dogs (Szivek et al., 1995 and Szivek et al., 1996).

The goal of the experimental study was to develop a method which caused faster bone bond to CPC coated strain gauges. It was hypothesized that a biochemical or biological surface enhancement would increase the rate of bone to CPC attachment and thus a more accurate strain reading at an earlier time. Transforming growth factor-β1, osteogenic protein-1®, extracted endothelial cells, PepTite2000™, calcium sulfate dihydrate and a combination of transforming growth factor-β1 and endothelial cells were used as surface enhancements. These surface enhancements were chosen for their ability
to induce bone formation due to their osteogenic potential, osteoconductive ability or their ability to increase vascularization (Becton, 1996, Sumner et al., 1995, Daluiski et al., 1999, Bosch et al., 1999, Bulstra et al., 1999, Villanueva et al., 1990, Williams et al., 1992, Tschopp et al., 1994, Sidqui et al., 1995, Peltier, 1961 and 1978). The chosen CPC particle blend had demonstrated secure bonding at six weeks in an earlier rat study (Battraw et al., 1999). A three week study was developed to evaluate the enhancements at half the time required for an unenhanced gauge to yield an accurate strain reading.

Evaluating the sensing accuracy of CPC coated strain gauges in vivo is difficult. A method of determining when an accurate strain reading may be recorded by examining the bone to CPC particle attachment would be beneficial. Optical Coherence Tomography (OCT) was examined as a tool for examining bone attachment to the CPC coated strain gauges.

Strain transfer between the bone and CPC coated strain gauges is not fully understood. Combined biomechanical and histological studies have illustrated that an accurate strain reading may be achieved even with partial bonding or irregular in-growth of bone around the CPC particles (Maliniak, et al., 1993; Szivek et al., 1996). The effect of bone growth over the edges of the gauge will also likely affect the measured strain. Factors such as waterproofing materials or the thickness of CPC and polysulfone layers may be important parameters to consider when designing gauge attachment protocols for
in vivo experiments. These variables are difficult to examine experimentally because of the small size of sensors and the large number of experimental parameters. The objective of the computational study was to create and validate a finite element model of strain transfer between cortical bone and a strain gauge. The finite element model was subsequently used to perform parameter studies on the effects of interface modulus, interface thickness, gauge geometry, waterproof coating, and partial gauge debonding. It was hypothesized that a thicker layer between bone and gauge would reduce strains, as would an interface that was too hard or too soft. It was also hypothesized that waterproofing layer thickness would affect the effective strain sensed by the strain gauge.
2 Background Research and Literature Survey

Bone is a living tissue that is continuously remodeling. This remolding process is affected upon by both internal and external stimuli. It is important to understand the basics of bone function and biology when evaluating a method of monitoring in vivo bone strain. This chapter examines bone physiology at the cellular level and discusses factors that affect rates of bone formation and resorption. Characteristics of the calcium phosphate ceramics and the surface enhancements are discussed followed by the motivation behind the finite element analysis.

2.0 Basic Structure and Function of Bone and Bone Cells

An internal skeletal system consisting of the systematic and well-evolved structure, bone, is present in all vertebrates. It provides the mechanical support for locomotion and offers protection for internal organs, such as the heart and lungs, and the nervous system, brain, spinal cord, etc. Blood cell formation (hematopoiesis) occurs in the bone marrow. Contained within bone are metabolic reserves for calcium, phosphate and minerals. Bone is an inhomogeneous, anisotropic material consisting of several layers. The structure of bone further varies over the lifetime of an animal or human due to growth and continuous remodeling.

The two primary types of bone structures, which are often intermixed, are compact (also known as cortical bone) and trabecular (also known as cancellous bone)
bone. Compact bone is the hard outer structure of long bones and predominates in the appendicular skeleton. The trabecular structure is predominately found in vertebra and the pelvis as well as the extremities of the long bones. Compact (cortical) bone has a 80-90% calcified matrix while trabecular or cancellous (spongy) bone has a 15-25% calcified matrix. Although compact bone makes up 80% of the total bone structure, skeletal metabolism is almost equally divided between the two bone types because trabecular bone is more metabolically active (Eriksen et al., 1994).

Compact and trabecular bone may possess either a lamellar or woven bone structure. Lamellar bone is formed from alternating collagen fiber layers giving it an organized, plywood like appearance which adds strength to the bone matrix. Woven bone lacks this collagen alignment and has a disorganized appearance. This type occurs during primary bone growth or during rapid, disorganized growth, as is the case with Paget's disease or highly stimulated growth. In non-diseased states, woven bone will be remodeled into lamellar bone (Eriksen et al., 1994).

Bone tissues are similar at the ultra-structural level. They contain an inorganic mineral phase composed of biological apatite crystals deposited within an organic phase of cross linked collagen fibers (Garner, et al. 1995; LeGross, et al. 1994). In its purest form the apatite is similar to calcium hydroxyapatite (HA), Ca$_{10}$(PO$_4$)$_6$(OH)$_2$. It accounts for approximately 60-65% of the bone by weight. The organic phase provides tensile
strength, while the mineral phase provides compressive strength. The composite structure of bone provides stiffness and resistance to fractures.

Bones may be anatomically subdivided into flat bones and long bones. Examples of flat bones are the mandible, scapula, skull bones and ileum. Examples of long bone include the femur, humerus and tibia. Long bones have a tubular diaphysis in the middle section, a metaphysis and then a rounded epiphysis at the ends. The diaphysis is formed from cortical bone while the metaphysis and epiphysis are predominantly trabecular bone. The periosteum is a thin highly vascularized layer of tissue surrounding the bone surface.

Osteoblasts and osteoclasts are responsible for bone development. Osteoblasts form a bone protein matrix (primarily type I collagen) that will calcify into mineralized bone. Osteoclasts are responsible for bone absorption during bone turnovers.

Osteoblasts are derived from undifferentiated primitive mesenchymal stem cells and differentiated chondrocytes. (Erlebacher et al., 1998; Simmons et al., 1990). The source of the stem cells has not been identified (Garner, et al., 1995). The stem cells become preosteoblasts which are unable to synthesize bone matrix, but they are able to divide and form osteoblasts (Garner, et al. 1995). Osteoblasts are responsible for regulating bone mineralization and for secreting collagen. These cells can become
osteocytes if walled in by mineralized bone and bone lining cells if they line the edge of mineralized bone.

The osteocytes and bone lining cells are not able to synthesize collagen. The osteocytes have enclosed themselves in small matrix cavities. They are connected to other osteocytes and bone lining cells by canaliculi (tubular channels). These channels are used for nutrient supplies by diffusion and may allow for intercellular communication.

Bone lining cells are only partially enclosed by bone matrix cavities, and have stopped forming bone matrix. These cells are responsible for maintaining the Ca+ level, possibly through ion pumps. They pump the calcium ions from the bone fluid compartments within the canaliculi to the extracellular compartment. Some bone lining cells may be formed directly from preostoeblasts and they have been shown to differentiate into active osteoblasts through stimulation, (Garner, et. al., 1995).

Osteoclasts are large multinucleated cells able to dissolve bone mineral and matrix (Garner, et. al., 1995). This process occurs at the interface between the cell and bone surface. The interface to the bone is elaborately folded and encompasses a microenvironment with a lowered pH and a large number of lysosomes. Osteoclasts are derived from the hematopoietic system (Erlebacher et al., 1998). Their development begins with a stem cell population (granulocyte macrophage). These cells are then
stimulated by granulocyte macrophage colony-stimulating factor in order to differentiate into premonocytes. The premonocytes, while under the stimulus of a local factor (cytokines), may either develop into a monocyte or an osteoclast. Monocytes continue on to form giant cells. An active osteoblast is then regulated by osteoblast stimulatory cytokines, and an inhibitory agent such as a circulating peptide.

There are intricate systems of channels and layers that make up the networking systems of bone. There are numerous intracellular gap junctions between the osteoblasts, bone lining cells, osteoprogenitor cells and osteocytes. These allow the cells to function as a group connected by a network of pathways. The gap junctions are often arranged in a side to side cell configuration, with cell membranes undulated. This increases the gap junction’s surface areas. The gap junctions are capable of transporting ions (electric couplings) and small metabolites. The bone cells are further connected to adjacent tissues, such as the endothelium layer, which surrounds the blood vessels of the bone matrix vascular supply (Cowin, et. al., 1991).

2.1 Bone Formation, Resorption, Remodeling:

The CPC coated strain gauges presented in the introduction are effective at monitoring in vivo strain only after adequate bone to CPC attachment. It is important to understand the bone development scheme in order to histologically study this bone attachment and thus evaluate the differences between CPC coated strain gauge surface enhancements.
Bone formation differs according to the type of bone being remodeled. However, both types of bone formation (intramembranous and endochondral) are formed by a deposition of hydroxyapatite crystals within a collagen matrix.

Intermembranous bone formation calcifies in patches rather than in the orderly fashion, common to bone remodeling. The mesenchymal cells differentiate directly into preosteoblasts during this process. Endochondral bone formation occurs by the calcification (mineralization) of a cartilaginous structure. The mesenchymal cells that create osteoblasts may also differentiate into prechondroblasts that form chondroblasts, which form the cartilage structure in the shape of the new bone. This cartilage is formed by a secretion of a collagen matrix, which encloses the chondroblast in a lacunae. Because this area has not yet calcified, it may continue to grow, stretch and divide in an appositional growth pattern. Fluids surrounding the fibers supply nutrients. When the cartilage begins to calcify, this nutrient supply is cut off. The chondroblasts then stop reproducing and die, leaving room for mineralization.

Bone mineralization involves the formation of hydroxyapatite crystals in calcifying cartilage (the organic matrix also know as osteoid) in new or remodeling bone. In laminar bone, this process occurs in tightly packed collagen fibrils and associated non-collagenous proteins. In randomly orientated collagen (and woven bone) the mineralization occurs in matrix vesicles. These vesicles are previously formed through
exocytosis from plasma membranes of osteoblasts. These vesicles are destroyed once the crystallization of the hydroxyapatite exceeds the allocated space. However, in remodeling bone, this crystallization process occurs without vesicle formation, directly in the collagen matrix.

Although the biology of bone cells has been well studied and documented, the various stimuli for cell activation and metabolism is not fully understood. Many details of this process are currently being studied. However, the general scheme or pattern of bone growth and development has been continuously observed in research.

The development of a Haversian canal in cortical bone and the development of a Howship's Lacuna in trabecular bone are processes with similar steps (Fig. 2.1).
Figure 2.1: Bone remodeling through trabecular and cortical bone.

Cement lines divide individual bone structural units. When in steps 2 and 3, osteoclasts become active and resorb preexisting bone matrix, the Haversian canal (or Howship's Lacuna) expands (steps 2-3). Osteoclastic activity can penetrate into adjacent bone structural units (step 3). The resorption process then receives a new cellular signal,
causing the absorption to cease and a cement line is formed followed by an osteoid seam. The resorptive seam is lined with active resorbing osteoclasts while the osteoid seam is lined with matrix forming osteoblasts. The osteoid seam advances as osteoblasts lays down new matrix, forming new bone (Steps 4-5). Interstitial bone may be formed between adjacent bone structural units. Once matrix formation ends the osteoblasts become quiescent bone lining cells (step 6) (Parfitt, 1983). In a healthy human, one cycle from activation to resorption and then to formation is completed in approximately 100 days in cortical bone and 200 days in trabecular bone. The formation period is approximately triple the resorption (erosion) period (Eriksen et al., 1994).

There are two different remodeling patterns within Howship's Lacuna in trabecular bone. For both cases, bone lining cells are first activated by an external stimulus which recruits osteoclasts to form a resorption pit. For the first case, the osteoclasts complete their resorption of the old bone before the osteoblasts begin to lay down new bone. For the second case the osteoblasts begin to remodel the bone after osteoclastic resorption but the osteoblasts follow the osteoclasts directly. In both cases, the osteoblasts become quiescent bone lining cells after filling the resorption site. Precursor cells are present in these remodeling events (Parfitt, 1983; Garner et al., 1995).

A bone remodeling unit within a Haversian canal not only progresses centrifugally (Fig. 2.1), but also longitudinally forming a cutting cone. The apex of the
cutting cone is made up of advancing multinucleated osteoclasts. They may advance in any direction. The perimeter is eroded from this action, causing the surface to roughen. Spindle shaped precursor cells are present just behind the cutting cone near a capillary loop supplying nutrients to the cells within the remodeling unit. Quiescent mononuclear cells that are at a stage between resorption and formation line the Haversian canal just behind the cutting cone. Behind this area are advancing mononuclear osteoblasts forming the osteoid seam. After these osteoblasts fill in the Haversian canal, they become flattened quiescent osteoblasts, or bone lining cells. They form the final resting layer between the Haversian canal and the newly formed bone (Parfitt, 1983; Garner et al., 1995).

2.1.0 Osteoclastic Resorption Phase

The osteoclastic resorption process (erosion) removes both mineral and organic matrix. In a healthy human this process takes about 30 days in cortical bone. The preosteoclastic phase lasts about 10 days, followed by 15 days of monocytic activity and then five more days for the osteoclasts to leave and the osteoblasts to arrive. This process of osteoclastic and mononuclear resorption creates a narrow tunnel of about 150 micrometers in diameter. In cancellous bone this process takes about 43 days: 7 days for osteoclast resorption followed by 36 days of mononuclear resorption. A resorption depth of approximately 60 micrometers is reached (Eriksen et al., 1994).
Within an active osteoclast carbon dioxide diffuses into the cell and reacts with water to form carbonic acid (H$_2$CO$_3$). This then separates into bicarbonate and hydrogen ions. The bicarbonate is pumped out of the cell through the chloride-bicarbonate exchanger. The chloride ions entering the cell allow it to maintain its membrane potential. The absence of bicarbonate ions pushes the previous reaction, producing more hydrogen ions. The hydrogen ions are then transferred to the "sealing zone" between the bone matrix and osteocyte by a proton pump. The chloride ion follows, again to maintain membrane potential. The hydroxyapatite is then broken apart by protein hydrolysis. The resulting amino acids are then removed from the resorption pit. The removal process is theorized to occur in one or more of three ways: "(1) Translation of individual components through the cytoplasm, (2) Bulk transfer of these products via endocytotic vesicles or, (3) release of products when the osteoclast detaches along the sealing zone (Garner et al., 1995).

2.1.1 Osteoblast Formation Phase

Osteoblasts produce Type I collagen and a variety of matrix components. These matrix components play an important part in the autocrine and paracrine regulation. They are involved with cell division, collagen production and turnover, bone mineralization and bone resorption (Simmons et al., 1990). Osteoblasts lay down a collagen matrix which forms an osteoid during bone formation. The osteoid will mineralize, possibly due to the alkaline phosphatase produced by the osteoblasts. The mineralization process
progresses in part as a result of supersaturation of calcium and phosphorous within the osteoid seam. (Simmons et al., 1990)

The formation phase for a healthy human lasts about 90 days in cortical bone. Osteoblasts will first synthesize the collagen matrix, which later mineralizes (calcification). There is approximately a 15-20 day time lapse between the bone matrix synthesis and the mineralization. Although the reason for this time delay is disputed, it has been attributed to the time required to remove crystallization inhibitors (i.e. pyrophosphate and phosphoproteins associated with calcified tissues) and/or the time necessary to synthesize the proteins necessary to initiate the mineralization (Garner et al., 1995) (Eriksen et al., 1994). This process will leave the haversian canal with a mean wall thickness of 40 to 60 micrometers and an inner canal diameter of about 30 micrometers (Eriksen et al., 1994).

In human cancellous bone, the formation phase occurs for about 145 days. Preosteoblasts migrate to the resorption site and differentiate into osteoblasts for approximately the first seven days. The osteoblasts form the collagen matrix for about 15 days before matrix mineralization. This creates approximately 40 to 60 micrometers of new bone (Eriksen et al., 1994).

Bone formation is normally coupled with resorption but when de nova bone formation occurs on a quiescent surface woven bone will initially form. De nova bone
formation can be caused by normal growth, hormone or protein stimuli, or by diseases. During formation of woven bone the osteoblasts lay down the collagen fibrils in a disorganized manner and the mineralized bone then lacks a lamellar pattern.

2.1.2 Histomorphometry

Histomorphometry is the quantitative study of histological sections using stereological and image processing techniques to study bone development and its responses to various stimuli. Gross measurements from bone sections such as total bone areas and cortical areas provide an overview of bone changes. Periosteal new bone growth as well as eroded perimeters provide some detail about surface activity. Labeling mineralizing bone presents a unique way to study the growth patterns and cellular activity during a specific time period. The number of unlabeled and labeled osteoid seams give an indication of the bone remodeling activity. Osteoid widths, interlabel widths and inner and outer labeled perimeters provide an indication of bone formation.

![Bone labeling example for histology measurements.](image)
The rate of progression of advancing osteoblasts and osteoid seams can be quantified using calcein or tetracycline labeling. These labels are deposited in areas of bone matrix mineralization (Fig. 2.2). For example, a tetracycline label given at time = 0 days and 10 days followed by explantation of the sample at time = 20 days provides label lines in bone (Fig. 2.2). The tetracycline labels the newly calcifying bone at the zone of demarcation. With a new label given at L₂, two tetracycline labels, separated by an interlabel width (ILW), are visible on bone sections with ultraviolet light. The interlabel time (ILT) for this example is 10 days. The average mineral appositional rate (MAR) is equal to the interlabel width divided by the interlabel time. For this case 5μm would be divided by 10 days or a MAR of 0.5 μm per day. The MAR for a young 214-339g Sprague Dawley or Wistar rat in lamellar bone near the periosteal surface in the region of the mid shaft on the femur has been reported at 3.2 μm per day (Hammond and Storey, 1970).

Another value of interest is based on the time necessary for the zone of demarcation to advance from L₁ to L₂ (Fig. 2.2). This time is known as the mineralization lag time (MLT). In the Figure it is 20 days. Only the last picture, H, can be viewed during histology. However if the osteoid seam width (OSW) is assumed to be constant, then the mineralization lag time is the same as the mineral appositional rate.
2.2 Load induced Stimulus

Load induced strains have a unique effect on net bone mass and morphology (Rubin et al., 1996) and have been studied for over a hundred years. However, the study of these stimuli independently is difficult because hormones and growth factors affect bone mass and morphologic changes. The complexity of the heterogeneous, composite structure of bone matrix makes it difficult to understand the effects of loading on bone remodeling.

In order to study individual cell types and their actions, they must first be released from the matrix by enzymatic digestion. Various experiments have been conducted by first isolating and then subjecting fibroblasts, chondrocytes and calvarial osteoblasts to mechanical stimuli in cell cultures. The cells have been stretched, compressed, loaded in bending and electrically stimulated to induce and study bone formation.

Many constraints and simplifications inherent to cell culture systems affect strain stimulus, bone growth and resorption. The enzymatic digestion may affect cell membrane proteins. In addition, separating cell types reduces the heterogeneity of the cell types. This eliminates different cell type interactions. A further problem is that communication channels between cells are damaged and cells can lose their network relationship. These modified conditions may change a cell's ability to respond to mechanical stimuli.
2.2.0 Mechanoreceptors

Stretch activated mechanoreceptors are present at the cellular level in fibroblasts and osteoblasts, and may be partially responsible for bone development. When a cell is deformed, stretch activated ion channels open (or close) allowing only certain size ions (such as K⁺, Ca²⁺, Na⁺, Li⁺, and Cs⁺) through. It is believed that this flow of ions is in part responsible for bone development.

The Brownian ratchet effect is one theory describing bone mechanoreceptors. This theory is based on the observation that the cell membrane is fluctuating in a thermal environment and that when actin monomer polymerizes between the space of the cytoskeleton and a patch of membrane, the cell is pushed slightly out of its original location. This hypothesis is supported by observation that ~20% local height increase is generated during a 15 Hz dynamic loading of MC-3T3-E1 osteoblast-like cells by a force with a 12 Angstrom amplitude after 90 seconds (Otter et al., 1999).

2.2.1 Responses to Stress / Strain

Stresses of 0.1 MPa applied at 60 Hz affect bone formation and resorption rates (Otter et al., 1999). Low intensity mechanical forces have also been noted to stimulate new bone growth. A relatively low load with a high frequency of application will induce bone growth. A one to ten microstrain applied for 10 or more minutes a day at above 10 Hz induces new bone formation. An optimal loading characteristic with a minimal loading duration was investigated by McLeod et al., 1988. They demonstrated that an
external loading regime of 0.3 g applied at 22 Hz, for 32 minutes resulted in a 4% bone mineral density increase in the sheep tibia (p<0.05). While loading regimes of 22Hz for 8 minutes per day resulted in a loss of bone mineral density (-5%, p<0.05). The control for this group lost approximately -3%, p<0.05. Loading frequencies of 45 and 84 Hz also proved ineffective at maintaining or increasing bone mineral density. This study indicated that a lower frequency loading is more beneficial for inducing bone growth or reducing osteoporosis. (McLeod, K.J. et al., 1998)

Not all studies of strain induced bone formation have produced bone formation. One study showed a loss in trabecular plate thickness in adult mongrels when subjected to a 1 Hz 35.6N trapezoidal load at 1800 cycles per day over a six month time frame versus a control group (Caldwell et al., 1999). They theorized that either the time frame was too short or that less bone was needed to support the loading conditions. Micro-CT scans of the loaded sample and control quantified the differences in trabecular bone density. Load induced bone growth remains inadequately understood.

Researchers have documented the range of strain required to cause bone formation or resorption (Cowin et al., 1991). They have examined bone tissue response to strain stimuli using strain gauged animal models. They noted bone formation at strain levels over 0.003 strain and bone resorption at strain levels less than 0.001 strain.
appears to experience a relatively constant remodeling rate at strains between 0.001 and 0.003 strains.

The deformation that a bone cell, 10 micrometers in diameter, must perceive is very small. A 10 micrometer cell, strained 0.001 units, will displace (10 micrometers \times 0.001\text{strain} = ) 10 nanometers or 100 angstroms. For a 0.003 strain deformation the cell will deform 300 angstroms. Bone cells have not been shown to be this sensitive in cell cultures. However, another mechanical factor in the bone matrix can affect cell deformation. The lacuna shape in a bone matrix produces a strain concentration and thus a higher cell deformation occurs. (Strain progresses linearly through a homogenous isotropic material, but in an abnormal shape or material, such as the lacuna in a bone matrix, it encounters non-uniform strains). It is suggested that this increases the strain by a factor of ten and increases the cellular level sensitivity 10-fold as compared to the gross tissue level sensitivity (Cowin et al., 1991).

2.2.2 Fluid and Pressure Stimulus

Studies have investigated the possibility of fluid flow, caused by pressure gradients inducing bone remodeling. Bone is a porous material that can absorb and release fluid while cyclically loaded. As bone is loaded and unloaded, various pressure gradients occur throughout the medium inducing fluid flow. The rate of flow will depend
on the pore size and geometry (permeability), the fluid viscosity, the pressure gradient and other boundary conditions.

Compressive loading produces a radial fluid flow in osteons (Cowin et al., 1991). The active fluid transport due to pressure gradients was noted to be higher than diffusive transport. The marrow in cancellous bone reflected the hydrostatic pressure caused by the vasculature, rather than what might be expected due to mechanical loads. So the hydrostatic pressure in cortical bone is influenced by mechanical loading whereas the hydrostatic pressure in more porous cancellous bone is relatively unaffected by mechanical loading.

Fluid flow will cause ion transport throughout the bone matrix inducing an electrokinetic effect. As the charged ions flow through the porous matrix, the charge is also translated. This produces a streaming potential. This streaming potential is estimated to be as high as 2mV. Otter et al. have shown that an electrical stimulus as small as 1mV/m affects bone growth. But the locations of the induced charges are not fully understood. Fifty percent of the fluid is located in the intracrystalline spaces of the bone matrix, which appear to have a transverse directional structure. This is susceptible to streaming potentials. There are several other larger pores containing fluid capable of connective fluid transport between the cell membranes of the osteocytes, the mineralized
lacunar walls, and the mineralized osseous canaliculi and between membranes of
enclosed osteocytic cell processes.

Current knowledge of the effects loading has on bone growth, resorption, and
formation are not fully understood. A method of studying long term in vivo strains on
bone surfaces resulting from a variety of loading conditions would enhance knowledge of
this behavior.

2.3 Calcium Phosphate Ceramic Coated Strain Gauges

Electrical resistance foil strain gauges are able to accurately measure small
changes in length for extended periods of time. A gauge contains strips of foil, which
change length when stretched. The foils resistance changes linearly with applied strain
over a defined range. Strain is determined by measuring the resistance change. A
voltage applied to a wheatstone bridge with the strain gauge in it allows measurement of
current flow when the bridge is unbalanced by a change in resistance.

Lanyon and Smith (1969), successfully documented the use of in vivo strain
gauging on sheep tibias. Gauges were attached with an isobutyl 2-cyano-acrylate. A
similar procedure was used by Lanyon to study strain behavior on sheep lumbar vertebrae
during gait (Lanyon L.E., 1971). This lead to strain mapping of the bone surface. Lanyon
et al (1981), studied the bone strain behavior on the proximal femur in sheep after a total
hip replacement. They were able to demonstrate a relationship between implant loosing
and reduced bone strains near the implant, which resulted in bone resorption and further implant loosening (Lanyon and Rubin, 1984). However they had to re-implant new strain gauges for additional readings throughout their experiment. The cyanoacrylate adhesive is quickly absorbed in vivo, causing inaccurate strain readings (Szivek et al., 1995). The glue will dissolve in a few hours to a couple of weeks.

In order to bond a gauge to bone for an extended period of time, a technique was developed to attach calcium phosphate ceramic (CPC) particles to the gauge surface (Szivek et al., 1985; Szivek et al., 1995; Szivek and DeYoung, 1997; Szivek et al., 2000). The CPC coating was attached to the back of the strain gauge with a heat treated polysulfone. The CPC coating encourages bone growth and attachment to the particles allowing a strain transfer from the bone surface to the strain gauge.

Calcium phosphate ceramics such as resorbable tricalcium phosphates (TCP) and bioactive hydroxyapatites (HA) have been used to coat orthopedic implants in order to induce bone ingrowth (Hench, L.L, 1998). Sumner et al. (1995) shown that CPC causes a 3-fold increase in bone growth after 4 weeks relative to non-coated control implants in a 3mm gap model between a 6mm diameter, 30mm long cylinder implanted in the proximal humerus of a canine. The strength of these ceramic coatings allows them to be used as a surface coating on loaded implants (i.e. knee and hip artificial joints). One disadvantage is that it takes some length of time for the bone to bond to the particles and
form a tight junction. The time required for bone bonding depends on biological and mechanical factors within the area but bone attachment to the CPC surface coating has been noted to take several weeks in both animals and human subjects (Spector et al., 1994)

Szivek et al. have used CPC coated strain gauges for \textit{in vivo} bone strain measurements in dogs (Szivek et al., 1997) and rats (Szivek et al., 2000). This technique allowed for strain readings for up to 18 weeks. Szivek et al. (1995, 1996) have shown that the time required for sufficient bone bonding to produce an accurate strain reading is at least 6 weeks in rats and 9 weeks in dogs. A six week study of CPC 6 (rounded spray dried amorphous TCP) in the dog model yielded a sensing accuracy of 30% (Szivek et al., 1995). Other CPC's such as CPC 1 (angular rocklike crystalline CPC) and CPC 2 (spherical crystalline CPC), examined after 6 weeks, gave a sensing accuracy of 31 ± 6% and 78 ± 35%, respectively (Maliniak et al., 1993). After twelve weeks CPC 1 and CPC 2 had a sensing accuracy of approximately 30% and 50% respectively (Szivek et al., 1995). CPC 3 (angular rocklike amorphous CPC) alone yielded a sensing accuracy of approximately 100% after 6 weeks in rats (Battraw et al., 1998).

The currently used particle blend for the CPC coated strain gauge application consists of 15 wt. % of a CPC previously designated CPC 6 and 85 wt. % of a CPC previously designated CPC 7. CPC 6 is a small rounded amorphous tricalcium phosphate (TCP) particle with an average long axis of 9 ± 7 μm and an average short axis of 6 ± 5
\( \mu m \) (Fig. 2.3). CPC 7 is an larger angular rock-like microcrystalline hydroxyapatite with a long axis dimension of \( 561 \pm 112 \, \mu m \) and a short axis dimension of \( 115 \pm 70 \, \mu m \) (Fig. 2.3) (Battraw et al., 1998). This blend has demonstrated a high CPC to bone interface shear strength after 6 weeks \textit{in vivo} with an acceptable CPC / gauge interface strength and reasonably rapid bone bonding characteristics. (Battraw et al., 1998; Battraw et al., 1999). CPC 3, which yielded a sensing accuracy of approximately 100% after 6 weeks had a gauge interface shear strength of only three-eight's that of CPC 6 (Battraw et al., 1998).

Figure 2.3: Scanning Electron Micrographs of CPC 6 and CPC 7.

The current method of attaching CPC particles to the back of the strain gauges is based on work by Battraw \textit{et al} 1998 and 1999. The first study showed that small rounded HA's bonded to the gauge backing better than large chip shaped HA's. The second study showed agreement with earlier work which showed that relatively
amorphous or microcrystalline CPC's bonded more quickly than crystalline ones. In the first study various surface roughening and heat treating techniques were evaluated. An optimal heat treating and gauge surface preparation techniques were determined for the highest interface shear strength between various CPC's, polysulfone, and strain gauge backing.

2.4 Rat Femur Model

CPC coated strain gauges have previously been studied in a rat model (Battraw et al., 1998; Battraw et al., 1999). Szivek et al. (1995), studied CPC coated strain gauges for 6 and 12 week time periods. Wilson et al. (1998), documented sensing accuracy's and histomorphometric results for various CPCs in the model while Battraw et al. (1998), studied the interface strength of CPC coated gauges in the rat model. Past work has established surgical protocols and augmented comparisons. Unpublished in vitro CPC coated strain gauge tests were conducted evaluating cell attachment to CPC particles. However the results were contradictory to in vivo studies. For these reasons the rat model was chosen for this surface enhancement study.

2.5 Surface Enhancements

A description follows of five surface enhancements which are proteins, chemicals and cells believed to enhance bone growth.
2.5.0 PepTite-2000™

Integrins are cell surface receptors primarily responsible for cell-matrix mediated interactions. Seven, possibly nine of the currently identified integrins contain an arginine-glycine-aspartic acid (RGD) sequence within their associated peptide (Tschopp et al., 1994). These peptides may be synthetically sequenced and used to illicit a cellular response.

PepTite-2000™ was specifically designed to non-covalently bond (carboxy-terminal) to an existing surface and to leave the RGD sequence accessible for cell attachment (Tschopp et al., 1994). It is believed to establish a material to tissue interface allowing normal tissue structural development. This peptide was developed to induce rapid vascularization when attached to the surface of an implant.

A vascular supply is responsible for supplying the organic and inorganic structures of bone with molecules and diffusible ions. For this reason the vascular supply is critical for bone resorption, vitality, and formation (Brookes and Revell, 1998). This specific peptide is noted to attach endothelial cells to produce a vascular matrix that would promote bone growth.

The amino acid sequence of this peptide is:
acetyl-G(dR)GDSPASSKGGGGS(dR)LLLLLL(dR)-amide.

It begins with an amino-terminal presentation sequence, acetyl-G(dR)GDSP-,
followed by a spacer sequence, -ASSKGGGGS-. Finally there is a carboxy-terminal
sequence (dR)LLLLLL(dR)-amide, which attaches the peptide to the material surfaces
(Tschopp et al., 1994).

2.5.1 Osteogenic Protein-1

Osteogenic protein 1 (OP-1) is a synthetic form of a naturally occurring bone
morphogenetic protein 7 (BMP 7) derived from the dimeric protein TGF-β superfamily. BMPs
have been noted to possess osteoinductive properties and to induce ectopic bone
formation (Daluiski et al., 1999). A ten-fold increase in alkaline phosphatase activity
(leading to osteocalcin production) after 48 hours in vitro has been noted when BMP-2
and BMP-3 were added to a murine bone-marrow stromal osteoprogenitor cell line.
BMP-2 has been noted to increase alkaline phosphatase activity in a subpopulation of
muscle derived cells, leading to osteogenic differentiation (Bosch et al., 1999).

Onishi et al., studied the presence of OP-1 during rat fracture healing through
immunostaining and found that on day 3, OP-1 was strongly evident in the thickened
periosteum near the fracture and levels remained high only during early intermembranous
and endochondral ossification. It was not present in proliferating or mature
chronodrocytes (Onishi et al., 1998). OP-1 was further noted to cause osteoclast differentiation and maturation in vitro (Hentunen et al., 1995). OP-1 is osteogenic in human patients.

2.5.2 Transforming Growth Factor-β1

Transformation Growth Factor-Beta (TGF-β1) is a multifunctional polypeptide. The precursor protein was originally expressed in a Chinese hamster ovary cell line. The particular strain used in this study was synthesized from human platelets into a 25kD homodimer (Becton, 1996). It affects many aspects of cell growth, differentiation and function, including the induction of osteoblast mitogenic activity and the proliferation of osteoprogenitor cells (Erlebacher et al., 1998). Osteoblasts and osteoclasts secrete TGF-β and all isoforms (TGF-β1, -β2, -β3) are present within the bone matrix (Erlebacher et al., 1998). Kabasawa et al. (1998) suggested that the target cells for TGF-β in normal young rat tibial bones are mainly extracellular matrix-producing chondrocytes and undifferentiated preosteoblasts in which matrix production and differentiation of these cells is influenced by TGF-β.

Published evidence indicates that it has produced a 3-fold increase of bone ingrowth into porous coated canine implants after four weeks (Sumner et al., 1995). In that study plugs 7 mm in diameter and 30 mm in length, were coated with either 120 or
335 micrograms of TGF-β1. The plugs were inserted into the proximal side of the
humorous of a canine model (3mm gap on all sides) for 4 weeks.

It is suggested by Erlebacher et al. (1998) that osteoblasts deposit TGF-β in the
bone matrix, later to be released by osteoclasts during resorption. This activates nearby
osteoblastic differentiation. They showed TGF-β highly increases osteoblast
differentiation, suggesting an increase in differentiation and proliferation of
osteoprogenitor cells, augmented by osteoclastic activity (Erlebacher et al., 1998). It was
further noted that the increased osteoblast differentiation and final development into
osteocytes lead to an increased osteocyte density. Over expression of TGF-β2 in
osteoblasts increases the mineral apposition rate and the osteocyte density (Erlebacher et
al., 1998).

2.5.3 Calcium Sulfate Dihydrate

Calcium sulfate dihydrate (CSD), has been used in clinical practice to fill bone
defects and to deliver antibodies (Sidqui et al., 1995). CSH dissolves quickly in vivo and
is followed by osteoconductive bone formation in the defect. The CSD releases calcium
as it is digested by giant cells leaving a reserve for osteointegration (Peltier 1961; Peltier
1978).
2.5.4 Cell Sodding

Villanueva et al. (1990) have suggested that angiogenesis resulting from implanted endothelial cells increases bone remodeling. They placed endothelial cells isolated from a rat liver and rat fetal calvarial cells together and separately into an implanted diffusion chamber for a period of one month. When the endothelial cells were implanted alone, areas of fibrotic tissue grew inside the chamber and rapid vascularization occurred outside and around the chamber. When the isolated calvarial cells were implanted alone, large areas of loose connective stromal tissue grew in the chamber, with an occasional mass of chondrocytes. However, when the endothelial cells were implanted with the calvarial cells, regions of lamellar and woven bone and cartilage were embedded within the loose connective stroma. This blend of endothelial and calvarial cells increased the calcium content by more than ten fold. However, both cells types alone did show signs of ossification with increased calcium after 30 days.

A method of isolating endothelial cells for re-implantation, was developed by Williams et al. (1992 and 1995). The endothelial cells are harvested from highly vascularized fat tissue. This tissue theoretically contains one million endothelial cells per gram of fat. A collagenase is used to digest the extracellular matrix and release the endothelial cells. The cells are then spun out of solution through a series of centrifugation and washing steps. The endothelial cells are then combined with a growth serum to
provide nutrients to the cells. This liquid is then used to soak implant(s) (termed "sodding").

2.6 Finite Element Analysis

Strain transfer between the bone and CPC coated strain gauges is not fully understood. Combined biomechanical and histological studies have illustrated that an accurate strain reading may be achieved even with partial bonding or irregular in-growth of bone around the CPC particles (Szivek et al., 1995). The effect of bone growth around the edges of the gauge will also likely affect the measured strain. Factors such as waterproofing material stiffness and overall thickness or the thickness of CPC and polysulfone layers may be important parameters to consider when designing gauge construction protocols for in vivo experiments. These variables are difficult to examine experimentally because of the small size of gauges and the large number of experimental parameters. A finite element analysis will allow for the rapid assessment of relevant geometric and material parameters of bone, CPC, polysulfone, strain gauges and waterproof coatings.
3 Materials and Methods

3.0 CPC Preparation

A particle blend of consisting of 15 wt. % of a rounded amorphous tricalcium phosphate previously designated CPC 6 and 85 wt. % of a rocklike microcrystalline hydroxyapatite previously designated CPC 7 were mixed for the experiments. Particles were obtained from Biointerfaces Inc. (San Diego, CA).

3.1 Strain Gauge Preparation

Uniaxial Type EA-06-015K-120 single element strain gauges (Micro-Measurements Group Inc., Raleigh, NC) were coated, on their sensing surfaces with the blended CPC (Fig. 3.1). CPC particles were attached to gauges using a published procedure (Szivek et al., 1996; Battraw et al., 1998). The sensing sides of the gauges were sanded with 600 grit carbide paper. The carbide paper was brushed 10 times across, to lightly roughen the surface. Next the gauges were trimmed. Approximately 1 mm was removed from each side to minimize the size of the gauge for implantation. A thin layer of 15 wt. % solution of medical grade polysulfone (Amoco, Huntington Beach, CA) dissolved in 1,1,2,2 tetrachloroethane (Kodak, Rochester, NY) was applied to the sensing surface of each gauge. Gauges were then baked for 1 hour at 90°C. This baked layer was sanded with 600 and then 1200 grit carbide paper as described above. A second layer of the polysulfone was applied. The CPC blend was sprinkled onto the surface. The gauges
were baked for 5 hours at 90°C to dry the solvent and strengthen the interfaces between the CPC, polysulfone and strain gauge. A weight on a glass slide was used to press the CPC particles into the polysulfone during heat treating. The non-sensing surfaces of the gauges were coated using a published technique (Szivek et al., 1992) with three water proofing polymer coatings: nitrile rubber, acrylic and a polyurethane (M coat B, M coat D, and M coat A respectively). The nitrile rubber coating was cured for 24 hours at room temperature and touched up the following day if needed. Next an acrylic coating was applied and cured for one day. Then a polyurethane coating was applied to this surface and cured for 7 days. All gauges were double packaged and ethylene oxide sterilized and aerated.

Figure 3.1: Strain Gauge dimensions and with CPC particles. The lengths in parenthesis are the trimmed dimensions.
3.2 Surface Enhancements Applied to CPC Coated Gauges

3.2.0 PepTite2000™

Six gauges were coated with a proprietary peptide (PepTite-2000™) which was bonded to the CPC particles. The peptides were attached by soaking the strain gauges in a solution containing the PepTite™ dissolved in dimethyl sulfoxide and phosphate-buffered saline. (The details of the attachment method is proprietary and was undisclosed). Gauges were ethylene oxide sterilized and aerated prior to peptide attachment. Following peptide attachment, the gauges were resterilized prior to implantation. To ensure that the carboxy-terminal of PepTite™ adequately attached to the CPC 6+7 gauges, selected gauges coated with the PepTite-2000™ were placed in cell culture and cell populations on the surfaces were evaluated using fluorescence microscopy.

3.2.1 Osteogenic Protein-1 (Bone Morphogenetic Protein-7)

During aseptic surgery, 0.5 mg of Human Recombinant OP-1 (donated by Creative Biomolecules, Hopkinton, MA) was re-hydrated with 2cc of sterile water. The OP-1 dissolved completely into solution, and was then drawn into a sterile syringe. Two drops of the OP-1 solution were placed directly onto CPC surfaces and two directly onto
the bone contact zone during surgery for a total dose of $0.05 \pm 0.01 \text{ ml}$ or $12.5 \pm 2.5$ micrograms of OP-1.

### 3.2.2 Transforming Growth Factor-β1

The TGF-β1 (*Collaborative Biomedical Products* Bedford, MA) was purchased in a one microgram pellet. Two samples were purchased to cover 6 gauges each with $\frac{1}{3}$ μg of TGF-β1. Samples were stored at -70°C until used.

After preparing and sterilizing the six gauges in standard fashion, the following steps were taken to add the TGF-β1 surface enhancement. All steps were completed approximately 18 hours prior to implantation. All steps except step 1 were performed under a Labguard (Plymouth, MN. Model # Nu408-600) sterile laminar flow biological safety hood with sterile equipment. Sample sets of 3 gauges were prepared (and implanted) separately. The following method was repeated twice for each set:

1) The TGF-β1 was reconstituted with a $4\mu M$ HCL solution. The $4\mu M$ solution was acquired with a common dilution of 1000 mM HCL by mixing $20 \mu L$ of 1000 mM HCL with 4980 μL of distilled H$_2$O.
2) Three Sterile Petri Dishes (~100 x 15mm) were opened in the sterile environment and a piece of sterile gauze was placed on one side. MIU-Q \( \text{H}_2\text{O} \) (sterile water) was added with a sterile pipet onto the gauze until lightly soaked. Then the CPC coated gauges were removed from the sterile packaging and placed one per petri dish (with CPC side up). The petri dishes were then covered for later use.

3) Next 3cc of 4\( \mu \text{M} \) HCL was filtered through a sterile 0.2 \( \mu \text{m} \) acrodisc filter.

4) The TGF-\( \beta 1 \) was shipped in a double container, an inner and an outer. Under the flow hood, the inner container containing the TGF-\( \beta 1 \) was removed and the cap was cleaned with 70% ethanol. Then the inner lid was removed and 12\( \mu \text{L} \) of the 4\( \mu \text{M} \) HCL solution was added. This container/solution was swirled slightly until the TGF-\( \beta 1 \) was fully dissolved. The container was tapped lightly until all the droplets rested in the bottom of the container.

5) The reconstituted TGF-\( \beta 1 \) was applied to the back of each of the three gauges onto the CPC surface. The exact volume of the reconstituted TGF-\( \beta 1 \) was unknown, so small steps of equal doses were added to each gauge with the petri dish lids removed: 6 \( \mu \text{L} \), 2 \( \mu \text{L} \) then 1 \( \mu \text{L} \). This process was continued until all of the reconstituted TGF-\( \beta \) was applied. The petri dishes were then recovered and labeled.
6) The TGF-β1 needed to remain hydrated throughout its usable life, so the samples were placed in a TSAutoFlow™ (Pacific Science, Mission Viejo, CA) CO₂ water-jacketed incubator at 37 C until used.

7) After 18 hours the gauges were carefully carried to surgery, without removing the lids or tipping the gauges. Once in the sterile surgery suite, the gauze was re-hydrated if needed. Implantation of the gauges were performed using protocols described later in this chapter. Care was taken not to touch or dry the CPC.

The second batch of three TGF-β1 enhanced gauges was prepared more than 18 hours before surgery due to a scheduling conflict. These gauges were wrapped tightly in parafilm and refrigerated for one week. It was believed that this would have no effect on the validity of the surface enhancement.

3.2.3 Calcium Sulfate Dihydrate

An OsteoSet™ bone void filler kit (Catalog number 8400-0211), containing calcium sulfate hemihydrate (CSH) (Wright Medical Technologies Inc., Arlington, TN) was mixed with 1 part by wt.CSH to 0.3 part by wt. sterile water to form a slurry. This slurry was then allowed to thicken for five minutes, forming calcium sulfate dihydrate. The CPC particles were covered with a thin layer of the mixture and then implanted.
3.2.4 Endothelial Cell Sodding, with and without TGF-β1

Three gauges were coated with TGF-β1 before applying an endothelial cell layer and three received only the endothelial cell layer. Endothelial cells were harvested and prepared according to a published technique (Williams et al., 1992). Two sections of approximately 2x1x0.5 cm³ of epididymal fat were removed from each rat following a midline abdominal incision (Each rat received its own cell's for implantation to reduce the risk of implant acceptance). The fat was placed in a sterile container with harvest media, minced and washed to remove red blood cells, then digested and centrifuged to separate the fat, collagenase and cells. The vascular endothelial cells which formed a pellet were re-suspended and spun to create a concentrated solution of 0.7 to 4.3 x 10⁶ cells per milliliter. The CPC coated strain gauges were soaked in this cell-containing solution for 20 minutes before implantation.

The steps used to culture and apply the cells (cell sodding procedure) are as follows:

1) Fat was measured and placed in a DCF-PBS with 0.1% bovine serum albumin (BSA).

   (1 ml of displaced fluid = 1 gram of fat).

2) Poured fat and DCF-PBS into a small beaker and minced with scissors.
3) Sucked up fat and fluid with pipette (tip broken off) and put in sieve. Washed with DCF + PBS with 0.1% BSA.

4) Put the fat in an Erlenmeyer flask with a stir bar, using an equal amount of filtered collagenase; i.e. 2 mg/ml collagenase with 2 mg /ml BSA in DCF-PBS.

5) Put the flask in a shaking water bath for 30 minutes.

6) Put slurry in tube and spun for four minutes at ~210g.

7) Poured off fat and re-suspended cell pellet in 5 ml DCF-PBS with BSA in a new tube.

8) Spun for 3 minutes at ~210g.

9) Poured off waste and re-suspended in 5 ml DCF-PBS with BSA – 3 minutes.

10) Poured off waste and re-suspended in 1 ml M199 with 0.1% BSA.

11) Drew up 0.1 ml of solution and suspend in 9.9 ml of filtered Isoton. Then took 1 ml of the 9.9 ml suspended cells and place in 9.0 ml of filtered Isoton. Repeated once. Counted cell suspensions with a Coulter Counter® (Coulter Scientific Instruments. Miami FL). Performed 3 counts per container of Isoton. Performed at both low threshold limits (THL) of 13.6 and 15.9. Found the number of cells per milliliter by taking the average number (n) times the dilution (1,000) times the volume counted (2) per .5ml.

12) Poured 1 ml of cell suspension on strain gauge in a sterile petri dish. Incubated for ~20 minutes.

13) The strain gauges were in solution approximately 20 additional minutes before implantation into the rat.
The midline abdominal incision was stapled closed after fat removal. Staples were removed after 15 to 20 days. There was no sham group for rats who went through the cell harvesting procedure.

3.3 Surgical Procedures

Groups of six 120-day-old male Sprague Dawley rats were used to test each surface enhancement, except the cell sodding. The cell sodded gauges were tested with and without TGF-β1 in two groups of three rats. A total of 42 rats were used. All surgical procedures followed the National Institute of Health guidelines for the care and use of laboratory animals (NIH publication 85-23, rev. 1985). The rats were anesthetized with 0.32 mL of an injectable anaesthetic consisting of 5 parts ketamine (200 mg/mL), 5 parts xylazine (100 mg/ml) and two parts acepromazine (10 mg/ml). They were also given 0.03 mL of torbugesic.

One leg was shaved and scrubbed on the lateral and medial sides from below the knee to above the hip in preparation for aseptic surgery. An incision was then made on the lateral aspect of the leg, below the hip and extending partially to the knee. The intramuscular plane was separated to expose the femur. The periosteum on the anterior lateral surface was removed and dried with gauze. One gauge was placed on the mid diaphysis with the CPC layer toward the bone and the sensing element orientated along
the long axis of the bone. Gauges were secured with three, equally spaced, 00 Poly-Vicryl® resorbable sutures (Ethicon, New Jersey). The skin was closed with subcuticular stitches after apposition of the muscle. Animals were ambulatory within 5 hours and were returned to their cages. They were allowed normal cage activity with food and water ad libidum.

Seven days and again at three days prior to sacrifice, the test animals were subcutaneously injected with a 0.40cc solution containing 18 mg/ml of calcein. The calcein was added to distilled water and buffered with NaOH to a pH of 7.2. The calcein was used to label the mineralizing bone.

3.4 Mechanical Testing and CPC / Bone Bonding Analysis

Animals were euthanized using CO₂ hypoxia, three weeks after gauge implantation (App.A3). Both femora were explanted and all tissues were removed. The waterproof coating was removed with a soldering iron. Care was taken to assure that gauges were not dislodged during these procedures. In addition, bones were covered with saline soaked gauze during preparation to prevent dehydration.

Left and right femora were potted in separate aluminum holders embedding the femoral condyles in Cerrobend®, a low melting point alloy (Scottsdale Tool, Phoenix,
Anatomic landmarks were used to align and position femora at the same depth in the holders. A strain gauge was attached using a cyanoacrylate adhesive to the control femur in the same position as the implanted CPC coated gauge (on the contra-lateral femoral diaphysis). Great care was taken during this process to achieve accurate gauge placement.

The error associated with gauge placement is estimated by assuming the bone to be a linear, isotropic cylinder of length 25 mm and of constant cross-sectional area. With these assumptions the strain will vary linearly across the long axis of the bone. A mismatch of gauge axial alignment of 1 mm will therefore result in an error of $1/25^\text{th}$ or 4%. The error in strain gauge rotational misalignment may be estimated by noting that a rotated strain gauge is equal to the aligned strain gauge's measured strain multiplied by the cosine of the angle between them. A gauge misaligned by five degrees will have an error of 0.38 %.

Gauges were wired to female DB9 connectors and their resistances were tested. If the wired gauge did not have a resistance of $120 \pm 1 \text{ ohm}$, tabs were cleaned and rewired. They were then connected to male DB9 connectors wired to signal conditioners which were interfaced to a data acquisition board with NiDAQ software, monitored with LabView 5.0 on a Macintosh G3 (App. 2). The bones were tested in cantilever bending using a published procedure (Szivek et al., 1992). They were loaded so that gauges were placed alternately in tension and compression, using a servo-hydraulic series 810 MTS
(Materials Testing Systems Corporation, Minneapolis, MN) at a rate of 4.905 N/sec to a peak load of approximately 5.4 N (Fig. 3.2)

![Figure 3.2: Mechanical testing of an explanted femur. This loading configuration placed the gauge in tension. It was rotated 180 degrees and re-tested loading the gauge in compression.](image)

Selection of this load rate was based on published in vivo strain measurements reflecting rat femur loading during normal gait (Keller and Spengler, 1982). Measurements were collected from 0 to 5.4N and the strain was interpolated at 4.905 N (0.5 kg). A percent sensing accuracy was calculated from the strain recorded for the CPC coated gauge divided by the glued gauge sensing accuracy = [strain CPC coated/strain glued] x 100). The glued gauge sensing accuracy is estimated to be approximately two to five percent. A Micro-Measurments rating for the CEA gauge series of "moderate" gives the gauges an accuracy of 2 to 5% (Micro-Measurements Division, Catalog 500)
The cantilever beam bending test data was recorded for each set of enhancements. Three runs each for tension and compression were averaged. The uncertainty of the averaged values was small (less than 3 micro-strain) as compared to the deviations between samples. Data outliers were eliminated using Chauvenet's Criterion by comparing the average of the combined tensile and compressive data sets (Holman, 1994). A t-test was used to determine the statistical significance of the measurements collected from the surface enhanced CPC coated strain gauges compared to the unenhanced CPC coated strain gauges. (SigmaStat 2.0, Chicago, IL).

The use of the contra-lateral femur as the control is valid based on the assumption that the mechanical properties and shape of the two femora are bilaterally symmetric. This assumption was previously tested in six 120-day-old male Sprague Dawley rats (Battraw et al., 1996). In this study there was not a statistically significant difference in the strain values or in the modulus at p = 0.05. This study showed that the femora are bilaterally symmetric in 120-day-old male Sprague Dawley rats.

3.5 Optical Coherence Tomography

The waterproofing material was removed from the CPC coated strain gauges but the gauges were left affixed to the femur. Scans were taken along transverse and longitudinal axes of the bone. Multiple scans were taken across the sensing element, solder tabs and polyimide. Images were also processed with clean glued gauges. The
sample depth was set to 1.0 mm and the width set to 3.0 mm. The image resolution was 512 by 450 pixels.

3.6 Histology and Histomorphometry

Bone samples were prepared for embedding by drilling a hole in the femur proximal to the knee which allowed fluid infiltration during dehydration and embedding. Ethanol solutions mixed with distilled water were used to dehydrate the bones. The dehydration steps consisted of placing the bones in 70% ethanol for 2 hours (2 changes), then 95% ethanol for 8 hours (3 changes), and then in 100% ethanol for 11 hours (3 changes) (minimum time limits). The bones were infiltrated and imbedded in polymethyl methacrylate (K-plast softener and initiator from Delaware Diamond Knives, Inc.), following a published technique (Emmanual, 1984).

There were three components used in the embedding solution. Part A was the pure methyl methacrylate monomer. Part B was the K Plast softener. Part C was the K Plast initiator. The K Plast (DDK-Plast) was purchased from Delaware Diamond Knives, Inc.

Two infiltration steps were required before embedding. The infiltrate was a solution consisting of 92% Part A and 8% Part B by volume.
Example: In order to fill 6 of the 15 ml vials used to infiltrate the samples, a total of \((6 \text{ ml} \times 15 \text{ ml} \times 2 \text{ ml})\) 180 ml is needed or \((.92 \times 180 \text{ ml})\) 165.6 ml of Part A and \((0.08 \times 150 \text{ ml})\) 14.4 ml of Part B.

This covered solution was mixed with a magnetic stir bar for 1 hour prior to use. Each pair of bones was then suspended in a vial containing the infiltrate for one hour while stirring. Then the infiltrate was poured out and fresh infiltrate added. The submerged samples were then placed in a vacuum overnight. This process removed the trapped air bubbles and helped the solution infiltrate into the sample.

The samples were then embedded. Again a solution of 92% Part A and 8% Part B was mixed and stirred for 1 hour. Then 1%, by weight, of Part C was added. The amount of Part C needed was found by multiplying the volume of Parts A and B by the specific gravity of C and multiplying this by 0.01.

Example: 180 ml (of A & B) \(\times 0.94 \times 0.01 = 1.69\) grams of Part C.

This solution was then stirred for one hour, completing the embedding solution.

The infiltrate was poured out of the vacuumed samples and then the embedding solution was added to the vials. The vials were topped off with embedding solution,
vacuumed for 20 minutes and capped or covered. An exothermic reaction occurs while the solution hardens but can be controlled by placing the vials in a temperature controlled water bath or a stream of cool air. For this reason the vials were separated to avoid heating of adjacent samples and boiling (bubbling) the solutions. If a sample became too warm to the touch, the vial was placed in a cool water or ice bath. Samples were left at room temperature to harden for 1-3 days.

After the embedding solution hardened, the glass vials were broken. Four to seven cross-sections were cut through each gauge perpendicular to the long axis of the bone (Fig. 3.3) using a diamond wafering saw.

![Figure 3.3: Cross sectional cuts for histology.](image-url)
Slides were glued to the samples before each cut, allowing a thin cut of 0.8mm. The sections were then sanded and polished to approximately a 10 micrometer thickness with a smooth finish. This was the equivalent of one to three cell layers thick. This was achieved by using a series of increasing fine grit carbide papers. These were 120, 240, 400, 600, and 1200 grit. Sections were polished with 5 μm alumina. The sections were then stained using Villanueva Mineralized Bone Stain (MIBS) (Harrington Arthritis Research Center; Phoenix, AZ) (Villanueva and Lundin, 1989 and Villanueva and Nimni, 1990). For this procedure, sections were soaked in MIBS at room temp and then rinsed and set in the following:

a) 0.25% HCL — four brief swishes  
b) 95% ethanol — 12 dips  
c) 100% ethanol — 15 dips  
d) 50% ethanol: 50% xylene — 15 dips  
e) 100% ethanol — 15 dips  
f) 100% xylene — 15 dips

Samples were dried using forced air. A thin layer of Permount® (Fisher Chemical) was applied to the sides of the slide and then cover slipped. The cover slip was held tightly to the sample and slide for 1 week with clothespins while drying.
Random samples were also studied using a scanning electron microscope (SEM) with a Robinson backscatter detector. Sections were cut to a 2 mm slice thickness and polished. Images were used to identify regions of bone apposition to the CPC coatings and to assess relative degree of calcification of healed regions compared to the remainder of the cortical bone, using techniques described by Skedros et al. (1993a and b).

Digital images of stained histological sections were taken using a Nikon Optiphot Microscope with a Sony Digital Photo Camera (DKC-5000). The information was processed on a personal computer. Images were taken with either a 10x or 40x objective. Final prints were made using a normal color printer using HP Premium Glossy Photo Paper. Digital images of the stained samples were collected to assess gross bone formation and surface structure resulting from the various surface enhancements.

Optical microscopy and histomorphometry were used to examine cellular activity, to view regions of new bone formation and to assess bone activity near CPC coatings. Histomorphometry was performed using transmitted and ultraviolet light. Total bone area and cortical areas were measured. Bones were then divided into 4 quadrants, the gauge being in quadrant 1. In quadrant 1 and within the area of new bone growth, the area of periosteal new bone, eroded perimeter, single and double label perimeter of calcein labels and the calcein interlabel widths were determined. Directly below the new bone in Quadrant 1 the number of osteoid seams, number of calcein labeled osteoid seams, osteoid
widths, calcein interlabel widths and inner and outer labeled perimeters of bone structural units were determined. The mineral apposition rate (MAR) was then calculated in both the new bone and existing bone under the gauge by dividing the interlabel calcein widths by the interlabel time (Garner et al., 1995).

3.7 Finite Element Model

3.7.0 Finite element model geometry.

The finite element mesh was constructed using a commercial preprocessing package (TrueGrid, XYZ Scientific, Livermore, CA). The mesh was parameterized to allow changes in overall geometry without altering mesh topology or element count. The rat femur was represented as a thick-walled cylinder with an inner diameter of 2.6 mm, outer diameter of 3.9 mm and a length of 25.0 mm (Fig. 3.4 A,B). These dimensions represented average values of gross and histomorphometric measurements obtained from the in vivo studies. The long axis of the cylinder was aligned with the global z-axis while the tangent of the strain gauge normal vector was placed in the x-y plane. The dimensions for the strain gauge (6.600 x 4.200 x 0.056 mm) were taken from literature supplied by the manufacturer, while the in-plane dimensions (0.50 x 0.35 mm) and position of the sensing element were taken from measurements made with digital calipers. The strain gauge was assumed to lie uniformly on the bone. The strain gauge is constructed of a top and bottom layer of polyimide, with the foil sensing wire sandwiched
between the polyimide layers. The two polyimide layers of the gauge were modeled separately so that strain readings could be obtained at the same radial position as the sensing wire. A highly refined mesh was created to represent the sensing element (Fig. 3.4C), so that the strains experienced by the lengths of the compliant foil sensing wire could be integrated to yield an average sensing element strain. Detailed transition regions were incorporated into the finite element mesh to integrate this refined portion of the mesh with the rest of the model.

Dimensions and regions of the interface between the bone and strain gauge were chosen to yield a finite element model geometry that would represent the bone-gauge composite after bone attachment to the strain gauge. Histological analyses of the in vivo model provided the information regarding the total interface thickness and the constituent layers of the interface. Based on histological measurements, the total interface thickness between the bone and the CPC coated strain gauge was 0.3 mm. The histological sections confirmed three distinct regions between the cortical bone surface and the gauge - a layer composed primarily of CPC particles and newly formed bone, a layer of CPC and polysulfone, and a layer of polysulfone. For the finite element analyses that incorporated all three layers, these layers were assumed to have equal thickness (Fig. 3.4 2A).
Figure 3.4: Finite element mesh used for parameter studies. A. Axial view of finite element mesh, illustrating the discretization used and the layers between the bone and strain gauge. B. Close-up view of the strain gauge. C. Close-up
3.7.1 Material properties.

All materials were represented with an isotropic hypoelastic constitutive model in the finite element code. Hypoelasticity is a generalization of linearized elasticity to large strains and rotations (see, e.g., Truesdell and Noll, 1992). Material properties were separately assigned to each material region indicated in Figure 2.4. The bone was assumed to be isotropic and homogeneous and the elastic moduli of the bone were based on experimental studies of 120 day old Sprague-Dawley rat femora by Battraw et. al. (1996). The bone moduli were 22.36 and 20.5 GPa for compression and tension, respectively. An elastic modulus of 2.8 GPa was used for the strain gauge material (type 6/6 polyimide, Park, 1987).

The material properties of the interface matrix were unknown. The modulus for pure hydroxyapatite has been reported from 180.5 to 41.5 GPa (Yoshimura et al., 1994) while pure polysulfone is 2.5 GPa (Maoch, 1979). However, the composite modulus will depend on the distribution and relative volume fraction of the CPC particles in the polysulfone. It will also depend on bonding between the two materials, and will be influenced by the ingrowth of new bone around the CPC particles. The CPC/polysulfone composite cannot be easily subjected to material testing, since it is both brittle and thin.
Thus, the moduli of the interface layers were introduced as parameters in the finite element studies, as detailed in the sections below.

The elastic modulus of the polysulfone was determined from a tensile test performed specifically for this study. A thin layer of polysulfone was created using standard protocols for gauge preparation (Battraw et al. 1998; Szivek et al. 1996). A dumbbell-shaped sample was punched from the polysulfone layer. The sample was gripped using a custom set of clamps and loaded in tension at 0.25 mm/sec until failure occurred. From measurements of stress and strain, the elastic modulus was calculated as 1.89 GPa, which was slightly lower than a reference value of 2.5 GPa (Maoch 1979). The value determined from this experiment was used for polysulfone in all finite element studies.

Poisson's ratio for all interface materials was set to 0.3. A parameter study of this material property was conducted during the early phases of the study. Variations of this parameter caused negligible changes in the average axial strain recorded at the sensing element.

3.7.2 Boundary conditions and solution method.

Because the end of the bone is typically potted in a low-melting point alloy for mechanical testing in bending, the z=0 end of the cylinder was modeled as built-in by prescribing fixed boundary conditions (Fig. 3.5). A load of 4.905 N was applied to the
cylinder at the opposite end in the same plane as the sensing element, placing the strain gauge in tension. Finite element analyses were performed using the three-dimensional, nonlinear, implicit finite element code NIKE3D (Maker et al. 1990). Trilinear hexahedral brick elements were used to represent all materials in the model. A quasi-static analysis was performed, with the load applied in increments. Because of the large deflections and rotations experienced by the bone-gauge composite during loading, a nonlinear, incremental-iterative solution strategy was used with a quasi-Newton procedure governing the iterative process (Matthies and Strang, 1979). Convergence at each increment in applied load was based on the norm of the change in incremental nodal displacements.

![Figure 3.5: Idealization of geometry and boundary conditions as used in the finite element studies](image)

A convergence study was performed to ensure that the mesh was sufficiently refined in areas of large stress gradients. The final mesh consisted of a total of 7,538 elements and 10,332 nodes, with 6 elements through the thickness of the interface separating the bone from the strain gauge. (Fig. 3.4A,B). A mesh of 9 elements through the thickness yielded the same average sensing element z-strain as 6 elements. Mesh
geometry was further refined by optimizing element aspect ratios and maximizing orthogonality of element edges.

3.7.3 Data reduction and analysis

For each analysis, the average axial strain value at the sensing element was found by averaging the nodal axial strains within the center of the sensing element (Fig 4.4C). The nodal locations were evenly spaced along the approximate locations of the sensing element wires. Because the bone and gauge underwent large deflections and rotations (but small strains) during loading, the Green-Lagrange strain components were used for strain comparisons. The Green-Lagrange strain components are equivalent to those of the infinitesimal strain tensor when the strains are small enough to justify application of the linearized theory of elasticity and are unaffected by rigid body rotations, unlike the infinitesimal strain tensor components (Spencer, 1980).

3.7.4 Model validation

Validation of the initial finite element model was achieved in several steps. First, the strains predicted by the bone model without any gauge or interface material were compared to predictions for the solution of a built-in cantilever beam using simple beam theory. Second, strains predicted for a perfectly bonded strain gauge were compared to those of the model without a gauge. Finally, the tip deflections and strains obtained in the experiments were compared to the finite element predictions for the case of a gauge bonded directly to the bone. To determine the optimal experimental configuration
needed to maximize strain transfer from the bone surface to the strain gauge sensing element, and assess the variability that would be induced by a number of experimental factors, a series of parameter studies were carried out as detailed in the paragraphs that follow.

3.7.5 Effect of interface properties and thickness

To examine the model sensitivity to overall interface properties, the predicted axial strain was determined for interface elastic moduli of 0.15, 1.0, 1.5, 1.89, 2.0, 10, 15, 20 and 50 GPa. This was conducted to develop an understanding of the general behavior of the model when the bone-gauge interface properties were varied. To simulate the effect of partial to full bone/CPC attachment, a parameter study of the bone to CPC interface was conducted using the same list of moduli. To study the effects of bone to CPC attachment and associated CPC/PS ratio, parameter studies of these interface layers were conducted as well. For the latter three parameter studies, the rest of the interface was left at 1.89 GPa (modulus of polysulfone). Finally, the total interface thickness was varied from 0.15 to 0.45 mm with (E = 1.89 GPa) to study differences in CPC coating thickness observed during the experimental study.

3.7.6 Effect of gauge debonding, waterproofing and trimming

Histological sections of the gauge-bone complex sometimes show regions of non-bonding between the gauge and bone. These regions are typically along the sides or at the ends of the gauge. The effect of gauge debonding was assessed by removing the CPC
to bone interface on the lateral sides and posterior and anterior sides of the strain gauge independently. The effect of a waterproof coating was studied by adding a 0.3 mm thick layer over the top of the gauge and varying its elastic modulus from 1, to 2, to 4 and to 10 GPa. In the experimental studies, the CPC coated gauges were trimmed to provide a better fit onto the bone. To understand the potential effects of this modification, approximately 1 mm was removed from each side of the modeled gauge,
4 Results

All implanted gauges were relatively uniform in dimensions and CPC coating thickness. All were prepared in a similar fashion and gauges with the poorest CPC coverings within a group were discarded. All rat surgeries except those with cell sodding lasted for 20 to 30 minutes. The rats that received gauges that were cell sodded were under anesthesia for an additional 1 to 1.5 hours. All rats awoke in less than an hour and were walking well within 4 hours. There were no complications during surgery. All rats behaved normally with no noticeable agitation resulting from the strain gauge implantation. Sacrifices and femur explanations were completed with no complications. The CPC coated gauges were easily found and not disturbed during explantation.

4.0 Mechanical Test Results

4.0.0 Unenhanced CPC Coated Strain Gauges

The first CPC coated strain gauge (UNEH4) was too loosely attached to the bone to mechanically test (Table 4.1). The sixth sample (UNEH6) was noted to have a thicker layer of CPC particles on the strain gauge prior to implantation. This gauge was accidentally pulled off during the second half of mechanical testing. The compressive values were not recorded and were not included for statistically calculations. The seventh (UNEH7) gauge had light CPC coverage and no coverage along the gauge edges and the gauge did not bond firmly enough to mechanically test (Table 4.1).
Table 4.1: Unenhanced Mechanical Test Data

<table>
<thead>
<tr>
<th>Rat Name</th>
<th>Compression Strain value at 0.50 kg</th>
<th>Load Distance (cm)</th>
<th>Ctrl Gauge Location (cm)</th>
<th>CPC Gauge Location (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ctrl</td>
<td>CPC6+7</td>
<td>% Accuracy</td>
<td>Ctrl</td>
</tr>
<tr>
<td>UNEN1</td>
<td>-489</td>
<td>-457</td>
<td>93</td>
<td>543</td>
</tr>
<tr>
<td>UNEN2</td>
<td>-520</td>
<td>-294</td>
<td>56</td>
<td>520</td>
</tr>
<tr>
<td>UNEN3</td>
<td>-629</td>
<td>-316</td>
<td>50</td>
<td>632</td>
</tr>
<tr>
<td>UNEH4</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>UNEH5</td>
<td>-526</td>
<td>-641</td>
<td>122</td>
<td>550</td>
</tr>
<tr>
<td>UNEH6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>728</td>
</tr>
<tr>
<td>UNEH7</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
</tbody>
</table>

Ave w/o Zeros: 76 ± 31%
Ave with Zeros: 52 ± 44%

The mechanical test data averaged for the attached gauges only was 76 ± 31% but with the unattached or loose gauges the average was 52 ± 44%. No data values met Chauvenet's Criterion as data outlier.

4.0.1 PepTite2000™ Enhanced CPC Coated Gauges

The functionality of the peptide coating was demonstrated by an elevated cell attachment of the treated versus non-treated CPC coatings in cell culture (Fig. 4.1)
Only two of the three sutures on the second gauge (PT2) in this group were secured. During femur explantation, PT2’s strain gauge debonded from the CPC particles while the CPC particles remained affixed to the bone. The fourth implanted strain gauge (PT4) also debonded from the CPC particles during femur explantation. Mechanical test data were ignored for both PT2 and PT4 assuming an error in gauge construction (Table 4.2).

Table 4.2: PepTite\textsuperscript{TM} Enhanced Mechanical Test Data

<table>
<thead>
<tr>
<th>Rat Name</th>
<th>Ctrl</th>
<th>CPC 6+7</th>
<th>% Accuracy</th>
<th>Ctrl</th>
<th>CPC 6+7</th>
<th>% Accuracy</th>
<th>Load Distance (cm)</th>
<th>Ctrl Gauge Location (cm)</th>
<th>CPC Gauge Location (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT1</td>
<td>388</td>
<td>202</td>
<td>52</td>
<td>-391</td>
<td>.95</td>
<td>24</td>
<td>2.53</td>
<td>1.54</td>
<td>1.4</td>
</tr>
<tr>
<td>PT2</td>
<td>NA</td>
<td>debonded</td>
<td>NA</td>
<td>NA</td>
<td>debonded</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PT3</td>
<td>577</td>
<td>284</td>
<td>49</td>
<td>-616</td>
<td>.207</td>
<td>34</td>
<td>2.5</td>
<td>0.51</td>
<td>0.44</td>
</tr>
<tr>
<td>PT4</td>
<td>NA</td>
<td>debonded</td>
<td>NA</td>
<td>NA</td>
<td>debonded</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PT5</td>
<td>587</td>
<td>379</td>
<td>65</td>
<td>-608</td>
<td>.253</td>
<td>42</td>
<td>2.5</td>
<td>0.65</td>
<td>0.76</td>
</tr>
<tr>
<td>PT6</td>
<td>687</td>
<td>445</td>
<td>65</td>
<td>-615</td>
<td>.371</td>
<td>60</td>
<td>2.5</td>
<td>1.11</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Average: \(49 \pm 15\%\)

No values were eliminated as data outliers.
4.0.2 Osteogenic Protein-1 Enhanced CPC Coated Gauges

Each implanted gauge received 2 drops of 250 micrograms/cc re-hydrated OP-1 onto the CPC particles and 2 additional drops were placed directly onto the femur. However the first gauge (OGPl) received 0.4cc of solution. OGP6 received 0.50 cc and all other rats received 0.6 cc of the re-hydrated OP-1 solution.

Gauge 1 (OGPl) had light CPC particle coverage. Gauge 2 (OGP2) lacked coverage on the gauge edges. The third (OGP3) had good heavy coverage. The fourth (OGP4) had a lighter coverage and lacked edge coverage. The fifth (OGP5) had no coverage on the edge closest to the sensing element, and last (OGP6) had good CPC particle coverage. All strain gauges yielded mechanical test results (Table 4.3).

<table>
<thead>
<tr>
<th>Table 4.3: OP-1 Enhanced Mechanical Test Data.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strain value at 0.5kg</strong></td>
</tr>
<tr>
<td>Rat Name</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>OGP1</td>
</tr>
<tr>
<td>OGP2</td>
</tr>
<tr>
<td>OGP3</td>
</tr>
<tr>
<td>OGP4</td>
</tr>
<tr>
<td>OGP5</td>
</tr>
<tr>
<td>OGP6</td>
</tr>
</tbody>
</table>

Average: 102 ± 27%
Average w/o outliers: 96 ± 18%

The OGP1 tensile value of 170% was omitted as a data outlier.
4.0.3 Transforming Growth Factor-β1 Enhanced CPC Coated Strain Gauges

Gauges TB1, TB2 and TB3 showed signs of bone growth over the proximal and distal ends of the strain gauges. The gauge for specimen TB4 touched sterile gauze just prior to implantation, possible absorbing a slight amount of solution. This gauge was not well attached for mechanical testing and assigned a percent accuracy of zero. The gauge for TB6 lacked CPC particles on all edges of the strain gauge and had a lower sensing accuracy than average (Table 4.4).

Table 4.4: TGF-β1 Mechanical Test Data

<table>
<thead>
<tr>
<th>Rat Name</th>
<th>Strain value at 0.5kg</th>
<th>Load Distance Location</th>
<th>CPC Gauge Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Compression: Ctrl</td>
<td>Compression: CPC 6+7</td>
</tr>
<tr>
<td>TB1</td>
<td>-556 -691</td>
<td>124 (116)</td>
<td>627 725</td>
</tr>
<tr>
<td>TB2</td>
<td>-492 -507</td>
<td>103 (102)</td>
<td>497 510</td>
</tr>
<tr>
<td>TB3</td>
<td>-640 -695</td>
<td>109 (105)</td>
<td>681 716</td>
</tr>
<tr>
<td>TB4</td>
<td>NA 0</td>
<td>0 (0)</td>
<td>NA 0</td>
</tr>
<tr>
<td>TB5</td>
<td>494 507</td>
<td>102 (118)</td>
<td>549 649</td>
</tr>
<tr>
<td>TB6</td>
<td>537 495</td>
<td>92 (77)</td>
<td>562 431</td>
</tr>
</tbody>
</table>

Average W/Zeros: 87 ±47%
Average w/o outliers: 105 ±14%

The tensile and compressive values of zero for TB4 were dropped as data outliers by the Chauvenet's Criteria.

4.0.4 Calcium Sulfate Dihydrate Enhanced CPC Coated Gauges

The second and third gauges (CA2 and CA3) had a light CPC coating that lacked edge coverage on the side closest to the sensing element. During femur explantation all of the gauges were noted to be loose or completely detached from the bone. No
mechanical test data were obtained. Some gauges were left held in place by soft tissue that was not dissected off from the sides of the gauges but all gauges were firmly held on the femur with suture for histology.

4.0.5 Endothelial Cell Sodded CPC Coated Gauges

The epididymal fat was successfully extracted from the rats and endothelial cells harvested. The concentration of cells per milliliter of solution used during gauge incubation is shown in Table 4.5.

Table 4.5: Number of endothelial cells per milliliter incubated on gauge.

<table>
<thead>
<tr>
<th>Fat collected (grams)</th>
<th>Cell Count</th>
<th>Average Cell Count</th>
<th>Millions of Cells per Milliliter</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1.5 g</td>
<td>374/15.9</td>
<td>443/13.6</td>
<td>384/13.6</td>
</tr>
<tr>
<td></td>
<td>340/13.6</td>
<td>397/13.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>325/13.6</td>
<td>425/13.6</td>
<td></td>
</tr>
<tr>
<td>CS2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>887/1059</td>
<td>1064/1035</td>
<td>1040/2</td>
</tr>
<tr>
<td></td>
<td>1198/1059</td>
<td>999/1035</td>
<td></td>
</tr>
<tr>
<td>CS3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>2156/2244</td>
<td>2132/2106</td>
<td>2145/4.2</td>
</tr>
<tr>
<td></td>
<td>2259/2244</td>
<td>1978/2106</td>
<td></td>
</tr>
</tbody>
</table>

The first gauge (CS1) was and no strain could be recorded. This value was ignored. The second (CS2) was too loose to test and assigned a value of zero (Table 4.5).
Table 4.5: Cell Sodding Mechanical Test Data

<table>
<thead>
<tr>
<th>Rat Name</th>
<th>Compression Ctrl</th>
<th>Compression CPC 6+7</th>
<th>% Accuracy Ctrl</th>
<th>% Accuracy CPC 6+7</th>
<th>Load distance Location (cm)</th>
<th>Load distance Location (cm)</th>
<th>Compression Location (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS1</td>
<td>NA</td>
<td>NA warped</td>
<td>NA</td>
<td>warped</td>
<td>2.5</td>
<td>1.02</td>
<td>1.02</td>
</tr>
<tr>
<td>CS2</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>2.5</td>
<td>0.74</td>
<td>0.80</td>
</tr>
<tr>
<td>CS3</td>
<td>-696</td>
<td>-200</td>
<td>29</td>
<td>695</td>
<td>2.5</td>
<td>1.02</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Average: 15 ± 17%

Chauvenet's Criterion was ignored because of the small sample size.

4.0.6 TGF-β1 and Endothelial Cell Sodded CPC Coated Gauges

The epididymal fat was successfully extracted from the rats and endothelial cells harvested. The concentration of cells per milliliter of solution used during gauge incubation is shown in Table 4.6. Visual examination of the cell sodded gauges suggested that they had a thinner layer of CPC after soaking in the endothelial cell solution.

Table 4.6: Cell Count for TGF-β1 Enhanced Cell Sodded Gauges

<table>
<thead>
<tr>
<th>Fat collected (grams)</th>
<th>Cell Count</th>
<th>Average Cell Count</th>
<th>Millions of Cells per Milliliter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Th, High</td>
<td>Th, Low</td>
<td></td>
</tr>
<tr>
<td>CS-TB1</td>
<td>358</td>
<td>424</td>
<td>386</td>
</tr>
<tr>
<td></td>
<td>355</td>
<td>406</td>
<td></td>
</tr>
<tr>
<td></td>
<td>338</td>
<td>432</td>
<td></td>
</tr>
<tr>
<td>CS-TB2</td>
<td>564</td>
<td>552</td>
<td>515</td>
</tr>
<tr>
<td></td>
<td>482</td>
<td>477</td>
<td></td>
</tr>
<tr>
<td></td>
<td>506</td>
<td>508</td>
<td></td>
</tr>
<tr>
<td>CS-TB3</td>
<td>126</td>
<td>205</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>124</td>
<td>197</td>
<td></td>
</tr>
<tr>
<td></td>
<td>103</td>
<td>165</td>
<td></td>
</tr>
</tbody>
</table>
The first implanted gauge (CS-TB1) could not be balance with the Signal Conditioner and no mechanical test results could be collected. This sample was ignored for the calculation of the average (Table 4.7)

Table 4.7: Mechanical test data for TGF-β1 and Cell Sodded gauges.

<table>
<thead>
<tr>
<th>Rat Name</th>
<th>Strain value at 0.50 kg</th>
<th>Load distance (cm)</th>
<th>Ctrl Gauge Location (cm)</th>
<th>CPC Gauge Location (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compression</td>
<td>Tension</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ctrl</td>
<td>CPC 6+7% Accuracy</td>
<td>Ctrl</td>
<td>CPC 6+7% Accuracy</td>
</tr>
<tr>
<td>CS-TB1</td>
<td>-484.2 dead ch.</td>
<td>NA</td>
<td>716.13 dead ch.</td>
<td>NA</td>
</tr>
<tr>
<td>CS-TB2</td>
<td>-597</td>
<td>-644</td>
<td>108</td>
<td>754</td>
</tr>
<tr>
<td>CS-TB3</td>
<td>-476</td>
<td>-128</td>
<td>27</td>
<td>544</td>
</tr>
</tbody>
</table>

Average: 64 ± 48%

Chauvenet's Criterion was again ignored due to the small sample size.

4.0.7 Surface Enhancement Compilation

Unenhanced gauges had a mean sensing accuracy of 52% ± 46%. Peptide coated gauges (49% ± 15%) displayed a lower sensing accuracy, but these results were not statistically significantly different from the unenhanced gauges. The OP-1 (96% ± 18%) and the TGF-β1 (105% ± 14%) showed the highest sensing accuracy, and both were statistically significantly different from the unenhanced with a 95% confidence interval (Fig 5.2). CSD gauges were too loose to test or fell off during necropsy. The sensing accuracy of all six of the CSD coated gauges was assigned a value of zero. The gauges coated with the endothelial cells displayed poor sensing accuracy (15% ± 17%). The gauges coated with TGF-β1 and then endothelial cells produced a wide range of sensing accuracy (64% ± 48%).
Figure 4.2: Mechanical Testing results. P-values are compared to the unenhanced group.

4.1 Histomorphometric Results

The unenhanced gauges had less new bone growth in quadrant one than other enhanced gauges (Table 4.8). OP-1 and TGF-β1 enhanced CPC coated gauges produced more new bone and more bone-to-CPC contact than other enhancements (Table 4.8). Both produced areas of woven bone, however the TGF-β1 produced tighter woven bone at the CPC-bone interface (App. 5). The gauges with the TGF-β1 enhancement also appeared to cause a slight (though not statistically significant) increase in total cortical
area (9.2 ± 1.3 mm² versus 8.8 ± .8 mm² relative to the unenhanced). There was visual evidence of a thicker calcein label on the periosteal perimeter. The OP-1 produced sporadic areas of new woven bone that often extended outward tangent to the bone surface. This resulted in a larger total bone area of 14.0 ± 1.1 mm² versus 13.3 ± .7 mm² for the paired unenhanced section. These patterns of sporadic woven bone were occasionally present on the medial side of the bone, opposite the gauge (App. 5).

Table 4.8: Total areas of bone and new bone growth for the enhanced and unenhanced CPC coated strain gauges. The OP-1 and TGF-β1 caused the most new bone to form adjacent to the gauge.

<table>
<thead>
<tr>
<th></th>
<th>Total Bone Area (mm²)</th>
<th>Total Cortical Bone Area (mm²)</th>
<th>New Bone Growth in Quad 1 (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unenhanced</td>
<td>13.3 ± 0.69</td>
<td>8.8 ± 0.80</td>
<td>0.16 ± 0.06</td>
</tr>
<tr>
<td>PepTite™</td>
<td>13.1 ± 1.28</td>
<td>8.8 ± 0.89</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>OP-1</td>
<td>14.0 ± 1.11</td>
<td>9.7 ± 1.08</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>13.8 ± 1.28</td>
<td>9.2 ± 1.29</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>CSH</td>
<td>13.3 ± 0.58</td>
<td>9.1 ± 0.87</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Cells</td>
<td>13.2 ± 0.59</td>
<td>6.7 ± 2.66</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Cells&amp;TGF-β1</td>
<td>13.0 ± 1.04</td>
<td>8.2 ± 0.49</td>
<td>0.3 ± 0.2</td>
</tr>
</tbody>
</table>

A vascularized layer was noted between the bone and CPC coatings on the histological sections of the PepTite™ and cell sodding enhanced coatings. Both produced large areas of erosion (0.6 ± 0.3 mm and 0.68 ± 0.39 mm in length respectively) versus 0.11 ± 0.07 mm for the unenhanced gauge on the periosteal surface beneath the gauge (Table 4.9). The PepTite™ enhancement produced a layer of new bone with 0.29
± .23 mm² area adjacent to eroded areas, that extended toward but not contacting the CPC particles. Vascularized areas with endothelial and fibrous cells surrounded the CPC particles. When TGF- β1 was added before the cell sodding procedure, areas of new woven bone were noted (Table 4.9), similar to those noted in TGF- β1 enhanced CPC coatings.

Table 4.9: Histomorphometric parameters measured in the new bone under the CPC gauges. The single / double label perimeter is the length of the calcein labels and the interlabel distance is the spacing between the calcein labels. The eroded perimeter is the length of bone that was absorbed under the gauge.

<table>
<thead>
<tr>
<th></th>
<th>Single/double label perimeter (mm)</th>
<th>Eroded perimeter (mm)</th>
<th>Interlabel Distance (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unenhanced</strong></td>
<td>1.7 ± 0.6</td>
<td>0.1 ± 0.1</td>
<td>16 ± 2</td>
</tr>
<tr>
<td><strong>PePTite™</strong></td>
<td>2.4 ± 1.3</td>
<td>0.6 ± 0.3</td>
<td>22 ± 4</td>
</tr>
<tr>
<td><strong>OP-1</strong></td>
<td>3.7 ± 1.7</td>
<td>0.2 ± 0.1</td>
<td>16 ± 4</td>
</tr>
<tr>
<td><strong>TGF- β1</strong></td>
<td>2.4 ± 2.0</td>
<td>0.1 ± 0.1</td>
<td>17 ± 4</td>
</tr>
<tr>
<td><strong>CSH</strong></td>
<td>0.9 ± 0.7</td>
<td>0.1 ± 0.1</td>
<td>18 ± 5</td>
</tr>
<tr>
<td><strong>Cells</strong></td>
<td>2.2 ± 0.9</td>
<td>0.5 ± 0.4</td>
<td>23 ± 5</td>
</tr>
<tr>
<td><strong>Cells&amp;TGF- β1</strong></td>
<td>2.5 ± 0.7</td>
<td>0.2 ± 0.2</td>
<td>25 ± 5</td>
</tr>
</tbody>
</table>

The CSD enhanced gauges produced similar histomorphometric results to the unenhanced gauges (Table 4.8-10 and Fig. 4.3) in all measured parameters except the single/double label parameter. However, with the CSD there was very little bone growth and attachment to the CPC particles. The CSD bone interface was clearly identifiable in the optical microscope but lacked osteoconductive activity as determined from a lack of calcein labeling. There appeared to be bone cells within the CSD matrix. The calcein labeled perimeter was only 0.94 ± 0.64 mm (Table 4.9). In some cases a micro-fracture
was observed at the CSD-bone interface. This was likely produced during bone removal or during slide preparation. There were areas of new woven bone in regions along the gauge edges where the CSD and CPC were exposed to soft vascularized tissues (App. 5).

The number of osteoid seams in the cortical bone of all groups were within a similar range (Table 4.10). The number of labeled osteoid seams in the cortical bone were higher for the PepTite\textsuperscript{TM} and the endothelial cells with and without TGF-\textbeta 1. The calcein label perimeters were largest for the TGF-\textbeta 1 group. All of the enhancements had a larger outer perimeter as compared to the unenhanced group, while only the PepTite\textsuperscript{TM}, OP-1, TGF-\textbeta 1 and CSH had a larger inner calcein perimeter (Table 4.10).

Table 4.10: Histology results for measurements made below the gauge and new bone in the cortical section. The higher number of labeled osteoid seams with the PepTite\textsuperscript{TM}, endothelial cells, and endothelial cells with TGF-\textbeta 1 enhancements indicate a higher bone turnover rate during the labeling period.

<table>
<thead>
<tr>
<th></th>
<th># of Osteoids</th>
<th># of Osteoids</th>
<th>Osteoid Width</th>
<th>Interlabel</th>
<th>Label perimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with label</td>
<td>(\mu m)</td>
<td>width (\mu m)</td>
<td>Outer (mm)</td>
<td>Inner (mm)</td>
</tr>
<tr>
<td>Unenhanced</td>
<td>32 ± 8</td>
<td>3 ± 2</td>
<td>6.7 ± 5.8</td>
<td>9 ± 3</td>
<td>0.15 ± 0.06</td>
</tr>
<tr>
<td>PepTite\textsuperscript{TM}</td>
<td>33 ± 21</td>
<td>13 ± 15</td>
<td>7.4 ± 2.3</td>
<td>12 ± 4</td>
<td>0.17 ± 0.06</td>
</tr>
<tr>
<td>OP-1</td>
<td>26 ± 18</td>
<td>4 ± 3</td>
<td>6.3 ± 2.7</td>
<td>10 ± 6</td>
<td>0.19 ± 0.06</td>
</tr>
<tr>
<td>TGF-\textbeta 1</td>
<td>25 ± 7</td>
<td>9 ± 7</td>
<td>8.2 ± 3.8</td>
<td>11 ± 3</td>
<td>0.22 ± 0.13</td>
</tr>
<tr>
<td>CSH</td>
<td>34 ± 17</td>
<td>3 ± 4</td>
<td>7.3 ± 2.3</td>
<td>14 ± 12</td>
<td>0.19 ± 0.06</td>
</tr>
<tr>
<td>Cells</td>
<td>21 ± 8</td>
<td>14 ± 8</td>
<td>7.0 ± 1.5</td>
<td>11 ± 3</td>
<td>0.16 ± 0.06</td>
</tr>
<tr>
<td>Cells&amp;TGF-\textbeta 1</td>
<td>36 ± 12</td>
<td>12 ± 10</td>
<td>8.9 ± 1.3</td>
<td>14 ± 10</td>
<td>0.26 ± 0.07</td>
</tr>
</tbody>
</table>

Mineral appositional rates (MAR) were calculated in both the new bone and the preexisting cortical bone (Fig. 4.3). The MAR for all gauges was higher in the new bone
in comparison to the preexisting bone. None of the MAR's in the preexisting bone were statistically significantly different. The MAR in the new bone was statistically significantly different for the PepTite™ and the endothelial cell sodding enhanced verses the unenhanced CPC coated gauges. Both of these groups had large areas of vascularization (App. 5).

Figure 4.3: Mineral Appositional Rates for the CPC coated strain gauge sets. Both the PepTite™ and the Cell Sodded groups had a statically significantly higher MAR than the control. Abnormally high vascular sections were noted in both (App. 5). (Mean ± Standard Deviation)
4.2 Optical Coherence Tomography Images of Bonded Gauges

The layers of a CPC coated strain gauge after removal of the waterproofing material were revealed by the Optical Coherence Tomography scan in both the cross-sectional scan (Fig. 4.4) and the transverse scan (Fig. 4.5). Although the scans were unable to see the CPC to bone interface, they were able to identify the location of the gauges on the bone.

Figure 4.4: OCT transverse image of a strain gauge and bone. The foil within the strain gauge reflects the OCT, producing a rippled image below any foil.
The foil within the strain gauges produced reflections that the OCT could not penetrate (Fig. 4.4). It was able to outline tissue and bone around the strain gauges.

Figure 4.5: OCT longitudinal image of a strain gauge bonded to bone. The layers and the curvature of the strain gauge are apparent from the image.

4.3 Finite Element Results

Finite element solutions were obtained without difficulty using 4 increments of applied bending load. Axial strains along the tensile surface of the bone varied linearly along the length, with zero axial strain at the built-in end and maximum axial strain at the loaded end, as expected based on linear elastic beam theory (Fig. 4.6A). The lower section of the bone was in compression while the top was in tension.
4.3.0 Model validation

Without a gauge present, the FEM tip deflection at the point of applied load and axial strain at the sensing element location were 0.14 mm and 512 με, respectively. Simple beam theory predicted values of 0.13 mm and 514 με. Strain transfer from the bone to the glued gauge was nearly complete (Figures 5.6A, B). However, there were subtle differences in the strain contours even for this case.

Figure 4.6: Contour/fringe plots of axial strain for the cases of A., glued (perfectly bonded) strain gauge and B, bone without a strain gauge. Results are fairly close, but there is some difference even for this idealized case of no interface layer between the gauge and the bone.
The average axial strain at the location of the sensing element was 539 με for the glued gauge, compared to 512 με for the bone in the same location. The higher strain with the perfectly bonded strain gauge is to be expected, since the gauge surface is farther from the neutral axis of the system than the case without the gauge. The predicted value for the glued gauge average sensing element strain was in good agreement with the value obtained from experiments (612 ± 101). The tip deflection under maximum load was higher (0.14 mm for finite element model, 0.32 ± .06 mm for experiments). This may be attributed to permanent deformation of the bone by the small loading point which left a noticeable dimple at the point of loading in the experimental model.

4.3.1 Effect of interface properties and thickness.

The parameter studies of the interface elastic moduli for simulation of a CPC coated strain gauge showed that maximum strain transfer occurs between the bone and gauge when the elastic modulus of the entire interface was approximately 2 GPa (Figure 4.7). The sensing element axial strain rapidly declined when modulus dropped below 1 GPa, and gradually declined for values above 2 GPa. This effect was more pronounced with the variations of the CPC/bone interface than with the CPC/PS interface. As the total interface thickness was varied, a thickness of greater than 0.15 mm began to reduce the sensing element axial strain (Fig. 4.7). As the total interface thickness was varied, a thickness of greater than 0.15 mm began to reduce the sensing element axial strains (Fig. 4.8)
Figure 4.7: Parameter study of the interface between the gauge and bone. Unless specified, modulus for other interface layers is \( E = 1.89 \) GPa. Results that were closest to the glued gauge axial strain values were obtained for interface values between 1 and 2 GPa. Changes in the total interface modulus had the largest effect on the measured strain, followed by changes in the CPC-PS interface, and finally the CPC-bone interface. As interface modulus was decreased, the sensing strain dropped off rapidly.
Figure 4.8: Effect of interface thickness on sensing element strain. There was a decrease in sensing element strain with increasing interface thickness greater than 0.15 mm.

4.3.2 Effect of gauge debonding waterproofing and trimming.

Reduction of the elastic modulus of the lateral sides of the CPC/ bone interface induced negligible changes in the average axial strain on the sensing element (504 versus 505 µε). When the interface along the sides was removed entirely, the sensing strain increased to 516 µε (Fig. 4.9). However by softening and then removing the proximal and distal ends of the interface, the strain was reduced to 487 and 380 µε, respectively. These results highlight the importance of adequate axial bonding in comparison to bonding of the lateral sides.
Figure 4.9: Effect of softening and debonding the CPC/bone interface on the lateral edges and the proximal and distal ends.

A waterproofing layer with modulus of 1 GPa reduced the sensing element recorded axial strain to 444 µε (a 12% reduction) (Figure 4.10). When the modulus was increased to 2 GPa, the strain was further reduced to 402 µε (a 21% reduction). A waterproof coating with an elastic modulus of 4 and 10 GPa further reduced the sensing element z-strain by 32% and 49% respectively.
Figure 4.10: Effect of the waterproof coating modulus on sensing element strain. The coating thickness was held constant at 0.3 mm. Even with a modulus of 1 GPa, the measured strain was only 444 με, still 50 με below the best results illustrated in Figure 4.7.

The effective strain transfer from the bone to the untrimmed strain gauge versus the trimmed gauge was 13% different (Figures 5.11 C,D). An area of high axial strain was present in the central regions of both gauges and the proximal side of the gauge (closest to the sensing element) was at a zero strain. By trimming the strain gauge, the sensing
element was moved into this region of lower strain reducing, the average sensing element z-strain from 583 to 510 με. Figures 5.10 C and 5.10 D also show the large axial gradients in strain across the strain gauge and sensing element, highlighting the importance of consistent placement of the gauges along the long axis of the bone for experimental studies utilizing a cantilever beam test configuration.

Figure 4.11: Comparison of an untrimmed gauge A., to a trimmed gauge B., used in the experimental model. The modulus of the entire interface was 1.89 GPa for both analyses.
5 Discussion

5.0 Discussion of Experimental Studies

The unenhanced CPC 6 + 7 produced a variable sensing accuracy of 52 ± 44% which was close to other CPC’s after a similar time frame (Szivek et al., 1995).

Two surface enhancements, TGF-β1 and OP-1, consistently accelerated the bone attachment to the CPC 6 + 7 coated strain gauges relative to the unenhanced gauges. Histology revealed extensive bone to CPC contact. However the OP-1 enhancement produced bone spurs extending into the adjacent muscle tissue. These spurs may have resulted from the enhancement application technique. A technique, similar to that used with the TGF-β1 may produce more consistent results with fewer areas of spur growth. Both of these enhancements produced woven bone in most regions (more pronounced with OP-1) and numerous osteoclasts and osteoblasts were evident indicating a high level of bone activity. A mixture of both TGF-β1 and OP-1 have produced a 2-3 fold increase in ossicle cross-sectional areas after 15 days in extraskeletal sites of primates (Ripamonti et al, 1997). A mixture of these proteins may also be useful as a surface enhancement for the CPC coated strain gauges.

The PepTite™ coating inhibited bone to CPC particle attachment relative to unenhanced samples by inducing a vascularized soft tissue layer between the CPC particles and the bone matrix. There was extensive bone growth up to the vascular layer
from the existing cortical layer. The calcein labels indicated that this was continuing from the 2 to 3 week time point with a statistically significantly higher MAR. This suggests that while a local increase in vascularization increases bone growth (during the calcein labeling period), this is a slower process than the process which formed woven bone when gauges were enhanced with OP-1 or TGF-β1.

The cell sodding technique produced similar vascularization and a statistically significantly higher MAR (as did the PepTite™) versus the unenhanced control group. However there were more areas with bone precursor cells adjacent to the CPC particles. Enhancing the CPC particles with TGF-β1 before the cell sodding process established significant bone to CPC particle attachment. This effect has been noted by Winn et al., (1999) when BMP-2 was used alone and in combination with human osteoblast precursor cells in a rat calvarial defect model. The sensing accuracy for the cell sodded gauges with TGF-β1 were lower and the scatter wider than might be expected from the amount of bone seen on histological sections. One gauge produced a nearly perfect sensing accuracy while another displayed very poor results. It is also possible that the harvest media used to digest the fat tissue and separate the endothelial cells partially dissolved the CPC or polysulfone coating on the gauges or that the gauges were flawed before being cell sodded.
The calcium sulfate dihydrate enhanced gauges showed very little bone attachment to CPC particles. Although there were precursor cells found within the CSH there was little osteoinduction caused by the CSD as noted from the lack of calcein labeling. Bone growth did occur along the edges of the gauges, where the CSD and the CPC particles were exposed to the surrounding tissues. The growth could have been due to the increased vascularization in these areas.

Two variables that could have affected the sensing accuracy in these experiments were attachment of the CPC particles to the strain gauges and relative alignment of the glued control gauges with respect to the CPC coated experimental gauges. The CPC particle attachment to the strain gauges has been well studied and characterized by Battraw et al, 1998. There were few cases of gauge debonding in this study. A previous study showed that a sensing accuracy error of less than 2.0% between the two contralateral femora can be achieved by accurate gauge alignment (Battraw et al, 1996)

The OCT images were able to successfully scan the layers of the gauge but had difficulty imaging through the metal foil of the gauge. This technique was unable to identify areas of bone bonding to the CPC particles.

5.1 Discussion of Finite Element Analysis

The finite element analysis analyzed strain transfer between cortical bone and an attached strain gauge. The geometry and material properties used in the model represented both published and experimentally determined values. The finite element
model was validated by a series of convergence studies, model refinements, and comparison to both theoretical predictions and experimental measurements. Deflection and strain values recorded experimentally closely matched those of the finite element model for the case of a glued gauge. Parameter studies were performed to assess the sensitivity of the strain field to changes in interface elastic modulus, gauge and interface dimensions, debonding and the waterproof coating material stiffness. All of these parameters had large effects on the strain transfer from the bone surface to the gauge sensing element.

The parameter studies of the bone-gauge interface elastic moduli demonstrated the effect of alterations of the interface modulus. Both the PS layer and the CPC/PS layer can be controlled experimentally. However, as the bone grows into the CPC coated strain gauges the effective elastic modulus will change. When the total interface thickness was increased above 0.15 mm the computed sensing element axial strain was decreased. The large CPC particles used were $561 \pm 112 \, \mu m$ by $115 \pm 70 \, \mu m$. These were blended with smaller particles which were $9 \pm 7 \, \mu m$ by $6 \pm 5 \, \mu m$. With the large particles orientated with their long axis parallel to the gauge surface and with a typical 0.1 mm thickness of polysulfone, the interface thickness is approximately 0.22 mm. Histology showed the large particles orientated with their long axis in a variety of directions and they were often 2 or 3 CPC particle layers thick. Analytically a 0.45 mm interface thickness reduces the sensing element axial strain by as much as 17% in comparison to the case of
a glued gauge. It is possible that this effect offsets the effect of bone growth along the proximal edge of the strain gauge in the experimental model.

Immediately after gauge implantation, there is no bonding between the bone and CPC particles. Fibroblasts, macrophages and giant cells cover the area between the gauge and the bone. Eventually osteoblasts will lay down a collagen matrix, attaching the bone to the CPC particles. This layer will have a low effective elastic modulus. This layer will begin to calcify into areas of woven bone infiltrated with cells such as fibroblasts, osteoblasts and endothelial cells forming capillaries. Woven bone, bonding the CPC particles to the preexisting cortical bone, was noted to produce an accurate measured strain in some cases.

Comparisons of the untrimmed and trimmed gauges demonstrated the importance of a constant gauge geometry between animals for accurate experiment comparisons. The large strain gradients along the length of the strain gauge highlighted the importance of proper gauge orientation and gauge placement to accurately measure in vivo bone surface strain.

In the in vivo experiments, bone growth on the proximal and distal ends of the gauge was often noted. By improving the bonding between these ends of the gauge and the bone, strain transfer is enhanced (Fig. 4.7). A 0.3 mm wide section of bone extending
just to the bottom surface of the strain gauge increased the sensing element axial strain by 21%. This surpassed the value measured from the glued gauge.

Debonding or softening of the lateral edges of the gauge had little effect on the predicted axial strain, while debonding or softening the front and back edges of the strain gauge reduced the axial strain in the sensing element. This demonstrates that CPC bonding in-line with the sensing element is more important for measuring axial strain. It also suggests that there must be sufficient bonding both in front of and behind the gauge sensing element to ensure proper strain transfer. To achieve bonding in vivo, the CPC coated strain gauge must be held securely against the bone surface while bone attachment occurs. In our experiments, resorbable sutures tied around the femur were used to achieve this. If the lateral edges of the strain gauge do not require bone to CPC bonding for long term in vivo strain measurements, it may be possible to glue the lateral edges of the strain gauge with an adhesive to affix the strain gauge to the bone long enough for the bone attachment to the CPC particles to occur. If a cyanoacrylate were used a gel form would be needed to keep the glue from being wicked into the porous CPC surface.

A stiff waterproofing layer was shown to be a potential source of strain sensing error. In our in vivo studies, a nitrile rubber, acrylic and polyurethane coating have been used, and these materials have a much lower modulus than epoxy. Further, we removed the materials before the experimental beam bending analysis. An epoxy coating is
attractive as a potential waterproofing layer. The results of this study indicate that an epoxy layer should be as thin as possible, or a softer material should be sought for the purpose of waterproofing.

The predicted strain gauge responses used in this finite element analysis are only valid for the specific material and geometric assumptions incorporated into the model. Homogeneous material properties were assumed for all material regions indicated in Figure 3.4. This assumption is likely appropriate for the bone and strain gauge materials. However, the local distribution of new bone, CPC particles and polysulfone in the interface layers will probably be inhomogeneous. The effects of this type of distribution can be incorporated in the finite element analysis by using micromechanical modeling techniques or homogenization theory however this was beyond the scope of this study. In addition, it is likely that such model refinements would not be warranted in terms of the change in the results. A more realistic geometry could readily be incorporated into the model by using micro-CT or histological sections to yield accurate representations for the bone internal and external surfaces. Again, these changes would provide more accurate strain predictions but would not alter the general trends demonstrated by the parameter studies.
6 Conclusions

6.0 Conclusions of Experimental Analysis

The unenhanced CPC 6 + 7 produced a sensing accuracy of 52 ± 44%, comparable to what was expected for a three week study. The bone to CPC particle attachment rate was increased with the use of both the TGF-β1 and the OP-1 surface enhancements. They produced sensing accuracy's of 105 ± 14% and 92 ± 12% respectively after three weeks although the OP-1 produced areas of erroneous bone formation. It is recommended that TGF-β1 be used to accelerate the bone attachment to the CPC coated strain gauges. The PepTite™ and the endothelial cell layer enhancements induced vascularization near CPC particles resulting in relatively slower bone to CPC particle attachment at three weeks but did have an elevated MAR indicating that this may be a viable solution at a slightly longer time point. The CSD inhibited bone to CPC particle bonding at three weeks and is not recommended as an accelerant. However it is not ruled out as a storage medium for a growth factor. The combination of TGF-β1 and endothelial cells produced a vascularized matrix enclosed in calcifying bone. This combination of enhancements is not recommend in young rats due to the difficulty of placing these enhancements and variable sensing accuracy. However this combination may prove advantageous for less active or malnourished bone surfaces.
6.1 Conclusions of Finite Element Analysis

Based on the results of this work, a number of recommendations can be made for the use of CPC coated strain gauges in experimental studies. The thickness of the interface layer should be minimized particularly when these gauges are used in an animal model with a small bone section. This can be achieved by altering the size of the CPC particles and the thickness of the polysulfone layer used to bind the particles to the gauge. Adequate gauge material should be present both proximal and distal to the sensing element. For long-axis bending studies, the sides of the gauge can be trimmed with little effect on the measured axial strain. Due to the variations in axial strain along the long axis of the bone during the cantilever bending experiments, an alternative test configuration such as four-point bending may provide a system that is less sensitive to gauge placement along the bone long axis.
7 Future Work

1. An alternative application technique for the OP-1 could be investigated. The growth factor proved well-suited to the production of rapid bone growth. However the sporadic growth of the bone is undesirable. A way to isolate the OP-1 to the CPC bone interface would greatly improve its usability in future work.

2. A dose response curve for the TGF-β1 would be useful. As observer on histological sections, bone growth occurred very rapidly and was excessive in some areas. It is possible that a smaller dose would produce the desired bone growth into the CPC particles.

3. Investigating the use of cell cultures to study bone cell responses to various CPC particles and growth factors may prove useful. However these studies will only allow investigation of single cell responses rather than entire system responses. Preliminary work in this area has showed contradictory information to that collected from in vivo studies.

4. The finite element analysis yielded valuable insight into geometric and material selection properties that need to be incorporated into gauge construction. Future models may yield needed knowledge for using CPC coated strain gauges at different anatomical sites in different species.

5. Variability between CPC and polysulfone coatings did exist. Preliminary trials of baking strain gauges in a vacuum bag appeared to reduce the number and size of trapped air bubbles in the polysulfone. Further development of this technique and/or
other techniques to increase the reliability and accuracy of the CPC coated strain gauges is needed.

6. The cell sodding with TGF-β1 produced a vascular matrix within calcifying bone. The small sample size did not fully account for the variability present within the mechanical data. This combination of enhancements may need to be reexamined.
Appendix 1: Animal Subjects Approval

All surgical procedures followed the National Institute of Health guidelines for the care and use of laboratory animals (NIH publication 85-23, rev. 1985). The research was approved by The Institutional Animal Care and Use Committee (IACUC 96-079-82-2 and IACUC 98-103-1)
Appendix 2: Data Acquisition Hardware and Software

At the beginning of this project the data acquisition configuration consisted of LabVIEW 2.0. The system was running on a PowerMac 7100/66 with 16 megabits of RAM. Both Data Acquisition (DAQ) boards were inserted in the available Nu-Bus slots. These boards were NB-MIO-16 L Rev E, PN 7013501-01, with the NB-DMA2800 Rev D, P/N 700794-01, 1991 Accelerator Board. The Termination Breadboard was the SC2070, Assy 180950-01, Rev D.

LabVIEW software allows the programmer to break apart certain user inputs/outputs into separate windows for viewing ease. The software was configured to perform the following windowed functions:

- **Introductory Window**: This is the top-level window. It allows the user to choose one of the eight secondary windows functions. The termination of all proceeding windows will return the user to this window. A secondary window is opened by clicking on one of the following:
  - "Channel Assignment": This allows the user to designate a specific name to each channel.
  - "Calibration Coefficients": This allows the user to assign calibration constants for specific channel inputs.
  - "Rates": These values are designated as a multiplier to the raw voltage inputs to obtain outputs in engineering units. These values may be manually set by the user or computed in the Calibrations: window.
  - "Calibrations": This window allows the user to zero out voltage offsets and calibrate inputs.
  - "Save Test Setups": This window allows the user to save setups from prior steps.
  - "Recall Test Setups": This window allows the user to recall previously completed steps from a file.
- "Monitor": This window allows the user to monitor all inputs in real time through a digital display.
- "Test": This window allows the user to record the data in engineering units. These can be saved to a file for later analysis.

All time based buffering of data was based on the internal CPU time. The main drawbacks of the past system were:
1) Only eight input channels were correctly configured in the software.
2) Data was double buffered to allow for a full sample collection resulting in a reduced data buffer.
3) Lacked a graphical output.
4) Analysis of data files was time consuming.
5) Screens offered only black and white user interfaces.
6) No data filtering was available.

Past procedures for data analysis involved data collection followed by opening all data in an Excel worksheet. Data offsets were then removed (often due to strain gauge drift between experimental runs). The data were then interpolated to find the result of interest. This task was tedious due to the noise fluctuations in the raw data and often took up to 1 hour per set of samples tested.

The hardware and software were upgraded to reduce data analysis time. A National Instruments PCI-MIO-16XE-50 replaced both DAQ boards. This board allows up to 16 channels of analog input with 16-bit resolution at a 20 kS/s (kilo-samples per second) sampling rate. The DAQ board is also equipped with two 12-bit analog outputs 8 digital I/O lines and two 24-bit built in counters. The board was factory calibrated to standards traceable to the National Institute of Standards and Technology.
The data acquisition system was configured to accept an input of +/- 10 V with the polarity set to bipolar and the gain set to one. These features were selected within the Apple Menu -> Chooser -> NIDAQ. With 16-bit resolution, the DAQ board records up to $2^{16}$ distinct values between the input range providing a resolution of:

$$\text{Resolution} = \frac{\text{Range}}{2^{16}} = \frac{20 \text{ V}}{2^{16}} = 0.305 \text{ mV}$$

When accounting for a large conversion factor of 900 με/V ("Rates") used in the software to convert the raw voltage values to values of microstrain, the resolution of the readings is noted at being:

$$(\text{Conversion Factor} \times \text{Resolution}), \quad (900 \ \mu\varepsilon/\text{V}) \times 0.305 \text{ mV} = 0.27 \ \mu\varepsilon$$

This is acceptable considering typical noise values after filtering are greater than 2-3 με.

The sampling rate is also acceptable for its predicted uses. Under a maximum usage configuration in which the stroke, load and all 10 available strain channels are recorded, the maximum number of samples per channel is:

$$(20 \text{ kS/S}) / 12 \text{ channels} = 1667 \text{ samples per second per channel}$$

A common sampling rate for testing strain gauges is 250 samples / second. Faster sample rates are possible for tests such as a destructive shear test. This test only requires stroke and load.

The newer generation DAQ boards were not designed to fit in the NU BUS slots available but rather the more current PCI slots. The board was placed in a Macintosh G3 with 64 MB of ram. The hardware upgrades dramatically upgraded the system, but
further allowed for software upgrades and re-coding of the software. *LabVIEW* 4.0.1 and finally version 5.0.1, serial number G10XS3047, was installed and configured. During the LabVIEW revision form 2.0 to 3.0 National Instruments restructured the way in which many of their input / output sub VI's (subroutines) performed. As a result, this was not a smooth upgrade. Upgrading the VI written for version 2.0 to 5.01 was additionally difficult. During the software upgrading procedure, the *Orthopaedic Research Laboratory's* primary VI was further improved and modified to adequately take advantage of the new hardware upgrades. The result was an increased data acquisition rate, decreased data analysis time, and an improved user interface.

### 1.1 *LabVIEW Test Program*

The software written for mechanical testing is the "Static Test Program LV vs5". It is located on the G3 computer and backed up on a JAZ 2G disk labeled G3 Backup. The program is located in the folder:

G3 Internal Hard Disk-> Applications -> Lab View 5.0:

To begin the program double click on the short cut labeled "Static Test Program LV vs5" located on the desktop. This links to the actually file located at:

G3 Internal Hard Disk-> Applications -> Lab View 5.0 -> user.lib:

*LabVIEW* 5.0 will load and the following screen will appear:
There are eight options that the user may click on; Channel Assignments, Channel Calibrations, Rates, Calibrations, Save Test Setup, Recall Test Setup, Monitor, and Test. The user begins by clicking on the RUN arrow located on the top left corner of the screen. If a previously used test setup was saved it may be recalled otherwise begin by clicking on the Channel Assignments button.
The channel assignments window allows the user to input the names of each input channel. The program will organize the output data file into columns with headings specified in this window. Channel 0 is always load and 1 is always stroke. Channels 2 - 11 are available strain inputs hardwired from the signal conditioners on the 810 Material Test System (MTS), Eden Prairie, MN from the NB-M10 Board. Channels 12 - 15 are free. Clicking on the EXIT button will return the user to the main window and allow for the next selection, Calibration Equivalents.
This window allows the user to assign calibration values to specified channels. The user should select -500 or +1000 for strain channels. The strain gauge signal conditioners have a calibration toggle switch labeled Cal A and B. The middle position is Cal OFF. When the switch is toggled to position A, a voltage value equivalent to 1000 micro-strain is produced. Position B produces -500 micro-strain. The LabVIEW program will use the input voltage and calibration value to calibrate each strain gauge channel to micro-strain. This is explained further in the Calibrate window. By clicking on EXIT the user returns to the main window. At this point the Rates window may be selected to continue the calibration process.
Figure 4: LabVIEW Rates Window

The rate values entered here are multipliers used to convert the raw voltages from the input channels to designated units. The Load and Stroke "Rates" need to be entered at this point. The rates for the strain values will be computed in the next window, Calibrations.

The rates for the load and stroke are based on converting the raw voltage (±10V) into engineering units. For example, when using the ±200 kg load cell with the Percent Load on the MTS 442 Load Controller set to 100 percent, the correct rate to enter into Channel 0 Load (kg) is 20:

\[(200 \text{ kg} \times 100\%) / (10 \text{ V}) = 20 \text{ kg} / \text{ V}.\]

Additional rates (conversion factors) are calculated by using the formula:

\[(\text{Load Cell Max Load}) \times (\text{Selected Percent Load}) / 10 \text{ V} = \text{Load Rate}\]

The Rate is then entered into this sheet.
The values should be tested by hanging a dead weight on the load cell and observing the converted value in either the Calibrations or Monitor window. An alternative technique is to let LabVIEW assign the Rate by putting the value of the weight (appropriate units) in the Calibrations Equivalents load input value and then following a similar procedure used to calibrate the strain gauge in the Calibration window.

The rate for the stroke is computed in a similar manner. The stroke full scale is 20 cm (±10), therefore the rate for the stroke in cm at 100% stroke percent is 1.

\[(10 \text{ cm}) \times (\text{Selected Percent Stroke}) / 10V = \text{Stroke Rate}\]

This value should then be entered as the stroke rate in LabVIEW.

Once the Calibration Equivalents and the Rates have been assigned the Calibrations window is opened. This window allows the user to zero the raw values and assign a multiplier to the voltage for conversion to usable engineering units. The channel numbers 0-15 are listed on the right. In the middle column is the user interface and on the left are the outputs. The user may select any channel to zero or set the rate. The load and / or stroke may be zeroed by double clicking on Channel 0 or 1 respectively and then clicking on the ZERO button. The box labeled "Engineering Units" should now indicate the correct value(s).
To set the strains, click on a strain channel (Channel 2-11). The voltage is averaged every 0.25 seconds and displayed in the "Steady Volts" output. The "Single Amplitude" output displays the maximum difference in raw voltage every 0.25 seconds. This is an indication of noise in the system. The user then clicks the "Zero" button to zero the strain gauge reading under a no load condition. The user then toggles the corresponding calibration switch to CAL A on the MTS 430 Digital Indicator. This produces a voltage comparable to 1000 ohms. By clicking on "Positive" then "New Rate" the user has effectively set the rate for the channel equal to: (Engineering Units, output) / (Steady Volts, raw voltage). Clicking Negative will invert the signal. This process is then repeated for all channels.

The load and stroke may also be set this way if a know weight or distance is applied to the system with a corresponding value entered into the Calibration Equivalents window.
Next, the user should exit this window and views the current setup in the Monitor window before continuing. This also assures the user that all values were passed to the main window and thus to the Save Test Setups window.

All of the data from the previous windows may be saved in the Save Test Setups window. Test setups are saved on the G3 folder labeled:

G3 Internal Hard Disk-> Applications -> Lab View 5.0 -> user.lib -> Test Setups:

The user saves by selecting "New", typing in the name of the setup and then selecting "File". If the user does not select "File" but pushes return a new folder with the name will be created. If at any time the user selects "Cancel" LabVIEW will present an error message. The program can continue by selecting "Continue" otherwise it will end and all work will be lost unless previously saved.
Test setups may be recalled at any time by clicking on the Read Test Setups button from the main window. If a test setup whose values were not correctly saved is recalled, the Monitor window will display zeros and LabVIEW must be restarted.

The Monitor window displays all channel assignments and their input values in engineering units. Data is received at the maximum allowable rate and then 30 consecutive data values are averaged and displayed. This provides a stable real time display for setting/checking loads, stroke and readable strains. Clicking EXIT will return the user to the main window.
The TEST button on the main window will bring the user to the test window. The user may input the temperature and humidity if required for record keeping. The user then enters the total number of samples per channel and the scan rate in samples per second. Typical values for cantilever beam testing are 500 and 250 respectively. This provides a total sample period of 2 seconds. The output labeled Number of Channels to Scan displays the total number of channels that will be recorded. This number should match the required value or an error in data entry has occurred.

A digital 3rd order lowpass Butterworth Filter was implemented into the system. This filter type has a smooth, monotonically decreasing frequency response. Increasing the filter order increases the transitional steepness of the cutoff frequency, however this is taxing to the CPU. The cutoff frequency is the half-power or 3-dB down frequency. (National Instruments Corp, Jan 1996).

The filter is calibrated with a cutoff frequency of 40 Hz with a sample rate of 250 samples per second. This setting is recommend for a typical 1 Hz mechanical test. This reduces the noise from 15-20 microstrain to ~5 - 6 microstrain, or the approximate noise
level with the MTS hydraulics off. This maybe modified or switched off in the digital filter sub-window.

Once all values are set, the user clicks on the Enable toggle and runs the test. The top graph shows all channels in engineering units (y-axis) plotted against the number of iterations on the x-axis. All recorded data may be saved or analyzed at this point.

![Image of LabVIEW Test Window](image)

**Figure 9: LabVIEW Test Window**

To locate recorded values (i.e. strain) corresponding to a given load or stroke, the user reads and edits the sentence in green to meet their particular requirements. For example (input values underlined, outputs bolded) the sentence reads:
When plot 0 with an offset of 0.00 when at a value of -0.5 or at iteration 265
Plot 2 with an offset of 16.22 is at a value of 694.39.

For this case plot 0 is the load and it was properly zeroed indicated by a zero offset. When this is at a value of -0.5kg it is at iteration 265. The offset for plot 2, the first strain channel is calculated and automatically rezeroed. It's corresponding strain value at the noted iteration is calculated from a second order polynomial curve fit. The curve fit is used to further reduce any noise in the system and the 2nd order polynomial allows the subroutine to be used with nonlinear tests. The curve fit is shown in the lower left graph. This sentence is saved to the data file. It further notes that the offsets are calculated from iterations 15 to 30. 0 - 15 is not used, anticipating and filtering error.

If the program defaults to an incorrect iteration (as maybe the case in a cyclic or unloaded test) the user may reduce the scan area in the second lower green box until the desired value is given. The usability of this subroutine allows the user to compare any two inputs at a designed value.

Testing may be repeated by clicking on the Enable toggle button. Termination of the program occurs by closing LabVIEW or clicking on the small red stop sign located on the top left corner of the LabVIEW window.

Reference:

# Appendix 3: Timelines for Surgeries and Calcein Labeling

## Control Group, Unenhanced

<table>
<thead>
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<th>Procedure</th>
<th>Date</th>
<th>Time</th>
<th>Total Time</th>
<th>ILT</th>
<th>OST</th>
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<td>4/19/99 (UNEN1, UNEN2, UNEN3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1st calcein</td>
<td>5/10/99, 9am</td>
<td></td>
<td>28? (disk shows 21 days)</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>2nd calcein</td>
<td>5/14/99, 9am</td>
<td>(use 21)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SAC</td>
<td>5/17/99</td>
<td>(efile, 5/10/99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
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<tr>
<td>1st calcein</td>
<td>6/10/99</td>
<td></td>
<td>21</td>
<td>4</td>
<td>3</td>
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<tr>
<td>2nd calcein</td>
<td>6/14/99</td>
<td></td>
<td></td>
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<td>SAC</td>
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## PepTite™

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<th>OST</th>
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<td>3</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>March 8, 1999 (PT4, PT5, PT6)</td>
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<td>21 days</td>
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## Osteogenic Protein - 1

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## Transforming Growth Factor Beta - 1

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<tr>
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Calcium Sulfate Dihydrate

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Cell Sodding with and without TGF-β1

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Staples removed 8/25/99 14 days
Staples removed 8/30/99 11 days
## Appendix 4: Histomorphometric Data

### Histomorphometric Results of the Unenhanced Group

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<tr>
<th>Rat Name</th>
<th>Slides</th>
<th>Total Bone Area (mm$^2$)</th>
<th>Total Cortical Bone Area (mm$^2$)</th>
<th>New Bone Growth in Quadrant 1 (mm$^2$)</th>
<th>Single/Double label perimeter (mm)</th>
<th>Inter label Distance (mm)</th>
<th>eroded perimeter (mm)</th>
<th># of Osteoids</th>
<th># of Osteoids with label</th>
<th>Ostoid Width (mm)</th>
<th>Interlabel width (mm)</th>
<th>Label per (mm)</th>
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### Unenhanced AVERAGES

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### Comments:
- UNEH1 had unusually high bone growth on the edges of the gauge.
- UNEH5 S2, 5 of the 8 osteoids are next to/in the new bone.
- UNEH3 S5 had very low bone growth.
- UNEH6 had excessive amounts of polysulfone in contact with the bone. Histological results will be ignored.
## Histomorphometric Results of the PepTite2000™ Coated Group

<table>
<thead>
<tr>
<th>Rat Name</th>
<th>Slides</th>
<th>Total Bone Area (mm²)</th>
<th>Total Cortical Bone Area (mm²)</th>
<th>New Bone Growth in Quad 1 (mm²)</th>
<th>Measurements in new Bone only</th>
<th>Measurements in Quadrant 3</th>
<th>Osteoid Width</th>
<th>Distance from label</th>
<th>Interlabel width</th>
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**Comments:**
- PT1 had a fair amount of erosion centered below the gauge.
- In PT1, the new bone growth looked like a porous spongy structure with many interior areas of growth. All of it flowed.
- PT2 had bone growth off of and away from the cortical bone layer. Some was to the side and even up and over the gauge.
- PT3 had the same woven (porous) bone on the sides. Lots of it.
- All gauges had the following descending layers: Gauge CPC tissue extremely large amounts of bone growth old cortical bone layer.
## Histomorphometric Results of the OP-1 Group

<table>
<thead>
<tr>
<th>Rat Name</th>
<th>Slides</th>
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<th>Total Cortical Bone Area (mm²)</th>
<th>New Bone Growth in Quad 1 (mm²)</th>
<th>Measurements in new Bone only</th>
<th># of Osteoids with label</th>
<th># of Osteoids without label</th>
<th>Osteoid Width (mm) with label</th>
<th>Osteoid Width (mm) without label</th>
<th>Interlabel width (mm)</th>
<th>Interlabel perimeter (mm)</th>
<th>Measurements in Quadrant 1</th>
<th># of Osteoids with label</th>
<th># of Osteoids without label</th>
<th>Osteoid Width (mm) with label</th>
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**OP-1 AVERAGES**

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**Comments:**
- OGP1 S3 had 3 of the 6 osteoids on the surface, and S4 had 5 of the 7 on the surface.
- OGP1 has areas of random or "floating" bone growth. Some appears to originate from the CPC and other sections of bone growth appears to have formed without preexisting bone cells.
- OGP2 has a strand of separated bone growth along the bottom side of the bone.
- OGP3 had areas of what looked like osteoclasts and osteoblasts formation zones, but was full of small bead-like black dots.
- OGP6 had large areas of woven bone growing out and on top of the gauge and 19 labeled osteoids in the new bone area.
### Histomorphometric Results of the TGF-β1 Group

<table>
<thead>
<tr>
<th>Rat Name</th>
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<th>Total Bone Area (mm²)</th>
<th>Total Cortical Bone Area (mm²)</th>
<th>New Bone Growth in Quad 1 (mm²)</th>
<th>Measurements in new Bone only</th>
<th>Measurement in Quadrant 1</th>
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<td>Single/double label perimeter (mm)</td>
<td>Inter label distance (mm)</td>
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<td>S3</td>
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**TGF-β1 AVERAGES**

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</table>

**Comments:**
- TB2 had a porous bone growth on the left side below the gauge and extending down.
- TB4 S3 had 3 large osteoids at the surface of diameter, 0.027mm
- TB4 S3 had a large ring of bone growth all the way around the cortical layer approx. 0.1 to 0.2 mm thick.
- TB5 S3: all 13 labeled osteoids were within the new bone growth.
# Histomorphometric Results of the Calcium Sulfate Group

<table>
<thead>
<tr>
<th>Rat Name</th>
<th>Slide</th>
<th>Total Bone Area (mm²)</th>
<th>Total Cortical Bone Area (mm²)</th>
<th>New Bone Growth in Quadrant 1 (mm²)</th>
<th>Single/Double label perimeter (mm)</th>
<th>Inter label perimeter (mm)</th>
<th>Eroded perimeter (mm)</th>
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<th># of Osteoids</th>
<th>Osteoid Width (mm)</th>
<th># of Osteoids</th>
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**Calcium Sulfate AVERAGES**

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*# of Samples* |         | 12    | 12   | 12   | 12   | 8     | 6     | 12    | 12    | 9     | 5     | 4     | 3     |

**Comments:**
- On CA1 there is only bone growth on the outer edges. There is osteoclastic activity into the Calcium Sulfate in these areas.
- CA5: all the labeled osteoids were closer to the bone marrow.
- CA4: Bone growth to one side of the gauge only.
- CA6 had a little strip of new labeled bone in the center of the gauge.
### Histomorphometric Results of the Cell Sodding (with and without TGF-

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<th>Inter label Distance (mm)</th>
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<td>10</td>
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<td>0.0288</td>
<td>0.3037</td>
<td>0.1326</td>
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<td>NA</td>
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</tr>
<tr>
<td></td>
<td>S5</td>
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<td>8.18</td>
<td>0.4248</td>
<td>2.75</td>
<td>0.019</td>
<td>0.087</td>
<td>17</td>
<td>3</td>
<td>0.0089</td>
<td>NA</td>
<td>NA</td>
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#### Cell Sodding with TGF-b AVERAGES

<table>
<thead>
<tr>
<th>Average</th>
<th>Standard Dev.</th>
<th># of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.977</td>
<td>1.039</td>
<td>6</td>
</tr>
<tr>
<td>12.977</td>
<td>1.039</td>
<td>6</td>
</tr>
<tr>
<td>13.32</td>
<td>0.138</td>
<td>6</td>
</tr>
</tbody>
</table>

#### w/o TGF-b AVERAGES

<table>
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<th>Average</th>
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<th># of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.23</td>
<td>0.583</td>
<td>6</td>
</tr>
<tr>
<td>12.23</td>
<td>0.583</td>
<td>6</td>
</tr>
<tr>
<td>13.32</td>
<td>0.138</td>
<td>6</td>
</tr>
</tbody>
</table>

#### Comments:
Appendix 5: Digital Images of Selected Histology

A5.1) Unenhanced: UNCH2, slide #2
A5.2) PepTite™ coated: PT2, slide #6
A5.3) OP-1 enhanced: OGP6, slide #2.
A5.4) TGF-β 1 enhanced: TB5, slide #2.
A5.5) CSD enhanced: CA5, slide #2.
A5.6) Cell Sodded: CS3: slide #3.
A5.7) Cell Sodded with TGF-β1: CS-TB1, slide #4.
Figure A6.1: The unenhanced CPC coated gauge (G) is shown using standard light photomicrography attached to the polysulfone (PS) and then to the CPC particles (C1-C3). New bone (NB) is shown extending from the preexisting cortical bone (CB) to particles C1 and C2. No growth is observed adjacent to particles C3.
Figure A6.2: The PepTiteTM enhanced CPC gauges (G) produced a vascularized tissue (V) layer between the CPC (C) / Polysulfone (PS) and the new bone (NB). A large amount of new bone grew from the preexisting cortical bone (CB).
Figure A6.3: Osteogenic protein-1 enhanced CPC coated gauge (G) is secured to the new bone (NB) through CPC particles (C1-C3). This interface bonds the gauge to the preexisting cortical bone (CB). Bone spurs (S) existed on the section of the OP-1 group. (A) shows areas of forming bone.
Figure A6.4: Transforming growth factor beta-1 enhanced CPC coated gauge (G) is shown attached to the CPC particle (C1, C2) by the polysulfone layer (PS). The new bone (NB) has extended from the preexisting cortical bone (CB) along the entire CPC-bone interface. There is a small amount of erosion (E) of the cortical bone. Osteoclasts (OC) and osteoblasts (OB) are readily observed.
Figure A6.5: The calcium sulfate dihydrate enhanced CPC coated gauge (G) failed to help bond the preexisting cortical bone (CB) to the CPC particles (C1, C2). The calcium sulfate dihydrate (CSD) is visible between the bone and the CPC.
Figure A6.6: The endothelial cell layered CPC coated gauges produced a structured tissue layer (T) rich in capillary vessels (V1, V2) between the CPC particles (C) and the preexisting cortical bone (CB). New bone (NB) and areas of erosion (E) or absorption are visible on the cortical layer, osteoid seam. V1 points to a single nuclei of an endothelia cell forming a capillary. Attached to the interior of the cell wall are possible four immune cells. (PS) polysulfone; (G) gauge; (OS)
Figure A6.7: A combination of transforming growth factor beta-1 and endothelial cells produced areas of new bone (NB) and vascularization (V) between the preexisting cortical bone (CB) and CPC (C) coated strain gauge (G). An air pocket (P) is noted within the (PS) polysulfone. (A) areas of calcifying bone.
Appendix 6: Example of a True Grid Input Deck

title Analysis of strain gauge bonded to SPLIT femur interface $E=1890\,\text{N/mm}^2$
(N,mm,MPa)
c convergence test

c === POST-TRUEGRID MODIFICATIONS TO NIKE3D INPUT FILE ===
c None at this time

c === CONTROL DEFINITIONS ===
nikeopts
  auto c enable automatic timestepping
  nsteps 4 c number of timesteps
delt 0.25 c initial delta-t
mxxs 0.5 c maximum allowable timestep size (negative = must point load
curve #
mnss 0.25 c minimum allowable timestep size
iprt 999 c printout interval (set to 999 for no output, 1 to get interface
forces)
iplt 1 c plotting interval (plot every step to n3plot files)
nsmd bfgs c use bfgs solution method
bwmo off c bandwidth minimization off (not needed when stifcore=3)
sw6 c enable sense switch 6 (verbose output of convergence info)
nbsr 1 c number of steps between stiffness reforms (every step)
nbeo 1 c number of steps between equilibrium iterations (every step)
nibr 7 c max number of equil (bfgs) iterations between stiffness reforms
msrf 10 c maximum number of stiffness reforms per timestep
opnit 50 c optimal number of iterations per timestep
igapfg 1 c output slidesurface gaps as y-velocity for plotting in griz
dctol 0.005 c displacement norm convergence tolerance
ectol 0.001 c energy norm convergence tolerance
anal stat c static analysis
maxmem 0 c store stiffness matrix in core (3 = incore + Boeing-Harwell
solver)
bfgscore c store bfgs vectors in core (always do this - default is out of
core
bfor 10 c brick element formulation (1 = bbar, 10 = 1 plus incore storage
of b
brstif c enable brick element geometric stiffness
lsolver fissle c use fissle linear equation solver (this is default)
nrest 999 c number of steps between restart dumps
nsbrr 0 c number of steps between n3drr dumps (0=every step [manual
wrong?])

;
c === PARAMETERS ===
c parameter a1 [.684/2] c major radius, outer ellipse
  b1 [.470/2] c minor radius, outer ellipse
  a3 [ta2-2\%off23/2] c major radius, innermost ellipse
  c

c === SURFACE DEFINITIONS ===
c define planes for simulating offset gauge mounting
sd 1 plan -1.2155373e+00 6.8936543e+00 1.0000000e+01 .99619 0 .08716
sd 2 plan 1.2155375e+00 6.8936539e+00 1.0000000e+01 .99619 0 .08716
sd 3 plan 4.065 6.988 8.015 -.035 0 .397
sd 4 plan 4.065 6.988 11.985 -.035 0 .397
c === MATERIAL DEFINITIONS ===

1. ... linear elastic bone in tension ...
   nikemats 1 1
   rho 8.54e-5  c density in lbm/in3
   e 20500.   c modulus in N/mm2
   pr 0.3  c poisson's ratio

2. ... linear elastic bone to CPC ...
   nikemats 2 1
   rho 8.54e-5  c density in lbm/in3
   e 1890.0    c modulus in N/mm2
   pr 0.3  c poisson's ratio

3. ... linear elastic CPC/Polysulfone ...
   nikemats 3 1
   rho 8.54e-5  c density in lbm/in3
   e 1890.0    c modulus in N/mm2
   pr 0.3  c poisson's ratio

4. ... linear elastic polysulfone to gauge ...
   nikemats 4 1
   rho 8.54e-5  c density in lbm/in3
   e 1890.0    c modulus in N/mm2
   pr 0.3  c poisson's ratio

5. ... linear elastic gauge ...
   nikemats 5 1
   rho 8.54e-5  c density in lbm/in3
   e 2800.0    c modulus in N/mm2
   pr 0.3  c poisson's ratio

6. ... linear elastic sensing element lower...
   nikemats 6 1
   rho 8.54e-5  c density in lbm/in3
   e 2800.0    c modulus in N/mm2
   pr 0.3  c poisson's ratio

7. ... linear elastic sensing element upper ...
   nikemats 7 1
   rho 8.54e-5  c density in lbm/in3
   e 2800.0    c modulus in N/mm2
   pr 0.3  c poisson's ratio

8. ... linear elastic bone in compression ...
   nikemats 8 1
   rho 8.54e-5  c density in lbm/in3
   e 22360.0   c modulus in N/mm2
pr 0.3  c poisson's ratio
;

c == FEMUR AND GAUGE ==

c master, sensing element CURVED

cyli 1 4 6 8 10 11 12;
ccyli 1 4 5 6 7 8 9;
   1 4 7 10 13;
   1 4 7;

1.3 1.95 2.05 2.15 2.25 2.278 2.306;
c radial coordinates (mm)
-4.97 -2.49 0 2.49 4.97;
c circumferential coordinates
(degrees)
14.1 14.35 14.6;
c axial coordinates (mm)

Set boundaries for element merging
bb 1 1 1 7 1 3 1;
bb 1 1 1 7 5 1 2;
bb 1 5 1 7 5 3 3;
bb 1 1 3 7 5 3 4;

c Assign Material Properties
mti 1 2; ; ;
mti 2 3; ; ;
mti 3 4; ; ;
mti 4 5; ; ;
mti 5 6; ; ;
mti 6 7; ; ;

c interrupt

c slave, gauge outer and bone CURVED

cyli 1 4 6 8 10 11 12;
c radial index progression
   1 4 6 11 12 13 14 15 20 22 25;
c circumferential index progression
   1 11 14 22 23 24 27 29 39;
c axial index progression

1.3 1.95 2.05 2.15 2.25 2.278 2.306;
c radial coordinates (mm)
-90 -43.5 -24.2 -4.97 -2.49 0 2.49 4.97 24.2 43.5 90;
c circumferential coordinates
(degrees)
0 10 12 14.1 14.35 14.6 14.95 15.3 25;
c axial coordinates (mm)

c delete unwanted regions of mesh
dei 4 8; 4 6;  c deletes area for sensing element
dei 2 7; 10 11; ;  c del on side of gauge
dei 2 7; 1 2; ;  c del on side of gauge
dei 2 7; 1 11; 8 9;  c del anterior to gauge
dei 2 7; 1 11; 1 2;  c del posterior to gauge
c radial cir axial ?

c region (rad7 cir71l, axial9), direction, amount
res 1 1 3 7 11 4 3 .75;  c posterior to SE
res 1 1 6 7 11 7 3 1.4;  c anterior to SE
res 1 3 1 7 4 9 2 2 .75;  c side of gauge
res 1 8 1 7 9 9 2 1.25;  c side of gauge
res 1 1 8 7 11 9 3 1.1;  c anterior to gauge
res 1 1 1 7 11 2 3 .95;  c anterior to gauge
trbb 1 4 4 7 4 6 1; c rad cir axial
trbb 1 4 4 7 8 4 2;
trbb 1 8 4 7 8 6 3;
trbb 1 4 6 7 8 6 4;

c Assign Material Properties
mti 1 2; ; 1
mti 2 3; ; 2
mti 3 4; ; 3
mti 4 5; ; 4
mti 5 6; ; 5
mti 6 7; ; 7

c point loads - along -x direction

c region

c load_curve number

c amplitude

c vector in cartesian coords
fci -2;4 8; -9;1 .981 -1 0 0

c end of bone is built-in
bi ; ; -1;dx 1 dy 1 dz 1 rx 1 ry 1 rz 1 ;

c BACK SIDE OF BONE FORM GAUGE

cyli 1 4; c radial index progression
   1 11; c circumferential index progression
   1 11 14 22 23 24 27 29 39; c axial index progression
   1.3 1.95; c radial coordinates (mm)
   90 270; c circumferential coordinates (degrees)
   0 10 12 14.1 14.35 14.6 14.95 15.3 25; c axial coordinates (mm)
res 1 1 3 2 2 4 3 .75; c region (rad7 cir8, axial7), direction, amount
res 1 1 6 2 2 7 3 1.4;
res 1 1 8 2 2 9 3 1.1; c anterior to gauge
res 1 1 11 2 2 2 3 .95; c anterior to gauge

mti 1 2; ; 8

c end of bone is built-in
bi ; ; -1;dx 1 dy 1 dz 1 rx 1 ry 1 rz 1 ;

endpart
c === MERGING ===
merge
c bptol 1 2 0.0
stp .001
c interrupt
c === WRITE NIKE3D DECK ===
nike3d
title Analysis of strain gauge bonded to femur (N,mm,MPa)
write
end
References


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Maoch. Design, Engineering, Data Handbook F47178, 1979 rev. 4th:2;2


