VII. Wound and Fracture Healing

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Electrical Stimulation for Augmentation of Wound Healing

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Sponsor: VA Rehabilitation Research and Development Service

Purpose — We propose to undertake an analysis to determine whether electrical stimulation can provide exogenous influences that augment wound (ulcer) healing. The analysis will consider the augmentation of wound healing resulting from the interaction of electric and/or magnetic fields with cellular structures and the desposition of heat in the damaged tissues. The field interaction analysis is important because of the possible influence of induced forces on the transport across cell membranes and the movement of charged particles within the damaged tissue. These factors can alter the electric potentials and currents associated with normal connective tissue repair processes. The heat deposition analysis is important because a vigorous inflammatory response appears to aid in controlling microbial contaminants and enhancing the rate of wound healing. This aiding effect is related to the diminished blood flow and concomitant reduction in oxygen delivery to the wound area. The lack of sufficient oxygen negatively affects cell replication, neovascularization, collagen synthesis, and leukocyte function.

Progress — The analysis will be conducted by a medical doctor and electromagnetics engineer working collaboratively to elucidate mechanisms by which electric and/or magnetic fields can augment wound healing. The analysis will include blood and its components, hemorrhagic responses, inflammatory responses, fibroplasia, epithelialization, scar maturation, etc.

Enhancement of Wound Healing Using Synthetic Skin, Electrical Stimulation, and Hyperbaric Oxygen Therapy

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Sponsor: Non-designated from Ethicon/Johnson & Johnson

Purpose — The beneficial effects of electrical stimulation in bone repair have been firmly established. Recent literature also documents increased collagen production and epithelial stimulation in the presence of electrical currents which, in early studies, have also demonstrated accelerated wound healing of decubitus ulcers. The mechanism of this action is believed to occur through increased tensile strength and organization of collagen production and cellular maturation. A variety of human and animal studies have shown the bio-compatibility of bovine dried collagen which is used in synthetic skin, already approved for human studies, and also in dietary consumption with commercial sausage casing. In the laboratory at UMDNJ/Rutgers Medical School, a unique crosslinking technique has been chosen that promotes maximal ingrowth of fibroblasts and angiogenesis as well as allowing collagen production.

Progress — The topical hyperbaric oxygen therapy has been routinely used for decubitus ulcer patients at VA Medical Center, Lyons for the past 2 years. This treatment is relatively simple, safe, and inexpensive. It is easily tolerated by patients. It shortens the patient's suffering, eases the nursing effort, and no adverse reactions have been observed. The combination of hyperbaric oxygen and the use of synthetic skin may or may not further accelerate the healing process of the decubitus ulcers, but is an area which needs to be studied.

Optimal wound healing in the proposed clinical circumstances may occur with utilization of both electrical stimulation and collagen sponge dressings. The goal of our studies would be to establish an initial pilot study over a year's duration of approximately 24 patients with use of the electrical stimulation, synthetic skin, and hyperbaric oxygen therapy.

We believe the techniques used, which have previously been approved for human studies and are commercially available, are of minimal risk either in terms of immunogenic or infectious complications. Electrical stimulation has been used in bone healing as well as pain control and similar levels proposed for the study. We anticipate no adverse effects. Careful clinical monitoring along with daily and weekly summaries would terminate any adverse chronically observed effects if they were to develop.

Electrical Stimulation of Mandibular Fractures (Rabbits)

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Sponsor: National Institutes of Health

Purpose — Electrical stimulation of bone growth has been demonstrated in animals, and has been reported to be a clinically useful modality for testing nonunions of long bones. We propose to evaluate the use of electrical osteogenesis in accelerating the healing of mandibular osteotomies. Sixty rabbits will receive a standardized unilateral mandibular osteotomy. In 30 rabbits, 10 microamperes DC will be administered to the fracture site from an implanted power unit using platinum wire electrodes. The remaining animals will be implanted with a dummy power unit. The rabbits will be sacrificed at 2 to 6 weeks post-fracture, and the degree of healing in the stimulated and sham-stimulated animals will be compared histologically using a numerical grading system with blind scoring. If successful, the proposed work would lead to a clinical study and, ultimately, to the possible use of electricity to reduce morbidity in patients with mandibular fractures by decreasing the healing time.

Acceleration of Fracture Healing Electrical Fields (Rabbits, Rats)

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Sponsor: National Institutes of Health

Purpose — The object is to continue research investigating the effects of applied electrical fields on the acceleration of fracture healing in laboratory animals. The proposed research is designed to determine the optimum parameters of applied (exogenous) electricity for accelerating fracture healing, to determine the role of stress generated (endogenous) electricity in fracture healing, and to determine the mechanism of electrically induced osteogenesis at the cell level.

Progress — Methods to be used include the comparison of the osteogenic response of *in vitro* fetal rat tibia and *in vivo* healing rabbit fibula to constant direct current, various pulsed unidirectional electric fields, and various electromagnetic fields. Osteogenesis and bone healing will be evaluated by incorporation of tritiated thymidine, Ca45, and 35S04 as well as maximum resistance to bending as determined by an Instron Testing Machine. Stress generated potentials will be measured in fracture calluses. Origin of stress generated potentials will be evaluated by altering collagen in tendon biochemically. The mechanism of action of electrically induced osteogenesis will be sought by determining pO2 and pH changes in the vicinity of a cathode, changes in surface of cell membrane, mitochondria release of calcium, cellular proliferation and migration, and collagen and proteoglycan biosynthesis and processing.

Electrical Stimulation of Osteogenesis Using Selected Techniques

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Purpose — This initial report is for a research project involving the use of various, selected electrical stimulation techniques for bone growth and repair. The overall goal is to determine an effective stimulation technique to be employed in research that will evaluate the appropriateness of electrical stimulation therapy to remobilize patients with loose prosthetic devices (trauma and irritation present) and patients with osteopenia (trauma and irritation absent). The specific aims are to: 1) define the dose response relationship in magnetic field amplitude for electromagnetic stimulation (EMS) produced by a sinusoidal waveform; 2) determine whether trauma and irritation are required with EMS produced by either a sinusoidal or a square-pulse burst waveform; and 3) compare the efficacy of direct current stimulation (DCS), EMS by a sinusoidal waveform, and EMS by a square-pulse burst waveform in the same animal model.

Throughout this project, the tissue site selected for electrical treatment will be the rabbit tibia medullary canal.

Progress — Three sets of studies will be performed.

1) *Dose Response Relationship in Magnetic Field Amplitude for EMS by Sinusoidal Waveform.* Three groups of 12 animals each will be employed. Group 1: One week of acclimation, bilateral intramedullary rod insertion, and implantation surgery, 4 weeks of healing, 3 weeks of unilateral treatment, sinusoidal EMS A versus no EMS, terminate; Group 2: As in Group 1 but with sinusoidal EMS B versus no EMS; Group 3: As in Group 1 but with sinusoidal EMS C versus no EMS. Sinusoidal EMS A (B or C) is a type of EMS produced by a specific sinusoidal waveform.

2) *Necessity of Trauma and Irritation with EMS by Sinusoidal Waveform or Square-Pulse Burst Waveform.* Two groups of 12 animals each will be employed. Group 4: One week of acclimation, unilateral intramedullary rod insertion, and implantation surgery, 4 weeks of healing, 3 weeks of bilateral treatment with sinusoidal EMS O, terminate; Group 5: As in Group 4 but with square-pulse burst EMS. Sinusoidal EMS O is optimal of sinusoidal EMS A, B, or C. Square-pulse burst EMS is a type of EMS produced by a specific square-pulse burst waveform.

3) *Efficacy of Three Different Electrical Stimulation Techniques in the Same Animal Model.* Three groups of 12 animals each will be employed. Group 6: One week of acclimation, bilateral intramedullary rod insertion, and implantation surgery, 4 weeks of healing, 3 weeks of bilateral treatment, DCS versus sinusoidal EMS O, terminate; Group 7: As in Group 6 but with DCS versus square-pulse burst EMS; Group 8: As in Group 6 but with sinusoidal EMS O versus square-pulse burst EMS.

For each individual study, the biological response will be evaluated by histomorphometry of new bone formation, necrotic tissue, and selected cell types within medullary canal tissue.

Electrical Osteogenesis—Mechanisms and Causes of Failure

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Purpose — The goal of this study is to investigate the mechanisms underlying the electrical stimulation of fracture healing by continuous direct currents. This is accomplished by carrying out several biophysical, biochemical, and biological measurements in a canine experimental model of fracture with and without electrical stimulation.

Progress — The experimental model is an osteotomized canine radius with implanted Ag/Ag Cl electrodes. Electrical field measurements and roentgenograms are obtained weekly. The dogs are sacrificed at 3, 6, and 9 weeks postosteotomy. In selected dogs, revascularization of the fracture is determined by microangiography performed using India ink just before sacrifice. After sacrifice, mechanical rigidity of the radius is measured using a 4-point bending apparatus. Osteogenesis may then be quantitated by histomorphometric and biochemical measurements.

Electrical measurements in a fresh fracture model and a minimum injury model have been used to determine the electrical field pattern in bone imposed by the exogenous direct current signal. Quantitative evaluation of a delayed healing model in canine radius using biomechanical, histomorphometric, and biochemical measurements has been completed. Mechanical rigidity has been found to correlate well with revascularization and callus remodeling in the fresh fracture model. An experiment to determine the optimum values of electric field in the fracture as well as the source current for direct current stimulation of osteogenesis in this model is in progress.

Studies on Factors Affecting Orthopaedic Infections (Human, Hamsters)

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Sponsor: National Institutes of Health

Purpose — This research addresses diagnostic and treatment problems associated with infection in three separate types of orthopaedic patients.

The first type have injuries in which the wound is open and probably contaminated by bacteria. The problem of infections in orthopaedic surgery is of special interest because of the use of foreign bodies in the management of fracture.

One decision involves whether or not to close a wound with sutures. The second decision is whether or not to use internal fixation devices in the presence of a possibly contaminated field. Laboratory procedures that would quickly inform the surgeon of the level of bacterial contamination would aid in these decisions. Scientific evidence of the role of internal fixation in high rates of infection would also help. Quantitative wound culture studies are proposed to answer the question of whether or not there are increased rates of infection with the use of internal fixation.

The second type are individuals who develop infections many months after an implant or total joint insertion. The question addressed in this situation is whether or not the existence of a sensitivity to one or more of the components of the implant might contribute to the likelihood of an infection.

The third type are patients in whom the diagnosis of the etiologic agent is difficult because the appropriate sample for bacteriologic culture is not easy to obtain or because there are antibacterial substances in the specimen. The use of rapid immunologic techniques is proposed to aid in the rapid diagnosis of septic arthritis and osteomyelitis. The studies proposed in this application should aid the orthopaedic surgeon in patient management decisions.

Clinical Study: Effectiveness of EBI Bone Healing System™ in Treating Femoral Head Necrosis

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Purpose — This study seeks to determine the degree of effectiveness of the EBI Bone Healing System™ in the treatment of avascular necrosis (AVN) of the femoral head over a 12-month period.

Progress — The study will be a multicenter (15 to 20) trial. Each center will be asked to submit 25 patients. Patient selection criteria determine that those included in the study are likely to suffer femoral head collapse and subsequent total hip replacement. Each patient will be supplied with the EBI Bone Healing unit. This study will require that patients wear this device for 8 to 10 hours per day (ideally during sleep), 7 days per week for a period of not less than 6 months. Patients with unilateral AVN involvement may be partially weightbearing (3-point gait) with two crutches for the first 3 months. After the first 3 months, patients may bear weight as tolerated. For the first 3 months, patients with bilateral involvement will be instructed to use two crutches with a 4-point gait. After the first 3 months, the patients may bear weight as tolerated. Each patient will be asked to return for follow-up assessment at 3 months, 6 months, and 12 months after starting treatment. AP and lateral X-rays and required forms will be submitted to EBI for each follow-up.

To assess the effectiveness of the EBI pulsed magnetic field in the treatment of avascular necrosis (AVN), patients will be evaluated functionally as well as radiographically: 1) *functional analysis*. Using Charnley modified D'Aubiqne and Postel numerical classification grading system, a 6-point scale for each of these categories (pain, function, and mobility) will be used. For each category a "1" will represent the most severe condition and a "6" will represent the least

severe condition; 2) *radiographic analysis*. Both anterior/posterior and frog lateral X-rays will be used to detect changes in the affected hip. In particular, joint space, collapse, avascular segment containment, and cystic lesions will be noted. Additionally, based on the X-ray evaluation, a classification of the lesion will be made using the Ficat classification.

Assessment of Nutritive Skin Blood Flow

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Sponsor: VA Rehabilitation Research and Development Service

Progress — *Fluorescein Studies*. The evaluation of skin blood flow using low dose intravenous fluorescein in patients with peripheral vascular disease is progressing smoothly. Two manuscripts describing recently completed studies are in preparation. The first one describes a retrospective study of fluorescein delivery to dysvascular limbs. In this study the relative delivery of fluorescein to the limb compared to a reference area was measured. The data were then used retrospectively to develop indices of fluorescein delivery to predict skin viability following amputation. A second manuscript examines prospectively the ability of low dose fluorescein delivery to predict skin viability using the indices developed in the previous study. This prospective evaluation has been found to be accurate and promises to be a reliable clinical test.

Helium Studies. The quantitative evaluation of skin blood flow using a helium flux technique is underway. Over the past 9 months, a helium mass spectrometer has been acquired, set up, and has been operational for the past 3 months. Currently, studies are underway examining helium flux/blood flow in normal volunteers. The effects of skin probe temperature, day to day and subject to subject variation are being determined and this study is virtually complete. Within the next quarter we will compare the helium flux method to radioactive Xenon washout in normal volunteers and compare these data with washout rates of intravenously administered low doses of fluorescein. This study will allow direct comparison of three independent measures of blood flow in the same subject.

Altered Collagen and Wound Metabolism in Non-Healing Diabetic Ulcers

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Purpose — This project is designed to test the hypothesis that potentially correctable metabolic abnormalities may interact with ischemia, neuropathy, and infection to obstruct healing of diabetic ulcers. Subjects include diabetic and nondiabetic patients admitted to the Seattle VA Medical Center Amputation Service who may require lower extremity amputation as a result of diabetes and/or vascular disease. We will test whether recent poor glycemic control, abnormal ascorbic acid metabolism, altered zinc availability to injured tissue, and increased nonenzymatic glycosylation of dermal collagen are associated with and potentially responsible for failure of wound healing that leads to amputation in diabetic individuals.

During the initial project year we have developed biochemical and vascular methods and established protocols for studying potential amputation patients

when they become available. Glycemic control in diabetic patients will be documented by measurements of fasting plasma glucose and glycosylated hemoglobin. Ascorbic acid levels will be determined in samples of plasma from all patients and in leukocytes and dermal tissue from selected patients, measured by high performance liquid chromatography. Zinc levels will be measured in samples of plasma leukocyte and wound tissue by atomic absorption spectrophotometry. Methods have been finalized for extraction of collagen from skin and wound tissue specimens from amputated limbs for subsequent measurement of the extent of glycosylation of collagen fractions. Nutritional status is being evaluated by a panel of laboratory nutritional indicators measured in fasting plasma. Vascular status of diabetic and nondiabetic amputation subjects is being documented by standardized measurements of limb transcutaneous oxygen tension (T_CPO_2) and segmental Doppler blood pressures.

Preliminary Results — During the first 10 months of this study we have studied 40 diabetic individuals who have been candidates for or actually received limb amputations. Twenty-eight nondiabetic amputees with peripheral vascular disease have been enrolled. In addition many of the biochemical and vascular measurements have been standardized in 10 healthy nonsmoking elderly males without diabetes or vascular impairment. Vascular and plasma metabolic measurements have been made in 60 diabetic individuals admitted to the same hospital but who have not had amputations or lower extremity ulcers. Preliminary data analysis confirms that zinc and ascorbic acid metabolism may be abnormal in diabetic and nondiabetic patients who require lower extremity amputation.

Future Plans — During the second year of the project we will continue to enroll subjects in these patient categories. A subgroup of patients will be given experimental iatrogenic microwounds inflicted on the limbs 7 days prior to amputation in order to derive a semiquantitative independent index of cutaneous wound healing to correlate versus the metabolic parameters. We hope to be able to identify metabolic abnormalities that may contribute to limb loss and wound failure in diabetic individuals.

Scientific Basis for a New Protocol in External Fixation of Fractures

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Purpose — This project is intended to advance the state of the art in the external fixation method of fracture management. The proposed strategy will use objective criteria to determine the earliest safe time to begin partial weightbearing and apply electrical stimulation as a preventive measure against delayed union.

Progress — We have developed a method of *in vivo* mechanical testing of fractures in a canine tibia model. This method obtains a stiffness parameter that characterizes the healing fracture by determining the rigidity of the bone-fixator composite as a function of post-fracture time. The influence of electrical stimulation at different, physiologically significant, stages of fracture healing on

this healing curve is investigated experimentally. The stimulation will be applied in two separate phases—middle and late phases of fracture healing. Stimulation of fractures in the early phase (i.e., fresh fractures) will not be attempted since there is no evidence to date of acceleration of healing of fresh fractures by electrical stimulation. Histomorphometric methods will be used to measure new bone formation. Methods for evaluation of the status of healing also will include roentgenography and microangiography and *in vitro* tensile testing of the tibia at sacrifice.

Noninvasive Quantification of Fracture Healing

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Purpose — The goal is to develop a noninvasive method to quantify fracture healing. Such a tool would be useful both clinically, in early diagnosis of delayed unions and clinical healing, and in research to diagnose various rehabilitation protocols.

Progress — Two and one-half years of clinical testing on 28 patients with closed tibia fractures has shown a statistically significant difference between the natural frequencies of the fractured tibia and the contralateral normal tibia. The natural frequencies of the fractured tibia have been shown to increase over time for normal healing, approaching the values of the contralateral normal tibia. Fractured tibia classified as nonunions have been shown to have different natural frequency healing curves compared to normal healing tibia.

The dog has been chosen as a model to find the correlation between the natural frequency of the healing bone and the breaking strength of that bone. This is being accomplished by producing oblique osteotomies in the radii of 21 dogs. Natural frequency testing is being performed weekly on each of the dogs. Two dogs are also being sacrificed weekly and both radii removed. The radii are submitted to Computerized Axial Tomography to find the cross-sectional area of the cortex. The radii are then subjected to torsional loads until the breaking strength is determined.

Morphological and Clinical Studies of Microwounds in Ischemic Human Tissue

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Purpose — The goal of this project is to study in a systematic fashion morphological and clinical features of small standardized wounds created on the lower extremities of patients with severe peripheral vascular disease (PVD) necessitating amputation.

Progress — The wounds are created with a Simplate II bleeding time device under sterile conditions at locations immediately distal to the planned site of amputation and at a variety of clinically and biologically relevant time periods for the study of wound healing. Wounds are examined clinically immediately prior to amputation and then excised from the amputation specimen fixed for morpholog-

ical studies. When normal control subjects are used, standard skin punch biopsies are used to remove the microwounds.

Standard techniques for tissue fixation, sectioning, and evaluation of all samples by light microscopy and of selected samples by transmission and scanning electron microscopy are used. Morphological events of healing are then compared with forearm wounds of young adults from previous studies and leg microwounds from age-matched controls. Transcutaneous oxygen tension ($TcPO^2$) at the site of microwounds, the clinical appearance of the microwounds, the outcome of the amputation, and a variety of risk factors for PVD, such as smoking, diabetes, and hypertension, are also correlated with microwound healing.

Preliminary Results — During the past year, seven additional patients have had microwounds created prior to amputation and ten normal elderly subjects have had microwounds created and biopsied to serve as age-matched controls for the amputation patients. In addition to the morphological studies of the normal and abnormal wound tissue, immunohistochemical studies also have been performed on all normal and selected abnormal wounds. The normal subjects have wound repair that is clearly more advanced than patients undergoing amputation, but it is significantly more delayed than the normal young adult forearm microwounds. The observations of normal elderly leg wound healing are unique and will provide an important point of reference for both past and future investigators regarding wound healing in diabetic and ischemic patients.