Morphological and Clinical Studies of Microwounds in Ischemic Human Tissues

John E. Olerud, M.D.
Veterans Administration Medical Center
Seattle, Washington 98195

Sponsor: Veterans Administration Rehabilitation Research and Development Service

Peripheral vascular disease (PVD) is a major cause of morbidity and mortality in this country. Approximately 30,000 lower extremity amputations are performed annually in the United States, primarily for PVD. Failure of wound healing is one of the principal problems encountered in these patients, either non-healing of leg ulcers or nonhealing of attempted surgical intervention, such as grafting, debridement, or amputation. To date no systematic studies of wound healing have been done in patients with PVD because of the invasive nature of the methods for the study of wound healing and the tenuous nature of the affected extremities.

The method used in this study involves the creation of standardized microwounds on the extremities of individuals awaiting amputation necessitated by PVD. The wounds were made under sterile conditions using a Simlate-II bleeding-time device at sites distal to the planned amputation at predetermined times prior to amputation. The microwounds were excised from the amputation specimens immediately after amputation, placed in Karnovsky's fixative, and embedded in Epon 812 for thin sectioning for light and electron microscopy. Morphological events of dermal and epidermal wound healing were expressed as a ratio of the morphologically apparent wound age divided by the actual wound age and were related to transcutaneous oxygen tension (Tc pO₂), amputation outcome, and clinical factors such as diabetic status.

Microwounds have been studied in 15 subjects with wounds created 7 days prior to amputation. In three of those subjects additional time points were studied at approximately 2, 12, 24, 48, and 72 hours as well as 10, 14, 21, and 28 days prior to amputation. There was no morbidity from the procedure. In general, the events of healing were considerably retarded relative to normal. The dermal events of healing were significantly more retarded than the epidermal events. While the method appears to be a safe and effective means of obtaining relatively standardized wound tissue for study of the healing process in ischemic human tissue, it did not appear to be consistently useful as a predictor of amputation outcome at the selected level.

Future studies include expanding the number of subjects so that more meaningful analysis of clinical factors such as Tc pO₂, diabetic status, and amputation level may be made. Electron microscopy of selected material will be done and immunohistochemical studies of human wounds are planned.

Effect of Stress and Motion on Repair of Hard and Soft Tissues

Savio L-Y. Woo, Ph.D.; Richard D. Coutts, M.D.; Steven R. Garfin; and Wayne H. Akeson, M.D.
Veterans Administration Medical Center
San Diego, California 92161

Sponsor: Veterans Administration Rehabilitation Research and Development Service

This research program is designed to pursue Wolff's Law on tissue healing and remodeling. Although initial immobilization after musculoskeletal trauma is necessary for tissue healing to take place, the deleterious effects of prolonged and/or over-imobilization on the repair process and of the disuse atrophy of the surrounding tissues are also well known. We have chosen two projects as areas of concentration: (i) to study the deleterious effects of large internal fixation plate systems used to immobilize long bone fractures, and (ii) to evaluate the effects of motion and stress on the repair of the medial-collateral ligaments.

In the internal fixation plate study it should be noted that by and large the currently used devices are effective in treatment of long bone fractures. However, direct osteonal bone union healing under rigid fixation must be viewed, conceptually, as a slow and unnatural way to mend fractures. In addition, the large fixation systems shield the underlying healed bone from physiological stresses and can cause atrophy or osteoporosis. At plate removal, refractures of bones are of significant clinical concern. In order to gain a better understanding and to improve the present internal fixation plate systems, we have developed new design criteria, i.e., moderate bending and torsional plate rigidities for adequate early immobilization in order to achieve callus fracture union,
and a low plate axial rigidity to minimize the stress (or strain) shielding of the underlying bone during the post-union remodeling process. A tubular cross-sectional plate, i.e., a flattened stainless steel tube filled with polyethylene, has been made. This plate has been applied to immobilized canine mid-shaft femoral osteotomy models and has been tested against the control plate (rigid plate made out of stainless steel). At 6 and 9 months postosteotomy, there were significant advantages both in terms of mechanical and structural properties of the healed bone underneath the low axial rigidity tubular plate. Morphometric evaluations of bones are in progress.

The next phase of this study will include the longer term (12 and 15 months) experimental animals as well as additional animal groups to study the effects of plate removal (recovery of bone). It is hoped our data will enable the clinician to choose a less rigid plate, e.g., tubular plate, and to select appropriate timing of plate removal on a rational basis.

In the ligament repair study, dogs and rabbits have been used as experimental animals, and a variety of treatment conditions, ranging from rigid immobilization and cage activity to immobilization plus cage activity, have been imposed to determine which set of conditions will enhance the speed and strength of ligament repair. In addition, ligament healing versus surgical repair studies have also been conducted.

The quality of the healing and repaired ligaments are being evaluated by morphological, biomechanical, and biochemical techniques. The advanced biomechanical testing procedures developed in our laboratory permit the studies of the properties of the repair line separately from, and in addition to, the bone-ligament composites. This is of great importance because the responses to the treatment regimens from various elements of the ligament composite, viz., area of repair, area of ligament proximal and distal to repair, and ligament insertion sites are different. In addition, we are measuring the joint laxity quantitatively using our newly developed apparatus. Cyclic and viscoelastic characteristics of ligaments are also being evaluated.

Currently, the healing MCLs of rabbits up to 40 weeks have been studied, but those for the dogs are limited to 6 weeks. Preliminary data indicate that early but limited motion is advantageous, and that the healed ligament is not comparable as the normal (even up to 40 weeks postoperative) in terms of strength, stiffness, and joint laxity. Additional sacrifice periods for dog MCLs up to 26 weeks are in progress. Biochemical measurements of water content, GAG, cell cellularity and collagen typing, and collagen cross-links of repairs and normal sites of the MCLs from sacrificed animals are also in progress. It is hoped that as a result of this data, additional treatment programs including exercise training can be incorporated so that determination of treatment programs on the healing ligaments will be possible.

Transcutaneous Oxygen Tension as Predictor of Wound Healing

Frederick A. Matsen III, M.D.; Ernest M. Burgess, M.D.; and Craig R. Wyss, Ph.D.
Limb Viability Laboratory
University of Washington
Seattle, Washington 98195
Sponsor: Veterans Administration Rehabilitation Research and Development Service

Transcutaneous oxygen tension has been studied for its usefulness in predicting wound healing potential in disvascular limbs. This noninvasive measure of local cutaneous perfusion has been correlated with the severity of peripheral vascular disease as determined by clinical symptoms. The relationship between local cutaneous perfusion, clinical symptoms, and sensory nerve function is currently under investigation.

Segmental transcutaneous PO\textsubscript{2} measurements were made on the limbs of diabetic and nondiabetic patients admitted to the Amputation and Vascular Services at the Seattle Veterans Administration Medical Center. These patients had varying degrees of peripheral vascular disease. Nondiabetic limbs with transcutaneous PO\textsubscript{2} values on the foot or below-the-knee of less than 20 mm Hg were significantly more likely to have rest pain or ulcers, to need an amputation, and to have failure of amputation healing than were those limbs with transcutaneous PO\textsubscript{2} values above 40 mm Hg. Diabetic patients show similar results, although many had ulcers coincident with transcutaneous PO\textsubscript{2} values greater than 40 mm Hg, suggesting factors other than inadequate cutaneous oxygen delivery may result in ulceration of diabetic limbs.

Healing of below-the-knee amputations was correlated with transcutaneous PO\textsubscript{2} values at that level, and amputation healing showed a strong correlation with below-the-knee transcutaneous PO\textsubscript{2}. All patients with below-the-knee transcutaneous PO\textsubscript{2} values above 40 mm Hg healed, 94 percent of those with values between 20 and 40 mm Hg healed, and the healing rate where the transcutaneous PO\textsubscript{2} value was
less than 20 mm Hg was 47 percent. Transcutaneous oxygen tension and laser-doppler velocimetry were compared over heated and unheated skin in which perfusion pressure was reduced by limb elevation. Results indicate that laser-doppler and transcutaneous PO₄₂ measurements do reflect changes in local perfusion pressure when made over warmed skin, but not when made over unwarmed skin.

The multiprobe transcutaneous oxygen tension monitor has allowed simultaneous measurements at eight sites and has enabled spatial mapping of cutaneous perfusion in disvascular limbs before and after amputation. These studies continue to elucidate the value of transcutaneous PO₂ measurement in the clinical assessment of the local circulatory status of skin.

Studies of Factors Affecting Orthopaedic Infections

Katharine Merritt, Ph.D.
University of California
Orthopaedic Research Laboratories
Davis, California 95616

Sponsor: National Institutes of Health
(National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases)

The research being undertaken is designed to investigate problems of infections in orthopaedic patients. One aspect of the study is to better understand factors leading to wound contamination in open fractures and any infections that might follow such contamination. Various factors, including the use of internal fixation devices, methods of irrigation, and time between injury and treatment, as well as underlying diseases, are being compared with infection rates and with level of tissue contamination. An animal model involving open fractures of the hamster femur using an ostectomy saw has been developed to better understand the role of internal fixation in infection. Studies have been done with Staphylococcus aureus and will be done with Proteus and an Anaerobic Streptococcus.

Studies also are being undertaken in the hamster to investigate host responses, such as sensitivity reactions, to the implant on infection rates. Finally, rapid diagnostic techniques are being investigated for diagnosis of osteomyelitis and septic arthritis using blood, joint fluid, and aspirates. These studies should provide information on the management of patients likely to develop infections.

Stimulation of Repair of Cortical Bone Transplants by Implantation of Piezoelectric Materials

George Van B. Cochran, M.D., M.Sc.D.; Marvin W. Johnson, Ph.D.; Wendell Williams, Ph.D.; and Bok Y. Lee, M.D.
Veterans Administration Medical Center
Castle Point, New York 12511

Sponsor: Veterans Administration Rehabilitation Research and Development Service

Introduction—Microampere DC currents have been used extensively to stimulate bone formation. Traditionally, these currents are delivered to implanted electrodes by external power sources or implanted batteries. The purpose of this project is to develop a novel approach to electrical stimulation of bone healing and regeneration (including incorporation of bone transplants) by employing direct delivery of faradic, microampere currents generated during physiologic loading by a piezoelectric material incorporated in a less rigid internal fixation device. From a theoretical standpoint, assuming simple tension or compression on a piezoelectric material, the charge generated is \( Q = A d_{31} S_1 \), where \( A \) is the material area, \( d_{31} \) is the piezoelectric constant, and \( S_1 \) is the stress. The current produced by application and release of load is bipolar, and the magnitude varies with frequency of loading. For 1 Hz loading, with the current output rectified by a full-wave bridge, the average current would be \( 2A d_{31} S_1 \). For PZT-5 (\( d_{31} = 270 \, \text{pC/N} \)), if \( A = 1 \, \text{cm}^2 \) and \( S_1 = 6 \times 10^{-6} \, \text{N/m}^2 \) (\( \equiv 100 \, \text{microstrain} \)), the average current generated would be 0.33 \( \mu \text{A} \).

Methods—As a pilot study in vivo, miniature (36 x 6 mm) replicas of three-hole internal fixation plates were constructed of delrin bonded to a non-electroded PZT-5 ceramic bimorph or a strip of polyvinylidene fluoride (PVDF). Four plates were implanted in each of eight dogs, one active plate fixing a 1.5-cm bone graft in the ulna, another overlaying two 4-mm drill holes in the radius, and two depoled (electrically inactive) plates on the contralateral bones as controls. The entire free surface of the piezoelectric material was in direct contact with bone; plastic screws were used for fixation. Results were assessed histologically after 8 weeks. In a later in vitro experiment, four-hole Kevlar/epoxy composite plates were constructed with a strain-gauged ceramic bimorph incorporated on top. For testing, the plate was attached to a canine femur with four insulated steel screws and the bone loaded in cantilever bending. Similar experiments were conducted with a bimorph bonded to a titanium plate. To generate current, repetitive loads producing 100 mi-
crostrain in the bimorph were applied at 1 and 2 Hz (load duration = 0.5 s; rise time, 0.2 s; plateau, 0.1 s; decay, 0.2 s). Bimorph output (surfaces versus vane) was connected to a full-wave diode rectifier. Average current was determined from voltage drop across a 1 MΩ resistor and a 0.47 μF capacitor shunting the rectifier output.

Results—The in vitro experiments showed that with rectification, continuous DC currents of 0.25 to 0.4 μA could be generated during a series of deformations. Without rectification, polarity varied with sign of the strain and bipolar currents were generated. The earlier in vivo study failed to show significant tissue reactions or differences on bone healing/remodeling beneath the free surface of active versus depoled bimorphs or PVDF.

Discussion and Future Plans—Our limited in vivo study suggested that the charged surface of a piezoelectric material, in itself, has no dramatic effect on osteogenesis, at least in a configuration in which charge density is low and fluctuations with stress are nearly symmetrical. Further work of this type is planned to confirm these negative results. Also, the effect of contact with piezoelectric materials on streaming potentials in underlying bone will be studied.

In the alternate mode, however, the in vitro tests demonstrated the feasibility of designing piezoelectric internal fixation plates that, during normal physical activity, could generate DC currents exceeding the 0.075 μA level identified recently as the minimum for faradic stimulation of osteogenesis. These experiments generated approximately 0.3 μA and an increase by a factor of 10 to 20 could be expected in clinical applications utilizing a proportionally larger area of piezoelectric material subjected to larger strains. This design concept offers considerable potential for research and development with respect to components, configurations, and applications for internal fixation. Materials ranging from titanium to fiber/plastic composites could be selected to enhance mechanical and stress coupling properties, while piezoelectric materials ranging from tissue compatible ceramics to polymers (PVDF) could be fabricated and poled to attain optimal current output and polarity in specific anatomical locations. Conceivably, electrical charge also could be generated by external ultrasonic energy during non-active periods.

In the next stage of this study, instrumented prototype piezoelectric plates will be implanted in dogs and current measurements made during walking. In the final stage, using rabbit and canine models, studies of effect of these piezoelectrically generated currents on bone formation will be made using implanted electrodes connected directly to the rectified plate output.

Effects of Immobilization and Motion in the Injured Tendon

Albert J. Banes
University of North Carolina
Chapel Hill, North Carolina 27514

Sponsor: National Institutes of Health
(National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases)

For the coming support year, the objectives are:
(i) to establish the pattern of tryptic peptides of collagen from tendons of exercised chickens and compare these to the established patterns for immobilized and control birds; (ii) to continue isolation of three major cross-link peptides that elute at the same position for collagens from normal, immobilized, and exercised birds, and to perform amino acid and sequence analysis to assign a location for these peptides within the collagen molecule; (iii) to continue isolation of tendon cells from sheath, synovium, and tendon fibroblasts.

The latter cells from immobilized and exercised birds will be subjected to tension and compression in vitro. The 35S-methionine labeling patterns will be compared to that of the normal birds. Particular attention will be given to the amounts of collagen, actin, and tubulin produced by the cells. The cell culture studies have taken an unusual turn. Populations of cells have been isolated: cells from sheath, synovium, and tendon proper are different in morphology, protein profile, rate of division, and adherence to substrate. On the other hand, tendon fibroblasts from a physically small tendon (flexor profundus) versus a large tendon (flexor to the gastrocnemius) appear to have minor differences in protein synthesis patterns. Consideration of physical stress on tendons led to the creation of a new culture plate that can accommodate up to 200 percent stretch. The latter has been in testing for 10 weeks as of this writing. Tendon cells in culture seem to respond to 0.1 percent compression in vitro by decreasing tubulin and increasing actin concentrations. Experiments with tension are in progress.

With respect to peptide sequencing, it is anticipated having at least three major peptides that contain either reducible or 3-hydroxyypyridinium (HP) cross-links quantitated and compared among the groups in the next 6 months. The absorbence and HP patterns have been completed. Currently being used is a
170-minute, 0.1M PO₄ 2.85 pH, acetonitrole gradient to elute collagen trypic peptides. As little as 10 ug of collagen need to be injected to establish absorbence and fluorescence patterns (HP), but more material is required for radioactivity detection. It is anticipated that the amounts and location of HP cross-link sites in tendon collagen from exercised birds will be different from those of the immobilized and control counterparts. The latter patterns are different; peptide purification and analysis are underway.

**A Study of Intertrochanteric Fracture Fixation Methods**


Bioengineering Unit
Wolfson Centre
Glasgow G4 ONW, Scotland

Sponsor: University of Strathclyde

This summary reports the findings of a three-part pilot project to determine the optimum technique for the fixation of unstable intertrochanteric fractures of the femur in elderly patients with osteoporosis. A total of 121 patients with unstable intertrochanteric fractures were studied. Eighty-two patients were treated with Kuntscher-Y nails (KY) and 39 were treated with a Honey Capener (HC) fixed-angle nail plate. Most complications were seen with the use of the HC nail plate. These were nail breakage (five), bending with loss of reduction (ten), and detachment of the plate (four). Most cases of failure could be related to a significant degree of osteoporosis which was estimated using Singh’s classification.

A similar study using Richards sliding screws was conducted on 86 patients with unstable intertrochanteric fractures. There was a 4.7 percent incidence of complications which was associated with a moderate degree of osteoporosis.

Clinical experience suggests the mechanical superiority of the Richards screws and KY nails but also highlights osteoporosis as a limiting factor in the use of these internal fixation devices.

Mechanical tests were therefore performed on these two implants using an Instron testing machine. In order to investigate the effect of osteoporosis, parallel tests were conducted with the use of adjuvant bone cement.

Paired femoral specimens with a standardized fracture were tested with and without the addition of cement. The degrees of osteoporosis were assessed for the specimens using Singh’s classification. The total strength of the Richards screw fixation ranged...
from 280 N to 1670 N for uncemented specimens and 760 N to 2750 N for cement addition. A similar twofold increase was also found for the KY nail. These results would appear to validate the use of adjuvant bone cement in the repair of fractures in osteoporotic bone.

The Effects of Pulsed Galvanic Currents on the Healing of Soft Tissue Injuries

Neil Spielholz, Ph.D.
New York University Medical Center
Department of Rehabilitation Medicine
New York, New York 10016

Sponsor: National Institute of Handicapped Research

About 2 years ago, a Ph.D. candidate working in this lab reported that pulsed galvanic stimulation seemed to speed the healing of surgically divided Achilles tendons in rats. This past year, using tensile strength as the measure of healing, we confirmed those observations. Equipment limitations, however, precluded our ability to measure tensile strength above 1500 gms. Since this value is well below the load needed to break a normal or fully-healed tendon, we must currently limit our comments about healing to only the early stages. We hope ultimately to be able to appraise the long-term effects as well.

In addition to the tensile strength studies, we accumulated considerable electron microscopic data concerning the ultrastructure of normal tendon and tendon subjected to immobilization by casting or denervation. The observations, which suggest strongly that tendon is far from being an inert structure, hopefully will be reported at an upcoming conference in Boston. Basically, the findings are that immobilization leads to atrophy of collagen fibers. This suggests not only a turnover of collagen but the ability of tendon to meet the demands placed on it in much the same way that bone and muscle do.

Plans for the coming year include attempts to upgrade this project’s materials-testing abilities (which will permit the monitoring of healing in more detail) and to continue with the electron microscope studies.

[See also VI. Biomechanics, A. Joint Studies, 2. Lower Limb, Biomechanics of Anterior Cruciate Repairs]