BIOMEDICAL ENGINEERING
Independent Research Projects
Class of 2002

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BMEN 490-491: Research & Professional Practice

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EFFECTS OF VARIOUS SURFACE COATINGS ON THE DIFFERENTIATION OF BONE MARROW STEM CELLS INTO NEURONS AND THEIR LONG TERM SURVIVAL

Student: Nelson Anderson
Advisor: Dr. Eric A. Nauman

Central nervous system injuries and diseases seriously affect millions of people every year in the US alone. It has been shown that neural stem cells can be very beneficial in the treatment of many of these injuries and diseases. But due to neural stem cells being highly inaccessible, bone marrow stem cells have become a possible substitute since they easily accessible have been shown not only to be able to differentiate into a variety of hematopoietic lineages, but also into many neuronal cell types. In this thesis the effects of various surface coatings on the differentiation and survival of bone marrow stem cells that have been differentiated into neurons will be studied and five long term neuron survival media will be tested for their effectiveness.

In this experiment fresh rat bone marrow stromal cells were cultured on plastic and gelatin. The cells were then differentiated into neuronal cells, with one group being trypsinized and re-plated on polylysine coated wells. The morphology of the differentiated cells was compared on the different substrates and effects of the different substrates and long term media on the survival of the neuronal cells were observed.

The culture medium derived from the work of Woodbury and his colleagues had an exponentially higher number of surviving neuronal cells after five days than any of the other long term media. No difference in survival or neuronal morphology was observed for gelatin and plastic substrates. The effects of the polylysine were inconclusive since the neuronal cells did not regain their neuronal morphology when re-plated onto the polylysine.

Given the ability to take bone marrow stem cells from an organism, differentiate the stem cells into neuronal cells and keep the neuronal cells alive until they can be placed back into the organism at a site of neural injury, we may be able to restore lost function to that organism. Ultimately, that is the goal to which this research strives. We can take the bone marrow stem cells out of the body and differentiate these cells into neuronal cells. We can keep the neuronal cells alive for five days, long enough to introduce them back into the body. Now the stage is set for the next step towards the goal: finding a three dimensional matrix in which the stem cells can be seeded, differentiated and placed back into the body at the injured or diseased area restoring function to the body.

THE EFFECT OF PH ON INTRACELLULAR CALCIUM IN ZENOPUS A6 TOAD BLADDERS

Student: Adanna Anyikam
Advisor: Lee L. Hamm, M.D.

Hypertension is a condition hallmarked by the elevation of blood pressures increasing the incidence of stroke and heart attack and is associated with many of the leading causes of death in America. The collecting ducts of the kidney are responsible for regulating final NaCl excretion, helping to conserve blood volume and blood pressure homeostasis. Although it is known that intracellular calcium levels [Ca2+]i exert positive feedback control on Na+ transport, [Ca2+]i regulation mechanisms are not fully understood. Awayda et al (2) have shown that in Xenopus A6 cells, transport of luminal Na+ through apical channels is pH sensitive by observing that acidification of extracellular pH, pHo, from 7.4 to 5.5, stimulated Na+ transport through apical Na+ channels. It was postulated that such effect was secondary to increases in [Ca2+]i. To gain a greater understanding of the mechanisms governing Na+ transport, and to ultimately determine methods to prevent or remedy hypertensive states and associated conditions, the role of extracellular pH (in vitro) on [Ca2+]i was investigated.

Changes in pH and [Ca2+]i were monitored using fluorescent probes BCECF-AM and Fluo 4, respectively, in Xenopus A6 cells. A positive control, using BCECF-AM and mammalian M-1 cells, measuring changes in pH by the testing software was performed. In addition, a positive control for measurable changes in [Ca2+]i was performed using both OK and A6 cells. Methods acidifying pHo did not alter pHi in Na+-free media. It should be noted that when referring to changes made to pHo, solutions were added or removed to only the apical side of cells. It was observed that decreases in pHo, from pH 7.4 to 6.4, caused increases in [Ca2+]i. Thus it is tentatively confirmed that the effect of pHo on Na+ transport is secondary to the overall regulatory control of [Ca2+]i.

PERIOSTEAL USE IN TENDON-BONE TUNNEL HEALING: AN IN VITRO STUDY

Student: Sarah Cohen
Advisor: Dr. J-K Francis Suh

Reconstruction of injured ligaments has long presented an orthopaedic challenge. Current ligament replacement techniques often involve attachment of a hamstring tendon graft into drilled bone tunnels by screws or endobuttons, which do not readily encourage integration of the graft tissue into the bone. Alternative methods may include the incorporation of periosteal tissue, with its naturally osteogenic cambium layer, at the bone-tendon interface. This project develops an in vitro model for investigation of that interface. Specifically, the hypothesis is that if placed with its cambium layer towards the bone, the periosteal patch will promote significantly greater integration and bone development at the interface than if its fibrous layer faced the bone. From New Zealand White rabbits, cancellous bone from the iliac crest and a periosteal patch from the medial proximal tibia were harvested. The periosteum was cultured for 3 or 6 weeks on iliac trabecular bone plates, with or without marrow and with either the periosteal cambium or fibrous layer facing the bone. Silk sutures were wrapped around the complexes to provide stability.

Histological analysis showed drastically less osteoid present in the periosteum as compared to a cross section of the tissue at time zero. The exception to this observation occurred directly underneath the anchoring suture material where osteoid was present, if not abundant, in all specimens. Many specimens showed close interactions and possible integration at the periosteum-bone interface, though no distinct differences were viewed between fibrous and cambium layer to bone interactions. Cambium layer tissue was observed to direct itself towards bone in one specimen, and in others, bone remained attached to periosteum despite forces that cracked supporting bone. Future experimentation with the addition of mechanical stimulation may lead to a complete understanding of the mechanisms for periosteum-bone tunnel healing. various thicknesses. Transmittance was also measured for agar slices of differing concentrations and thickness. To better compare thickness data, the change in thickness was measured for hippocampal slices during anoxic depolarization. In all both the hippocampal and the agar slices, transmittance decreased as thickness increased. Transmittance also decreased as the duration of hypoxia increased. The change of thickness during anoxic depolarization was very little (1-5mm) compared to the change in thickness needed to significantly lower transmittance. Therefore it is determined that the change in transmittance during depolarization is not primarily due to the change in thickness.

ASSESSING CRIMP LENGTH VARIATION IN PORCINE KNEE LIGAMENTS

Student: Luke C. Dixon
Advisor: Dr. Glen A. Livesay

The knee is among the most complex joints in the body and millions seek medical care for it each year. In fact, the anterior cruciate ligament (ACL), a ligament frequently injured, is unable to heal and an alternative repair is necessary. As need for tissue engineered ligament replacements increases, there is a prerequisite to characterize a functional ligament. Collagen, the structural element of a ligament, exhibits a crimping that may be responsible for the "toe" region in the structural response of a ligament. The crimping is "pulled out" during the initial stages of loading, before it becomes taut during the linear phase of the response. Additionally, past studies have indicated that the peripheral regions of a ligament elongate more than center region. There has been no attempt to determine if the crimping length (period) fluctuates as a function of position along the collagen specimen. 3 ACL and 3 medial collateral ligaments (MCL) specimens were obtained from a porcine knee. Using polarized light microscopy and a computer with frame grabbing capabilities, images were taken along each specimen. Each image was measured 10 times randomly in an image analysis program. For the ACL, the average difference in the proximal and center region was 6.35 μm while the average difference in the center and distal region was 7.35 μm. For the MCL, the average difference in the proximal and center was 10.31 μm and the average difference in the center and distal region was 8.88 μm (for comparison of middle of thirds). These results provide a rational for the additional elongation at the periphery compared to the center of a ligament.

COMPARISON OF THE EFFECTIVENESS OF AN AESTHETIC EXPERIENCE VERSUS TECHNICAL DRAWING IN THE VISUAL TRAINING OF AN ENGINEER

Student: Chris Fussell
Advisor: Dr. Ronald C. Anderson

Even with all the similar technical training engineering students receive, it is not difficult to realize that each individual engineer will view a problem, object, or image differently than his colleagues. The purpose of this study is to compare the drawings of freshmen biomedical engineering students before and after they have either an aesthetic drawing experience or have completed an appropriate number of technical drawing exercises in order to determine which activity will provide them with better drawing and visualization skills that they can use throughout their engineering careers.

The freshmen biomedical engineering students were given two minutes to memorize a drawing and given another eight minutes to reproduce the image to the best of their ability. They were then asked to complete a technical drawing workbook or reproduce the M.C. Escher lithograph entitled Waterfall. The students were also given a short questionnaire to determine their artistic/drawing background and to determine whether they are visual or verbal learners. Upon completion of this assignment, the students were then given another image to memorize for two minutes and another eight minutes to reproduce this image. Due to non-compliance from some students, a small number of subjects completed all the assigned material but no significant differences could be seen in the before and after drawings when comparing the drawings of the students who were assigned the technical drawing workbook and those who were assigned the Escher reproduction. The questionnaire provided valuable information about each student's drawing and visualization background and it also helped to determine that 84% of the students that completed the questionnaire were visual learners. The information obtained from this study will indeed help Dr. Anderson to provide more accurate instruction in the BMEN 203: Drawing and Visualization class. However, further studies over a longer time frame will provide a better

assessment of the types of experiences necessary for engineering students to improve their drawing, visualization, and communication skills.

GLUCOSE CONTROL IN DIABETIC PATIENTS USING A COMPUTER AIDED CARBOHYDRATE COUNTING/INSULIN BOLUS CALCULATOR

Student: George A. Hartel
Advisor: Karen E. Friday, M.D.

Carbohydrate counting combined with an insulin pump or regular insulin dosages can achieve better glucose control in Type 1 diabetic patients. Eating carbohydrates causes an increase in glucose levels in the blood. Blood glucose levels are very controllable with just two simple tools, carbohydrate counting and proper insulin dosages. The problem for diabetics lies with the actual counting of carbohydrates and then calculating the proper insulin bolus dosage. Diabetics often make mistakes when calculating their insulin bolus dosage or even use dosages from previous injections despite a change in the types of food they eat at a particular sitting. This improper dosage can lead to hypoglycemia, hyperglycemia, and even ketoacidosis. More importantly, this consistent fluctuation in patient blood glucose levels can lead to major microvascular complications in the heart, kidney's, and eyes. Our goal is to develop a study to test whether or not using a personal digital assistant (PDA) along with diabetes diet software to count carbohydrates and calculate insulin bolus dosages, will improve blood glucose control in Type 1 diabetic patients.

RESEARCH AND INITIAL DESIGN OF AN ACTIVE LEARNING EXERCISE FOR AN INTRODUCTORY CELL AND TISSUE ENGINEERING CLASS

Student: Jennifer Heddings
Advisor: Dr. Kay C Dee

The purpose of this thesis is to introduce an active learning exercise into the introductory course on cell and tissue engineering at Tulane University. Many courses in the department of Biomedical Engineering at this university are currently being revised to include opportunities for students to be active learners. In this case, active learning refers to any activity for students to engage with the course material, rather than passively listening to a lecture and taking notes. One of the goals of this thesis will be researching and summarizing current literature on the purported benefits of active learning to incorporate an active learning exercise in Dr. Dee's junior level cell and tissue engineering class. Also, a literature review of research of diabetes dealing with xenotransplantion of pancreatic islet cells will be done, with the purpose being the identification and justification of the major educational objectives relevant to that field of research. This area of research has such potential for improving the quality of life for the millions of people affected with this disease that it should be included in college courses on cell and tissue engineering. After becoming familiar with the computer program Visual Basic, an interactive computational model will be developed to reinforce the class lectures relating to transport issues such as encapsulated islet cells and xenotransplantation. This active learning exercise will be bench-tested with a representative group of students and revised according to results of the test. The final goal is the incorporation of this exercise into BMEN 340, Introduction to Cell and Tissue Engineering during the spring 2002 semester.

ANALYSIS OF EEG FROM PATIENTS TREATED WITH TRANSCRANIAL MAGNETIC STIMULATION

Student: Dan Jung
Advisors: Dr. Paul Nunez, Dr. Boutros

Transcranial Magnetic Stimulation (TMS) has become a new research and therapeutic tool in neuropsychiatry. In order to truly understand the effects of TMS on behavior and mood, it is important to know how it affects brain activity. One way to measure the effects of TMS on electrical activity in the brain is using electroencephalography (EEG). EEG records electrical potential from the scalp, which is related to the underlying current sources in the cortex of the brain. The sources in the cortex create an electric field that spreads passively through the brain and skull to the scalp based on the conduction properties of the head. The purpose of this work is to measure quantitatively the effects of TMS using EEG analysis.

Mathematical analysis of EEG signals can reveal relationships between different areas of the brain and how each region responds to TMS. Specifically, amplitude spectra of EEG signals and coherency estimates between channels are two useful quantitative measurements of brain activity from the EEG. These are computed using a Fourier Transform of the original EEG signal.

The results of this study show the changes in coherency before and after treatment. Most of the subjects had overall decreases in coherency, while two subjects showed increases in average coherency. In addition, a few of the subjects showed very little differences before and after treatment.

INVESTIGATIONS INTO THE PROLIFERATION AND DIFFERENTIATION OF HUMAN ADULT STEM CELLS FROM BONE MARROW STROMA

Student: Benjamin Larson
Advisor: Dr. Darwin Prockop

In the following thesis, we have investigated adult stem cell biology as it pertains to in vitro expansion and differentiation. Stem cells expanded in vitro were observed to be highly sensitive to plating density. Stem cell multipotency was observed to be highly dependent on the undifferentiated stem cell morphology. It was observed that stem cells expanded in vitro began as small spindle shaped cells and then transformed into medium spindle shaped cells followed by large flat cells. Differentiation from small or medium cells proved relatively successful, whereas differentiation from large flat cells proved less effective.

The main focus of differentiation in the present study was done with regards to chondrogenesis. During the condrogenesis of stem cells a gradual loss of cells and proliferation potential was observed. The gene expressions of many cartilage related genes were observed to increase over the course of differentiation as assayed by a microarray, providing useful information as to the development process as well as providing further verification of our in vitro chondrogenesis system. Our system of in vitro differentiation was also improved by finding that exogenous bone morphogenic protein, BMP2, further induces chondrogenic differentiation. A protocol was also developed for the differentiation of stem cells to hypertrophic chondrocytes.

USING NONLINEAR DYNAMICS TO ANALYZE SPATIOTEMPORAL DYNAMICS OF REETRANT ACTIVATION IN SIMULATED MYOCARDIUM

Student: Claire Larson
Advisors: Dr. Natalia Trayanova, Dr. James Eason

Introduction: The underlying mechanisms of fibrillation are not completely understood, but the concept of reentry is thought to be involved. In previous studies, transmembrane potential maps of reentrant activation are used to determine electrical characteristics of a simulated model of cardiac tissue, but the pathways have been difficult to follow. We will represent the transmembrane potential distribution in the heart during and after the application of a strong electric shock as maps of a unique variable, the cardiac phase. Using non-linear dynamics, maps are created based on phase trajectories and phase singularity history so that the sequences of events that sustain reentry can be followed more precisely. This study seeks to accomplish the following goals: 1)examine the spatial distribution of phase resetting in the myocardium as a result of a strong electric shock, 2)investigate the spatiotemporal dynamics of the response of the tissue to this resetting, and 3)dynamically track the movement of and interaction between the shock-induced phase singularities.

Methods: Using transmembrane potential maps created by S1-S2 protocol to induce reentry, we created plots based on phase, having axes (Vm(t-?),Vm(t)) with ? being a time embedded delay between two subsequent voltages. We calculated the inverse Arctangent of the angle created between the old voltage, Vm(t-?), of a point and new voltage, Vm(t). Transmembrane potential maps to localize the formation of phase singularities by integrating over a closed area for values of -2? or 2?.

Results and Conclusions: Using swarm plots (SPs) and phase singularity traces, we are able to pinpoint phase resetting due to the shock and the formation of spatially independent reentrant activation sequences that exist in the tissue. Information based on these plots is validated by information provided by transmembrane potential maps. We have developed tools based on non-linear dynamics that will further elucidate specific mechanisms behind reentrant circuits. Swarm plots and phase singularity traces show detailed activation patterns of quatrefoil and figure-of-eight myocardium. We can use these tools to better understand the spatiotemporal dynamics of a reentrant circuit, and understand the role that phase resetting and phase singularity existence play in the initiation and sustenance of fibrillation.

LIGHT TRANSMITTANCE IN RAT HIPPOCAMPAL SLICES

Student: Aaron Lill
Advisor: Dr. Norman Kreisman

Hippocampal slices respond to anoxia by depolarizing. Cells swell and darken during anoxic depolarization. Changes in light transmittance accompany injury and could therefore be used to quantify it. Light transmittance was measured for slices of various injuries (duration of hypoxia) and compared to slices of various thicknesses. Transmittance was also measured for agar slices of differing concentrations and thickness. To better compare thickness data, the change in thickness was measured for hippocampal slices during anoxic depolarization. In all both the hippocampal and the agar slices, transmittance decreased as thickness increased. Transmittance also decreased as the duration of hypoxia increased. The change of thickness during anoxic depolarization was very little (1-5mm) compared to the change in thickness needed to significantly lower transmittance. Therefore it is determined that the change in transmittance during depolarization is not primarily due to the change in thickness.

STRESS-RELAXATION OF EXTRUDED COLLAGEN SCAFFOLDS

Student: Todd Luka
Advisor: Dr. Glen A. Livesay

Currently, there are no optimal reconstructive strategies for anterior cruciate ligament injuries. Researchers seek to reproduce the structure and the function of naturally occurring ligaments in tissue-engineered ligament analogues. Before these analogues can be used in vivo, their viscoelastic properties must be well defined. This study rigorously characterized viscoelastic properties, through stress relaxation testing, on extruded collagen scaffolds (a biological material commonly used for ligament analogue constructs). Collagen fibers were produced using established protocols, generating fibers of consistent dimension and composition. Fibers were chemically crosslinked via carbodiimide coupling. Scaffolds consisting of ten fibers were assembled and clamped to a length of two inches. The stress relaxation tests were completed using an Instron 1122 with computer control. The response curves were typical of those seen in naturally formed collagen relaxation tests, even though a clear difference in strength is apparent. Studies such as the present constitute a critical step toward the eventual in vivo use of collagen-based ligament analogues.

TESTING THE VIABILITYOF VISUAL CHOICE REACTION TESTING AS A METHOD OF DETERMINING THE SEVERITY OFA HEAD INJURY

Student: Colin M. McDonagh
Advisor: Dr. David A. Rice

Head injuries, specifically concussions or mild traumatic brain injury, are a frequent occurrence in sports settings and athletic events, especially contact sports, such as football. Currently, there is no proven safe, effective test for an on field determination of whether an individual has received a concussion, except by a physician trained to do so. The purpose of this study is to determine the degree with which exercise, of varying types, effects reaction time. Multiple choice reaction time has been shown to increase in persons following a head injury.
We measured reaction times using a machine developed to test reaction time using four visual stimuli. Four lights lie atop four buttons. The user holds down the "Ready" button until one of the four lights illuminates. The user then depresses the button underneath the light as fast as they are able. Three subjects were tested both before and after anaerobic and aerobic exercise; four members of the Tulane Rugby Team were also tested both before and after practices on days when there was contact and no contact. One member of the rugby team was tested after he had received a concussion and his results compared to the average reaction times of the other testing subjects.
Moderate aerobic and anaerobic exercises (such as jogging and weightlifting, respectively) caused a general decrease in reaction time among the subjects. However, there was some variability among individuals. Among the rugby players, there was a general decrease in reaction times after having practiced. The rugby player suffering from a concussion had scores that were significantly higher then the average of the other subjects. This leads to the conclusion that multiple choice reaction time might be used as a standardized method for on the field testing of athletes for head injuries. Problems surfaced in the subjects finding it difficult to accurately use the machine following intense exercise. Therefore, possible methods for correcting this are to use simple reaction time in which the subject uses a hand-held button to respond to single stimuli.

IN VITRO TESTING OF TITANIUM DIOXIDE USING AC IMPEDANCE TESTING AND THE STUDY OF SURFACE TOPOGRAPHY

Student: Mohammad Imran Mehmood

Advisor: Dr. Kirk J. Bundy

Titanium based materials are widely used as implants in the human body. The microscopic surface characteristics of these implants are critical for compatibility with human tissue since they influence biological response. One important characteristic of the surface is its topography. A titanium dioxide sample was subjected to in vitro AC impedance testing, which in principle can give insight into surface topography. The data obtained was then fit to a circuit model using mathematical analysis. This model may be capable of representing the surface topography. In the future, this circuit model should be refined using other corrosion measurement methods. Also, surface science techniques should be used to gain a better microscopic understanding of the surface topography to determine the predictive ability of the circuit model. Potential surface analytical techniques to investigate the surface include x-ray photoelectron spectroscopy and ion scattering spectroscopy for determination of the stoichiometry of the top surface layers. Also, Scanning Tunneling Microscopy and Atomic Force Microscopy can be used to study the surface topography. The information obtained from these tests will aid future researchers in developing surface modification procedures for titanium based materials.

DEVELOPMENT AND APPLICATION OF A COMBINED MODELING AND IMAGING METHOD FOR DETERMINING BIOMECHANICAL RESPONSE OF ROLLER COASTER PASSENGERS

Student: Richard Morency

Advisor: Dr. Glen A. Livesay

One of the most popular amusement devices throughout the world today is the roller coaster. As design technology allows the roller coasters to be built that are much bigger, faster, and taller than the roller coasters of the past, care must be

taken to ensure the safety of the riders. The amount of forces placed on the riders can potentially cause injury, especially if the rider experiences a large change in force over a short period of time. Most roller coasters that invert riders use an over the shoulder harness to securely hold the passenger in an upright sitting position inside of the roller coaster car. This effectively fixes the torso of the passenger relative to the car but still allows the head to move freely. However, the head may experience an 'amplification' of accelerations experienced by the torso during the ride; this 'amplification' can be caused by an action similar to whiplash. This project develops an approach for analyzing the forces and accelerations experienced by a passenger in a roller coaster using modeling and imaging. Demonstration of this method was performed for a common design of roller coaster, and velocity and acceleration profiles were calculated for certain track elements. By using the Serret-Frenet equations the amount of tangential and normal accelerations experienced by a passenger were calculated. A basic model of the head and torso was then analyzed to assess whether the head experiences any amplification of the forces and accelerations that are experienced by the torso of the passenger.

ERGONOMIC ANALYSIS OF A CLASSROOM: BOGGS 242

Student: Rubén Alejandro Rodríguez Muñoz
Advisor: Dr. Glen A. Livesay

Ergonomics can be broadly defined as the scientific study of the interaction of humans and their surroundings with the intent to design for 'ease and efficiency' of use. One of the environments students routinely use is the biomedical engineering classroom in Boggs 242. It is desirable to have an environment that is conducive to learning, although numerous complaints have been made regarding uncomfortable chairs and lack of natural lighting. Both these situations may be detrimental to the learning environment and distract students from focusing their

attention on class. The classroom has an efficient amount of artificial lighting, yet recent studies indicate that the lack of natural light in a classroom decreases the retentive capability of the students. The chairs themselves are responsible for lower back pain and reduced flow of blood in the lower extremities. In addition, the chairs suffer from high levels of instability, which lead to them moving back once students are sitting in them. This causes students to be distracted from class, since they have to physically impede the chairs from moving. The overall goal of this thesis is to seek out and filter through applicable codes, (e.g, OSHA, universities and others develops) in regards to classroom design, and conduct measurements of the current design of the classroom. Measurements of the classroom and its environment will be conducted. These measurements (e.g area, volume, wall area and others) are compared to the existent relevant codes. Students will fill a survey where they will rate the room according to what they think needs improvement. A combination of the existing codes and the student's idea will result in a new ergonomic rating system for Boggs 242. Results obtained from the new system will determine what improvements, if any, our engineering

MECHANICAL PROPERTIES OF A NEW TRACHEAL INTUBATION TUBE INTRODUCER

Student: Joseph Olechowski
Advisor: Dr. J-K. Francis Suh

Tracheal tube intubation can be a difficult task. To aid in this life-saving procedure, the Eschmann tracheal tube introducer provides a method to intubate the trachea by locating the trachea through the larynx and then sliding the actual tracheal intubation tube over the guide. Though useful, this device is very expensive and has a short clinical life. This presents a problem for medical physicians in developing countries and the United States alike. This study examines the effectiveness of a new and much less costly tracheal intubation tube

introducer developed by Dr. James Riopelle of New Orleans' Charity Hospital. The use of this Teflon device has shown promising results in tracheal intubation and has been praised by physicians around the country and abroad, but has not been tested and approved for clinical use. This results in a liability for the hospital or clinic that uses the device. Through previous material and mechanical data about Teflon already known through company testing, and the results of a thorough investigation of the mechanical properties of other possible Teflon counterparts, it was determined that perfluoralkoxy (PFA), and not the original Teflon that was initially thought to be the answer, is the ideal replacement for the costly Eschmann device. Though many more tests will have to be performed to verify this result, the future of PFA in this application seems promising.

CARDIORESPIRATORY FITNESS PARAMETERS OF ELITE WOMEN BASKETBALL PLAYERS

Student: Jayme Ormiston
Advisor: Dr. Loretta Quinnan-Wilson

Physiological fitness tests in the lab include measures of running economy (RE), maximal oxygen uptake (VO2 MAX), and Lactate Threshold (LT). These tests measure cardiorespiratory fitness levels, as well as anaerobic and aerobic thresholds.

Since the onset of women's competitive basketball in 1950, the importance of women's basketball as well as the athlete's skill level have been growing without question. The uncertainty comes with trying to determine the fitness levels of these higher-level athletes. Women now, since Title IX, begin their training early in life and maintain a high level for longer than previous generations. Has the increased and prolonged participation of individuals, due to the repercussions of Title IX, increased their cardiorespiratory fitness level? Little research has been done on the physiological fitness level of the players since before Title IX, and it

is unknown if and how much fitness of women athletes has increased since then. Performance variables can also be studied to see if any correlation arises between performance and lab variables.

There are two objectives to this study. One is to define the fitness status of a current women's university basketball team and compare it to the fitness status of women basketball players prior to Title IX. Second, is to determine if a relationship exists between fitness parameters and field test results for a women's elite basketball team.

A current women's university basketball team performed a treadmill graded exercise test, body composition analysis, a timed mile run, vertical jump test, and maximal lifting test. The VO2MAX, LT, percent body fat (%BF), weight, height, mile run time, and vertical jump height were the most pertinent variables. The VO2MAX and size parameters were compared to pre and other post-Title IX players. The post-Title IX players were all taller and heavier than the pre-Title IX players. The current players, even though had an obvious increase in skill, has no improvement of VO2MAX, and had an increase in %BF in addition to the increase in height and weight as compared to pre-Title IX players.

It has been suggested this is due to the anaerobic nature of basketball and the women's game increasing competitiveness, the players are beginning to resemble the men's game, the players need to be bigger and stronger and have more anaerobic capacity. This result also resembles a trend in Americans of the population as a whole becoming bigger, fatter, and less aerobically fit.

LEUCINE METABOLISM IN SMALL-FOR-GESTATIONAL-AGE BABIES

Student: Shawn Ragbir
Advisors: Dr. Kim H. Parker, John Lever

Deaths due to nourishment-related causes accounted for 23% of all fetal deaths in the United States in 1998. This project aims to investigate the behavior of leucine in maternal, uteroplacental and fetal circulation.
Subjects of four various diagnoses bearing clinical significance to fetal nutrition, plus one set of controls (average-for-gestational-age fetuses), were first tested to determine their eligibility for this study. 13-C leucine carbon tracer was administered and blood samples were taken thereafter. Flows were measured using ultrasound and Doppler color mapping of blood velocity. Leucine and keto-isocaproic acid (KIC) concentrations were obtained via mass spectrometry.
Intrauterine growth retarded (IUGR) fetuses consistently showed lower birthweight and fetal blood flow, but also higher KIC concentrations. The umbilical vein, which supplies the fetus with nutrients, was consistently the vessel richest in leucine. Tracer concentration was highest in the umbilical artery.
IUGR is a serious condition that results in significantly decreased fetal weight, possibly caused by reduced fetal and maternal (uterine) blood flow. Keto-isocaproic acid production in the umbilical vein increases, but is significantly higher in IUGR victims. The equations gotten from the compartmental model are not appropriate if left as is to represent concentrations of leucine with respect to time.
We have concluded that IUGR fetuses do not take up maternally originating nutrients efficiently and that some changes to the four-compartment model are necessary to better reflect the time varying changes in concentrations at pregnancy.

PHASE SINGULARITIES AND TERMINATION OF REENTRY

Student: Ashley Schneider
Advisors: Dr. James Eason, Dr. Natalia Trayanova

Introduction: The improved efficacy of biphasic waveforms as compared to monophasic waveforms is a well-known experimental fact. There are observable differences in the virtual electrode polarization (VEP) induced by the two waveforms in the end-shock state. However, the dynamics of reentry termination are not well understood for either waveform. We examine the dose response behavior in the termination of reentry with these waveforms and the creation of postshock phase singularities by biphasic and monophasic defibrillation shocks.
Methods and Results: We constructed a bidomain model representing a simplified left ventricular cross section with fiber architecture, blood cavity, and a perfusing bath. We initiated a spiral wave reentry which completes a single rotation in 80 ms. To terminate the reentry, the tissue was subjected to a strong shock consisting of either a monophasic square wave or a 4 ms/1ms biphasic square wave. The shock is delivered between a catheter in the cavity and an electrode in the bath. We estimated the efficacy for a given shock strength by testing sixteen shock coupling intervals throughout a single rotation of the wavefront. The probability of success of the shock strength is defined as the fraction of the sixteen trial shocks which terminate all reentrant activity within 300 ms. The 50% effective dose (ED50) for the monophasic required 18% less energy than the ED50 for the biphasic. However, at ED95 the two waveforms required similar energy levels. Accordingly, the slope of the dose response curve for the biphasic was steeper than the monophasic. The biphasic waveform was also more likely to elicit a Type II termination of the reentry and induced an average of 20% fewer postshock phase singularities at the ED50 than the monophasic waveform.
Conclusions: Our results introduce for the first time a technique to analyze defibrillation mechanisms and present data describing monophasic and biphasic shocks through the examination of dose response behavior and phase singularity analysis. Further, we compare for the first time the probability of success for both monophasic and biphasic stimuli. Our results using a simplistic two-dimensional model do not match experimental defibrillation results. Additionally, our techniques reveal differences in defibrillation mechanisms between the monophasic and biphasic stimuli.

A QUANTITATIVE EXAMINATION OF THE FORCES ON THE CERVICAL SPINE DURING FOOTBALL TACKLES

Student: Nader A. Shourbaji
Advisor: Dr. David A. Rice

Football evolved to become an inherently violent sport. The technique and the nature of the game became focused on speed and strength. Additionally, equipment underwent a rapid transformation with the advent of new materials technology. Undeniably, both technique and equipment development influenced each other, yielding the high-impact modern game of football. Modern football helmets, with high-tech impact-resistant materials, afford players more protection than ever. The added protection, however, enables players to use their heads as battering rams to block or tackle opponents. This technique is commonly known as spear tackling. As this practice gained popularity, the incidence of cervical spine injuries increased, offsetting the benefits of improved equipment design. After this phenomenon was noted in epidemiological studies, the NCAA and all high school sports associations banned the spear tackle, and began penalizing those players employing it. Following this change in regulations, the number of cervical spine injuries decreased to the relatively low levels of today. Despite these changes in equipment, cervical spine injuries persist in football at all levels. Although several groups have studied the epidemiology or qualitative biomechanics of tackles that lead to injury, nobody to date has published quantitative data characterizing the actual forces to which the head and neck are subject during tackling. We designed a circuit to measure the axial force on the crown of the head using an air pressure sensor tied into a helmet's air bladder. We then developed a calibration curve relating the voltage output of the air pressure sensor to the force the crown of the helmet experiences. Once the output voltage is correlated to the load, the resulting calibration curve can be used to take force measurements from live players in the field.

NEURAL ADULT MARROW STOMAL CELL CULTURE IN COLLAGEN GELS: THE MECHANICAL EFFECTS OF CROSS-LINKING WITH CHONDROITIN-6-SULFATE

Student: Crystal M. Simon
Advisor: Dr. Eric A. Nauman

Spinal Cord Injury (SCI) is defined as any kind of trauma to the spinal cord that results in a loss of function such as mobility or feeling. There is currently no cure for SCI, but a variety of new therapies utilizing bone marrow stromal cell culture have shown promise. The broad objective of this study was to construct a suitable three-dimensional scaffold for the in vitro culturing of neurons that could eventually be transplanted into a transected spinal cord.

Collagen gels were designed that would be suitable predecessors for spinal cord replacements. A proteoglycan-based cross-linker, Ch6SO4 was added to the gel solutions in an attempt to increase the stiffness of the gel. Adult marrow stromal cells were cultured in gels both with and without the cross-linker to determine whether they were able to support neuronal differentiation. The bulk modulus, stiffness, and permeability of both types of gels were then determined and compared.

Collagen gels made with cells showed a significant decrease in size, indicating that the matrix was suitable for cell survival. Cross-linking with chondroitin-6-sulfate was not successful in increasing the stiffness or bulk modulus of the collagen gel, but it did cause the samples to have a higher permeability. Neural morphology was visible in cross-sections of the gel, signifying that differentiation did occur.

The cells in the gels contributed most significantly to the mechanical properties. Therefore, future testing should focus on regulating the stiffness, bulk modulus, and permeability of the collagen gels by varying the cell concentration. With increased optimization and control over this technique, a treatment for SCI may be made available.

USING PERSONAL DIGITAL ASSISTANTS IN CONJUNCTION WITH VIBRATING TECHNOLOGY TO INCREASE MEDICATION ADHERENCE IN THE ELDERLY

Student: Leland Godric Smith
Advisor: Dr. David A. Rice

Prospective memory failure, which is the ability to perform planned actions at appropriate times, prevents many of the elderly from performing their daily tasks. Sophisticated medicine protocols increase the demand on prospective memory and therefore promote a positive feedback cycle in the elderly that promotes medication nonadherence. Medication nonadherence can cause declining health in the elderly and consequently deprive them of the ability to remain independent. Our research uses a Palm based reminding system to combat the problem of Medication nonadherence. Another problem incurred by the elderly is the inability to distinguish between high and low frequency sounds. Therefore, researchers have decided to use a second enunciator to combat this hearing deficiency. The idea is that this second enunciator will stimulate more senses and raise both the degree and timeliness of the response of the elderly to the reminding system. In the study we will determine if using a Palm based reminding system accompanied by an attachable vibrating unit will prove effective in prospective memory failure.

PHOSPHORYLATION DEPENDENCE OF THE FUNCTION OF GABA RECEPTORS IN RAT HYPOTHALAMIC NEUROENDOCRINE CELLS

Student: Hoang Tran

Advisor: Dr. Jeff Tasker

The hypothalamic supraoptic nucleus (SON) contains magnocellular neuroendocrine neurons, which secrete oxytocin and vasopressin, hormones which regulate female reproductive functions and body water content. The release of these hormones is controlled by the electrical activity of the neurons. The neurons receive both excitatory and inhibitory synaptic inputs from other neurons. In the SON, as in the brain in general, the main inhibitory neurotransmitter is GABA. GABA exerts its effects by binding to postsynaptic GABA receptors. It has been shown that currents evoked by exogenous GABA decrease in amplitude over time, or run down, if ATP is depleted from the cell interior, but spontaneous synaptic GABA-mediated inhibitory postsynaptic currents (IPSCs) do not run down, or decrease in size over time, even when intracellular phosphate sources were depleted by intracellular perfusion of a patch solution lacking ATP and GTP. This suggests that constitutive phosphorylation is not required for synaptic GABAA receptor function in the SON. Using whole-cell patch-clamp electrophysiology, we found that the rundown of IPSCs caused by action-potential induced release of GABA is not dependent on phosphorylation. We also found that blocking kinase activity causes a rundown in exogenous GABA mediated current, even with ATP present in the cell, consistent with a dependence on phosphorylation. This difference in phosphorylation dependence may be due to the action of GABA on two distinct populations of receptors.

LIPID ANALYSIS OF THE TEAR FILM LAYER USING CAPILLARY ELECTROPHORESIS

Student: Michael Valetutti
Advisor: Dr. Jean T. Jacob

Dry eyes can be summarized as a constant pain from irritation in the eye and a sandy or gritty sensation and are defined as an insufficiency, qualitative or quantitative, of tear fluid that results in nonwetting of the eye and instability of the tear film. It has been hypothesized that when the lipid content within the tear is disturbed due to disease, hormones, drugs or environment the tear film is destabilized. The tear film is a micro-multilayer aqueous structure that depends upon its lipid content to maintain its delicate structure and reduce evaporation. However, in the normal resting eye the tear film is approximately 2 microliters in volume. It has not been possible in the past to completely profile the tear lipid content do to constraints in analytical methods. Through the use of a new technology, mainly capillary electrophoresis we will be capable of determining the content of the natural eye tear film. Capillary Electrophoresis is an analytic technique that is able to take small amounts of sample volume and separate them in small amounts of time at a relatively low cost. Samples from normal patients were taken only after a base line reading of possible lipids is found using samples of Lecithin and other standard phospholipids. The peaks from the Lecithin samples were compared to the samples taken from normal patients. Some peaks show to have similar migration times and similar amplitudes. These peaks will be fraction collected out and run using Thin Layer Chromatography to confirm that they are the same molecules.

EPITHELIALIZATION OF POLYMERS IN CORNEA ORGAN CULTURE

Student: Christopher Vandenberg
Advisor: Dr. Jean T. Jacob

Attempts to augment and replace the cornea with synthetic materials have been met with limited success, primarily because these materials have been unable to support and maintain a normal, stratified epithelium. The fundamental problem is

that the host epithelial cells do not recognize the polymer surface as stroma or "self" and, therefore, a degree of foreign body response from the immune system is present throughout the life of the implant causing rejection by the body. De-epithelialized rabbit corneas in organ culture re-epithelialize within 2-4 days in the laboratory, but polymer surfaces implanted in rabbit corneas in organ culture have been reported to take up to 3 weeks for epithelialization. This delay in the re-epithelialization creates a problem since without epithelia present the normal functions of the cornea are severely compromised. The goal of this project is to investigate whether the re-epithelialization rate of novel surface modified polymers.Contact lens shaped test surfaces modified with tethered proteins were implanted into the cornea of New Zealand white rabbits. Immediately after implantation, the corneas were removed and placed in organ culture. The rate of epithelialization was determined by the length of time required to close the defect. The defect was considered to be the de-epithelialized area. The size of the defect was determined by staining the cornea with fluorescein and taking photographs every other day for 15 days or until the defect is healed. The photographs were digitized using a scanner and analyzed to determine the area of the defect.
While the surgical procedure allowed for the defect to be made, the lenses possessed insufficient tensile strength to be implanted successfully. This issue prohibited the analysis of the rate of healing of the epithelium over the lenses containing the tethered proteins. The control de-epithelialized corneas healed in between 3-5 days.
It has been determined that the tensile strength of the lenses needs to be increased in order to implant the lenses by a reformulation of the composition of these lenses. The strengths should then be tested and compared to that of the original lenses and normal lenses.

DETERMINATION OF CORNEAL EPITHELIAL CELL ADHESION STRENGTH TO NOVEL POLYMER SURFACES USING A JET IMPINGEMENT TECHNIQUE

Student: Chris Wallace
Advisor: Dr. Jean T. Jacob

Millions of Americans today suffer from vision problems due to damage to or irregularities of the cornea. Artificial materials to aid the keratoplastic procedures used to treat these corneal defects have been under development for many years; although much progress has been made recently, the optimal material has yet to be achieved (Wang et al., 1999). The development of a material that allows corneal epithelial cells to attach and form a normal epithelial structure is the ultimate goal. A key step in identifying such a material is to identify the surface that allows the strongest bond to form between the epithelial cells and the surface. Many techniques to determine cellular adhesion strength to biomaterial surfaces have been described, including peel tests and ultrasonic methods. In this study, we used a jet impingement technique to study the adhesion strength of primary rabbit corneal epithelial cells to specifically surface-modified biomaterials.
Four different protein and/or peptide surface-modifications to the hydrogel were investigated: one grafted with laminin, one grafted with fibronectin, another grafted with 50% laminin and 50% fibronectin, and finally one grafted with fibronectin promoting peptide (FAP). Control samples were tested on glass surfaces with each cell culture to account for culture differences. Each type of surface-modified hydrogel was tested in replicates of 12. Primary rabbit corneal epithelial cells were isolated based on techniques described by Stocker et al (1958) and Gipson and Grill (1982). The isolated cells were seeded onto the protein and/or peptide surface-modified hydrogel samples within 35-mm wells of a 6-well plate and placed in an incubator at 37°C with 5.0% CO2 for 15 days, at which time confluence was achieved. The culture plates were removed from the incubator and subjected to a submerged, perpendicular laminar jet of 37° C phosphate buffer solution for 30 seconds at a Reynolds number of 2000. Immediately after impingement, the remaining cells were fixed and stained with Coomassie blue to allow easy definition of the lesions. Using the radius of the lesions along with the stress vs. radial distance calibration curves published by Deshpande and Vaishnav (1982), the shear stresses generated by the flow required to erode the cells at the perimeter of the lesions were calculated.

ACOUSTIC PROPERTIES OF NORMAL AND OSTEOARTHRITIC HUMAN ARTICULAR CARTILAGE

Student: Torrence D. J. Welch
Advisor: Dr. J-K Francis Suh

Articular cartilage plays an important role in allowing a smooth articulating motion while minimizing the mechanical impact at the joints during daily activity. The most common of orthopaedic diseases, degenerative joint disease (DJD) causes gradual deterioration of the mechanical strength of articular cartilage through wear and tear, resulting in joint pain and discomfort. The alteration of the mechanical properties of animal articular cartilage specimens during the course of pathological degeneration has been studied in depth, but ample data for actual human tissues at various disease stages are not currently available. Knowledge of these properties will provide insight to the currently unknown mechanism of articular cartilage degeneration.

It is hypothesized that osteoarthritis alters the attenuation of ultrasound waves through the affected cartilage. The objective of this study is to develop a relationship between the acoustic and mechanical properties of degenerative cartilage specimens. This relationship can be then used to evaluate the severity of osteoarthritis non-invasively. In the current project, the speed of ultrasound waves was measured through articular cartilage specimens collected from patients of varying stages of osteoarthritis and compared to mechanical data from the same specimens. The results of these tests were analyzed to obtain the correlations taken from tissues exhibiting various degrees of disease severity.

ELECTROGRAPHIC SEIZURES AND NEW RECURRENT EXCITATORY CIRCUITS IN THEDTATE GYRUS OF HIPPOCAMPAL SLICES FROM PILOCARPINE TREATED MICE

Student: Ronald Winokur
Advisor: Dr. Bret Smith

Seizures are an abnormal, excessive, paroxysmal, synchronous discharge in a group of neurons that may result in symptoms such as jerking of a limb, or interruption of normal behavior and brief amnesia. Epilepsy is a chronic condition in which spontaneous seizures occur repeatedly. In the particular case of temporal lobe epilepsy, the neuron loss and mossy fiber sprouting that occurs contributes to seizure generation. These characteristics of temporal lobe epilepsy have been modeled using a kainate-treated rat and can be modeled by treating mice with pilocarpine, a muscarinic agonist for acetylcholine that induces stereotypical temporal lobe epilepsy (TLE). For several hours following injection, the mice have generalized seizures and eventually develop spontaneous seizures. Timm's staining has revealed that robust mossy fiber sprouting occurs in pilocarpine-treated mice that developed status epilepticus. Consequently, electrophysiological recordings using antidromic electrical stimulation and glutamate photolysis have shown that pilocarpine-treated, SE mice with robust Timm's staining (mossy fiber sprouting) exhibit an increased excitability. The majority of pilocarpine-treated, SE mice had a population spike followed by a long DC shift, while controls and pilocarpine-treated mice that did not reach status epilepticus only had one population spike. This result correlated with previous studies performed in rats. Addition of the NMDA receptor antagonist, AP-5, and the AMPA/kainate receptor antagonist, CNQX, were found to block the secondary response of SE animals together and on their own. These results indicate that mossy fiber sprouting in the dentate gyrus is related to new synaptic circuits being formed in mice. Therefore, robust mossy fiber sprouting correlates to increased excitability and seizure susceptibility.

STRENGTH OF ADHESION OF LUNG EPITHELIAL CELLS

Student: Jadrien Young
Advisor: Dr. Kay C Dee

Infants that are born prematurely often lack adequate amounts of pulmonary surfactant. Surfactant is essential in lowering the surface tension within the lungs and a deficiency in this biochemical causes the airways to collapse. The most common treatment for this malady is mechanical ventilation to reopen the airways. We hypothesize that the high pressures associated with mechanical ventilation causes damage to the epithelial lining of the airways. The aim of our laboratory is to create model systems of airway reopening. Knowing the strength of epithelial cell adhesion to various substrates will allow us to design future studies (utilizing fluid shear stresses and air bubble passages over cell layers) within feasible parameters.

The goal of this project was to compare cell adhesion characteristics of freshly isolated type II rat alveolar epithelial cells to those of a commercially-available, transformed rat lung cell line (L2 cells). The study analyzed the cell types at both six-hour and three-day intervals to assess the affects of time on adhesion. To examine these characteristics, subconfluent layers of the two cell types were cultured on glass coverslips in both the presence and the absence of serum proteins. Microjet impingement was then used to create lesions on the surface. The remaining cell layers were fixed with formalin, stained with Coomasie Blue, and analyzed using Optimas imaging software. The average lesion radius was correlated to a wall fluid shear stress on the coverslip, using published correlations. The maximal wall shear stress withstood by the cells on the coverslips is a measure of the strength of cell adhesion to that substrate.

This study showed that between six hours and three days after seeding, the average shear stress that the L2 cells could withstand decreased and that the cells adhered more strongly in the absence of serum proteins. Results that could be correlated with previously published data could not be obtained with the freshly isolated alveolar type II cells. The results of this study suggest that the transformed cell line may be an acceptable experimental substitute for normal rat lung cells. However, further studies are needed to validate the comparison. Validation of the interchangeability will help us compare the potential contributions of fluid shear to that of other hydrodynamic forces when investigating epithelium denudation due to air bubble progression.

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