Summer 2013

Enhancing Global Research and Education in STEM at Spelman College: Abstracts 2013

Spelman College G-STEM

Summer 2013

G-STEM Cohort

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Fall 2013

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Evaluation of *In Vitro* Organoid Crypt System and Contribution of Fibroblast-Derived Wnt and R Spondin to Stem Cell Niche and Crypt Maintenance

Written by: Charmiah Amie

The goal of the present study was to optimize protocol of murine intestinal crypt 3D culture in preparation for future studies involving 3D co-culture with murine cancer cells, and ultimately with human tissue cultures. More specifically, this study analyzed the role of fibroblast cells in epithelial cell physiology and in stem cell maintenece, as well as investigated the contribution of fibroblasts to the development of inflammation induced cancer. Finding out which signaling ligands are donated by fibroblasts is helpful in assessing the potential success of future stem cell niche studies and contributes to the better understanding of tumor microenviroments. The fibroblast role in stem cell support was evaluated as well as the Wnt ligands which they donate to the system. Wnt signaling controls many aspects of intestinal physiology including crypt development and proliferation. 3D culture systems were used for crypt culture and 2D monolayer culture for fibroblasts. Transgenic mouse models of esophageal adenocarcinoma (L2-IL-1b mice) were used to conduct *in vivo* and *in vitro* experiments. RNA isolation and Reverse transcription followed by Real Time-PCR were essential in verifying gene expression and primer optimization. Fibroblast from intestinal cultures showed that fibroblasts expressed ligands Wnt 2, Wnt 3, and Wnt 4 on a RNA level. In contrast to fibroblast from normal tissue, Carcinoma Associated Fibroblasts (CAF's) express Rspondins 1, 2, and 3. H&E staining showed that the crypts present in the mouse models were successfully harvested and cultured. These results indicate that the system is effective. It was also concluded that fibroblasts do indeed express key molecules for stem cell maintenance and they play a role in the intestinal stem cell niche, especially in conjunction with Rspondins. Identification of the genes highly expressed in colon crypts allows for further identification of what supports colon carcinogenesis and potentially other gastrointestinal tumors.

Catalytic Test of Pt Deposited on ZnO Doped with Al, Ce and Zr on Valorization of Glycerol

Written by: Kevona Belcher

The selective reduction of glycerol to 1,3-propanediol was studied using an array mixed mineral catalyst. Biodiesel is an environmentally friendly product that derives from waste cooking oil, non-edible oils and algae oils. It is produced through the process of transesterification that results in glycerol as a byproduct; in many Europe countries, it is seen as undesirable. For approximately every 9 Kg of produced biodiesel there is 1 Kg of glycerol produced. Although glycerol finds several industrial applications as additive/starting material in many products including cosmetics and pharmaceuticals; unfortunately the demand for glycerol has been less than the amount of glycerol currently produced. Disposal of the excess glycerol by burning leave astounding negative effects on the environment. In order to overcome these shortcomings glycerol can be converted into a more economically desired 1, 3-propanediol. The plan is to use Pt deposited on various ZnO supports (ZnAlO, ZnOME, ZnCeO, ZnZrO, and ZnOcomer Aldrich) as a catalyst for the conversion of glycerol to 1,3 PDO through the process of hydrogenolysis. The catalytic reduction of glycerol was performed in a metal reactor under reducing conditions using hydrogen (or nitrogen) under pressure. The results shows that glycerol is converted to hydroxyacetone (acetol) and 1,2 PDO. The final reaction mixture was analyzed by GC-FID, TPR, EDAX, TPD, XRD, and TEM. The GC-FID results found formation of 1,2-propanediol, glycerol, and acetol with the highest conversion rate being 40%.

Tumor Necrosis Factor and Tumor Necrosis Factor Receptors in Coelacanth genes

Written by: Kierra Brown

Protein superfamilies incorporate any of a group of proteins having similar structure and functionality which descend from the same ancestral gene. The purpose of this study were to investigate the Tumor Necrosis Factor, TNF, superfamily composed of Tumor Necrosis Factor, TNF, and Tumor Necrosis Factor Receptors, TNFR by utilizing bioinformatics software. In this study, protein families were characterized by analyzing the similarities and differences between groups in the TNF and superfamily. The three species used in this study were Coelacanth, Fugu, and Homo sapiens. Bioinformatics techniques were utilized to analyze genes and characteristics of different organisms. A computer database, ENSEMBL, was used to collect data for the Coelacanth, Fugu, and Human TNF and TNFR proteins. ENSEMBL browser provides a variety of genomes with complete explanations using an automated genome annotation system. After determining whether the protein was the TNF or TNF receptor subfamily different groups were analyzed to determine similarities and inconsistencies in each group. Several genes were hypothesized to be artifacts or pseudo genes after analyzing the collected data. Computational tools or gene prediction tools mistake similar proteins that are not functionally related so they require manual manipulation to verify actual genes and not artifacts. The South African Bioinformatics Institute, SANBI, will conduct further analysis to verify all member of the TNF superfamily. By producing a phylogenetic tree, TNF superfamily can be further analyzed and its function in different organisms understood.

Analysis of C-type Lectin – Like Domains of the African Coelacanth Genome

Written by: Niwa Coleman

A modern fish known as the African Coelacanth, latimeria chalumnae or the “living fossil” is seen to closely resembles the fossilized skeletons of its ancient 300-million-year-old relatives. One important characteristic noted about the coelacanth was its lack of Immunoblobin-M (IgM). One possible link to this observation may be due to the role of the lectin super family for immunity. Recent gene duplication events were found in the C-type lectin protein families of the coelacanth. The superfamily of protein containing lectins known for their immunity abilities are referred to as C-type lectin –like domains (CTLD). CTLD are a large group of extracellular metazoan, proteins with diverse roles. The ensembl database was used to search for information about the various Celocanth genes, so that the genes could be manually curate through annotation. A database search was done for the 81 C-type lectin genes found within the latimeria chalumnae (L.C.) to be compared to 10 C-type lectin genes found in the takifugu rubripes (T.R.) and 10 found in homo sapiens (H.S.). In order to compare the Latimeria chalumnae (L.C.) genomes, an analysis of the orthologous groups (OG) number of exons, superfamily domain, print domain, start/stop location, protein length and split variants was examined. It is noted that there is a conserved number of exons within the L.C. to be 3-7. The typical splice variants for these genes are one and the average protein length is a 100-1000aa. The majority of the print domains for the Latimeria chalumnae are seen to be Antifreezell. The large abundance of these antifrezell proteins are not only seen in the L.C., but also the Takigufu rubripes as well. The orthologous group of this organism were seen to have a much higher splice variants and higher locations start and stop locations. It is also noted that print domain of the L.C. was not available for a large amount of the genes. The domain of these genes may have slightly evolved, yet still retains the overall structure to the c-type lectin. In the future, the Latimeria Chalummnae C-type Lectin genes will undergo BLASTing to be further examined through a complete sequence alignmentA phylogenetic tree will be constructed to further understand the C-type Lectin genes within organism such as the Latimeria Chalummnae.

Analyzing In Vitro the Mechanisms of Trastuzumab in Breast Cancer Cells

Written by: Jasmeka Colvin

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths. Breast cancer develops from tumors located in the mammary glands. There are three types of breast cancer: Luminal A and Luminal B, Triple Negative/Basal Like, and HER2. The targeted form, expressed in 20-30% of breast cancers, involves overexpression of the HER2 receptor. HER2 can only be activated by itself and other forms of the receptor therefore it has to be in close proximity with other forms of itself. Trastuzumab, also known as Herceptin, is a monoclonal antibody that binds to HER2 and acts by disrupting the downstream proteins that function in proliferation involved in the HER2 signaling pathway. We aimed to determine the effects of Trastuzumab on HER2 receptors and other proteins in BT474 cells in order to better understand the mechanisms behind the drug to improve recovery rates from this disease. Using cell culture and lysate with BT474 cells in DMEM media, Polyacrylamide Western Blot analysis, cell cycle analysis by DNA flow cytometry, and MTT cell proliferation assays, we assessed the overall effect of Trastuzumab. Our results showed in some instances, such as in those involving pHER2, HER3 and Erk ½, an apparent difference between the control and the treated samples can be seen at approximately 4 days, while in others the effect can be seen either immediately or not after 4 days. In addition, in Trastuzumab treated cells, there is an arrest of BT474 cells in the G1 stage of the cell cycle compared to control. In conclusion, Trastuzumab is a slow acting drug whose proliferative effects differ in regards to time as the protein of interest differs. Moving forward, more proliferative proteins may be analyzed and they can be exposed to Trastuzumab over a wider range of time. Results from this study can better aid in our understanding of breast cancer biology and pharmacologic therapies aimed at inducing apoptosis.

Red Blood Cell Ageing and the Ability of their Microparticles to Protect Human Endothelial Cells from Oxidative Stress

Written by: Llewellyn Delsarte

Red blood cells (RBCs) have various roles that are important in making sure that the body functions properly. They are responsible for delivering oxygen and removing carbon dioxide and other waste gases to and from the body. Studies have shown that storing blood for extended amounts of time leads to more oxygen entering the blood, causing oxidative stress and also formation of microparticles. Small in size (<1 micron), microparticles are membrane vesicles that are released by RBCs and other types of cells such as platelets, leukocytes, and endothelial cells. It has been shown that microparticles are increased in the plasma of patients with cardiovascular disease, however conflicting evidence has lead to the idea that the increased release of microparticles may be harmful to patient care. Other research findings suggest that microparticles derived from RBCs are actually protective against pro-inflammatory signals in endothelial cells. The aim of this project was to test the hypothesis that microparticles from younger red blood cells protect against oxidative stress signals in human endothelial cells, and as they age, if they enhance the oxidative stress response.

The primary research methods used for this study were the staining of RBC membranes with antibodies and analysis with a flow cytometer, measuring reactive oxygen species production through reactive oxygen species (ROS) assays, and observing the structural changes of the red blood cells using bright field microscopy. Results suggested that there was only an observed increase in the microparticle events among the RBC samples that contained only calcium ionophore. The other data for the RBC samples do not seem to support the initial hypothesis that was made. Further research needs to be completed to prove whether microparticles contain protective properties or if they contribute to cardiovascular diseases.

Detection of Salmonella using Nanoparticle-Based Hybridization Probes

Written by: Kameron Duncan

Fluorescence In Situ Hybridization (FISH) is a cytogenetic technique used to detect and localize the specific DNA sequences on chromosomes. FISH uses fluorescent probes that bind to the region of the chromosome where they share a high degree of sequence complementarities. Fluorescence microscopy is then used to find out where the fluorescent probe is bound to the chromosomes. In the given study, FISH is a protocol used to detect and localize ribosomal RNA targets of salmonella cells using florophore-tagged oligonucleotide DNA probes. Once detected, the DNA probes hybridise to their targeted rRNA sequences within each bacteria cell. Often times, the bacterial cell walls are permeabilised to increase hybridization probe uptake. The danger in permeabilisation is that it may cause cell burst or apoptosis. Consequently, nanoparticles known as happyfect, were studied to assist in the DNA probe delivery process. It was hypothesized that the facilitation of oligo probe delivery, uptake and hybridization using nanoparticles would increase fluorescent signaling. Furthermore, the washing process was predicted to reduce fluorescent background; increasing the signal to noise ratio. Various washing conditions were optimized such as: pH of buffer, salt (NaCl) concentration, temperature, time, Sodium Dodecyl Sulfate (SDS) concentration and Mueller Hinton Broth (MHB) amounts. Microscopic results proved that happyfect-facilitated probe uptake was successful and hybridization occurred best at two hours. The washing buffer was optimized at pH 7.2, 0.7M NaCl, 0.01% SDS, 50◦C and for 30 minutes; minimizing excess fluorescent background. In summation, nanoparticles known as happyfect were used to increase probe delivery into Salmonella cells.

Owl Butterfly Oviposition Patterns on Banana Leaves Santa Elena – Monteverde, Costa Rica

Written by: Tayler Elam

While butterfly eggs are more frequently found to be laid singly, a few species seem to oviposit in clusters, including the owl butterfly *Caligo memnon*. Though *C. memnon* egg clusters are relatively small, between five to ten eggs, groups of clusters occur that appear clumped. Number and nearest neighbor distances of *C. memnon* egg clusters on banana leaves were measured in an enclosure. Group sizes of early instar caterpillars were counted, as well. Butterflies lay egg clusters next to one another on 85 percent of banana leaves examined (n = 8 leaves). As early instar groups consisted of over 900 individuals, it appears that early instars benefit from larger groups than a single female lies in a single cluster. Therefore, clumping clusters from multiple females may be a means to increase group sizes of early instar caterpillars while still spreading eggs among many leaves, hence avoiding risk. Relevance of this topic is related to discovering more information about species survival and evolution. Furthermore, the purpose of this study is to determine whether or not *Caligo memnon* are selective in their oviposition choices and the survival benefits that come along with its structure.

Role of Wnt Signaling and SULF1/SULF2 in Muscle Growth

Written by: Asha Farmer

Wnt signaling is relevant to all methods of cell proliferation. Previous research deduced that the over-expression of the Wnt1 protein promotes cell proliferation, while the overexpression of the Wnt6 protein inhibits cell proliferation. This previous experiment dealt with satellite cells and transfected those cells with Wnt1 and Wnt6 protein. The current experimental topic deals with a C2C12 mouse muscle cell line. These cells were transfected with Wnt1, Wnt6, and eGFP (which served as the control). Over a 120 hour period, the cells were observed and counted using a haemocytometer to determine the proliferation over time. Another aspect demonstrated in this experiment was the relationship that certain sulfatases (SULF1 and SULF2) had on muscle growth. These sulfatases were tested throughout the different transfected cells and stained to determine whether or not they were present. If the cells stained red, then the test was positive. Knowing these results would determine and prove that sulfatases are necessary for the cell signaling and proliferation process. The hypothesis was that the cell line results would be no different than that of the satellite cells. The results show that after the cell count was completed, it was found that the hypothesis was proven to be true. The cells over---expressed with the Wnt1 protein had a higher cell count than those that were over---expressed with the protein Wnt6. SULF1 and SULF2 were also proven to be found positively stained in all over---expressed proteins.

Comparing Efficacy of a Hydrogel Based BMP-2 Delivery System to Vital Bone Grafts

Written by: Kalah Haley

Segmental bone defect surgeries in large bones have proven difficult to treat for orthopedic surgeons, and often require multiple surgeries to close the defect gap. Bone Morphogenetic Protein 2 (BMP-2) has been used to help bridge the defect and to regenerate the surrounding cells. In this study, experiments were designed to observe BMP-2's role in promoting cell migration towards filling a defect gap, created in segmental bone defect surgeries. Our group has developed an Alginate-based BMP-2 delivery strategy and established excellent healing in a rodent model. More recently, advances have been made to improve the alginate delivery system by using melting electrospinning techniques rather than the solution electrospinning methods. The alginate based delivery system has never been compared with healing induced by live bone grafting, which is a routinely used clinical approach to treat large bone defects. Our objective was twofold: [1] to compare vital bone grafts (bone chips) and the hybrid delivery system (nanofiber mesh, alginate and rhBMP-2) and [2] to compare solution electrospinning to melting electrospinning. Bone volume and bone density were calculated using Micro CT image analysis at Day 0 and weeks 2, 4, 8 and 12 to find the most efficient therapy method. We hypothesized that at 12 weeks, the hybrid system will be the more efficient system. The objective of this study was to compare the healing efficiency of these two systems by comparing volume of bone mineral deposited and the density over time. Data analysis showed that the hybrid system proved to be as efficient if not more so as the traditional bone chip delivery system.

Characterization of Atmospheric Aerosols

Written by: Brittany Jackson

Atmospheric aerosols have a significant impact on terrestrial radiation by absorbing and scattering radiation. The goal of this work was to characterize biomass-burning plumes traveling from North America and Saharan dust particles passing over the Andalusian Center for Environmental Research (CEAMA) on June 23, 2010. Biomass burning aerosols are the result of the burning of living or dead vegetation; they absorb and scatter shortwave radiation thereby having an influence on the radiative budget of the atmosphere. Mineral dust is an aerosol scatters and absorbs incoming solar light, which has an impact on long-wave terrestrial radiation. The instruments included in this research were the sun photometer and the elastic backscatter lidar. From these two instruments data was obtained that further assisted in the characterization of aerosols within the atmosphere. This data included color plots of lidar range corrected signal and time series of aerosol optical depth and Angstrom Exponent. In addition to these, other methods were used including back trajectory plots from the HYSPLIT model, NAAPS model, fire web map plots, and BSC DREAM8b model. Through analyzing all results, characterization was then achieved. The altitude, Angstrom Exponent, and depolarization values found for these particles were 7-10 km above sea level, 1-2, and 0.01 respectively. The altitude, Angstrom Exponent, and depolarization values calculated for these particles were 1-5 km above sea level, 0-0.9, and 0.08-0.12 respectively. Thus these results revealed that biomass burning aerosols and non-pure Saharan dust particles were present in the atmosphere above Granada, Spain on June 23, 2010.

Investigating Microglia in Zebrafish Model of Late Infantile Neuronal Ceroid Lipofuscinosis

Written by: Syundai Johnson

Ceroid Lipofuscinosis, Neuronal 2 (CLN2) is a disease that is caused by Tripeptidyl peptidase 1 (TPP1) deficiency. Blindness, ataxia, and epilepsy are seen in patients with this disease. CLN2 causes nerve cells in the brain and retina to die. Once those cells die, the resident phagocytic cells of the brain, microglia, become activated. A tropical freshwater fish called the zebrafish shows similar symptoms when modeling the disease. For this reason, zebrafish are used to study this disease. This experiment focused specifically on identifying the earliest signs of microglia activation. Neutral red treatments and *in situ* hybridizations were performed on zebrafish ranging from 24 hours, 48 hours, and 72 hours in order to observe the earliest signs of microglia activation. It was hypothesized that changes in the time course of microglia activation would be different throughout embryonic development of zebrafish modeling cln2 disease and zebrafish that were healthy. Images were taken using an inverted microscope to observe microglia activation. It was found that there was a difference in microglia activation throughout embryonic development; therefore, the results supported the hypothesis. There was, however, a difference in the microglia activation of the two protocols that were done. There seemed to be more microglia activated at an earlier stage in the Neutral Red Treatment experiments than in the In Situ Hybridization experiments. The experiments will need to be replicated to ensure that similar results are obtained. These findings suggest that based upon the assumption that microglia becomes activated prior to 72 hours, there is a possibility that a medication can be developed to stop microglia activation at an earlier stage; thus, decreasing the severity of the disease.

Alteration of Mitochondrial Bioenergetics and Apoptotic Signaling with Aging in Mice Kidney: The Effects of Calorie Restriction and Dietary Fat

Written by: Jasmine Mason

A primary mechanism to maintain cell homeostasis is apoptosis, a programmed or regulated form of cell death that controls the accumulation of defective cells. An imbalance of this process may result in the onset of several diseases, particularly those associated with aging. Calorie restriction has been proven to increase longevity and influence healthy aging by improving tissue performance and delaying the onset of age-related diseases. The underlying mechanisms of these effects likely involve decreased oxidative stress and optimization of mitochondrial bioenergetics in a tissue specific manner.

This study investigated the effects of calorie restriction and those changes induced by the predominant fat source (lard, soybean oil, fish oil) in calorie-restricted diets on kidney apoptosis in relation to aging in mice fed experimental diets for 6 or 18 months.
Markers related to apoptotic signaling, apoptotic protease activating factor 1 (APAF-1) and voltage-dependent anion channel 1 (VDAC-1), were measured for the different experimental conditions by following a Western blot methodology. Due to the role of ubiquinone (coenzyme Q, CoQ) in energy metabolism, alterations in CoQ biosynthesis may modify CoQ levels in tissues. Thus, the levels of coenzyme Q (CoQ) were determined by reversed-phase HPLC separation; also, mitochondrial cellular respiration was measured via citrate synthase mitochondrial marker enzyme.

Results indicated that the distribution of APAF-1 in the cytosol demonstrates no significant effects in dietary fat source in the control groups of mice fed under calorie restriction with the exception of those in which lard is the primary source of dietary fat. These findings suggest that apoptosis signaling is deceased in calorie-restricted diets in which lard is used as the primary fat source. The distribution of VDAC-1 in mitochondria demonstrates no significant effects in dietary fat source in the control groups of mice fed under calorie restriction. Lastly, results of the levels of coenzyme Q suggest an increase with age in mice. In conclusion, the results of this study suggest that calorie restriction and the predominant fat source in calorie-restricted diets indeed induce effects on kidney apoptosis in relation to aging.

Multi-step Protein Purification Technique for Crystallization

Written by: Albryona Pope

For years crystallization has been used to understand and identify the molecular structure of proteins. In order to obtain the best and most useful crystals from a particular protein and to properly identify its structure, it is necessary to purify the protein. The goal of this work is to develop a multi-step purification technique for the purification of two specific proteins, mGO, modified glycolate oxidase, and SL06. This purification aims to isolate the protein from a complex mixture. Each protein is expressed in bacteria, His-Tagged, sonicated, and dialyzed in order to purify. SL06 is a dimeric receptor protein that is involved in the stress response of a tomato. When exposed to abscisic acid, a hormone known to induce plant stress, SL06 forms a complex and is known to exhibit differences in crystal structure. By identifying the crystal structure of SL06 and SL06-ABA complexes, the differences and similarities between the two structures can be examined. mGO, also known as modified glycolate oxidase, is a protein involved in the production of glyoxylate and subsequently oxalate in humans. In people who suffer from hyperoxaluria type 1, AGXT, enzyme acting in the breakdown of oxalate is defective and causes a buildup in oxalate. When mGO reacts with a ligand, CCPST, the crystal structure of mGO is altered and oxalate production is inhibited. By examining the structures of mGO and mGO-CCPST, scientist can identify conformational changes that may aid in the treatment of hyperoxaluria. The use of this simplified multistep process aided in the crystallization process by producing a higher concentration of protein.

Education as the Best Medicine: A KAP study of Tuberculosis in Ha Makuya

Written by: Kwadernica Rhea

Tuberculosis (TB) is an infectious airborne disease. Due to its increasing resistance to medical treatments, it is becoming especially problematic in rural South African communities. This study aimed to assess the knowledge of, attitudes toward, and perceptions of (KAP) TB in Ha Makuya, South Africa, through the recognition of certain key terms pertaining to symptoms and modes of transmission. This study also analyzed the relationship between education level and TB knowledge in addition to any associated stigma. Twenty individuals were interviewed about their knowledge of TB. To rank understanding, a scoring system was created. These scores were then compared to education level and source of TB knowledge to determine the most effective manner to disseminate knowledge throughout the community. Clinics and schools were cited as the most common sources of TB knowledge. Those who learned at schools had the highest TB symptom knowledge scores. Additionally, higher levels of education correlated positively with greater TB symptom awareness. Participants showed an acceptance of those affected by TB and demonstrated a willingness to increase awareness about this disease. In order to develop TB understanding in this community, we advocate for improved TB awareness programs through education in schools. With a willingness to treat the infected and a strong desire to learn about TB, the community shows potential for improved TB understanding and prevention through optimized educational programs.

The Role of CD38 in the Expression of Regulatory B Cells in a Murine Model of Lupus

Written by: Gabrielle Richardson

Systemic Lupus Erythematosus (SLE) is a chronic inflammatory long-term autoimmune disorder that can affect the skin, brain, kidneys, lungs, and many other organs in the body. Some connections between CD38 and autoimmune disorders, including SLE, have been made, however the role of B cells in relation to CD38 has not been adequately examined. This study examined the role of the transmembrane protein, CD38, in the expression of regulatory B cells in a pristane- induced murine model of lupus. Pristane was allowed to incubate in the mice's bodies for 24 hours and two weeks. CD38 KO and WT mice injected with pristane were examined for the presence of regulatory B cells. The mice were also tested for the presence of dendritic cells, monocytes, and neutrophils. Cells from the peritoneal area and spleen were taken from each mouse and marked with various surface expression markers. The following surface expression markers were used to identify regulatory B2 cells: CD19-APC, CD5-PerCP, CD1d-PE, and CD38-FITC. The regulatory B1 cells were identified using the markers: B220-FITC, CD11b-APC, Gr1-PE, CD5-PerCP. Using FACS acquisition and Flow Jo Analysis, the percentages of those cells were found. All cells found under certain subsets do not display regulatory function and functional assay is required to confirm the regulatory function of those cells. Functional assay was not performed in this study; however the results showed promising increase in percentage of $CD1d^{hi}CD5^{+}CD19^{+}$ cells in the spleen of CD38KO mice, a known subset for regulatory B cells in the spleen. In future studies, functional assay will be performed to confirm the results of this study. Discovering the effect of CD38 on the presence of regulatory B cells can lead to better techniques in relation to B cell targeted treatment of lupus.

Multi-step Protein Purification Technique for Crystallization

Written by: Lydia Ruffner

MicroRNAs (miRNAs) play a vital role in gene translation by providing a faster way of silencing or amplifying genes whenever necessary. MicroRNAs have been found to help a cell recuperate from environmental stress caused by a lack of calcium that leads to an unfolded protein or chemical oxidative stress, which deprives the cell of oxygen. Once this type of stress is introduced to the cell, the cell must overcome it and miRNAs direct this process by amplifying the genes needed to repair the cell and silencing the genes that cause the cell to deal with growth. Without the small units of RNA cell function, the cells ability to repair would be little or non existent, because of the lethality that would arise in embryonic stages. To observe a semi MicroRNA deprived environment an important enzyme needed for miRNA maturation is deleted. The purpose of this project was to establish stress conditions in fibroblast cell lines by characterizing oxidative stress and unfolded protein response in 3T3 fibroblast cells, as well as to set up conditions to examine the deletion of dicer in a murine embryonic fibroblast cell line.

The approach in stressing the cells was to use three stressors: Sodium Arsenite, Cobalt Chloride, and Tharpsigargen at varied concentrations for less than six hours on 3T3 fibroblast cells. Western blot analysis showed no significant diffence in phosphorylation of eIF 2α between stressor concentrations and the control. To delete Dicer, a tamoxifen induced Cre-loxp PCR strategy was used, which showed positive results at all concentrations of tamoxifen. These preliminary results showed that dicer had been deleted from the murine embryonic fibroblast cell line after three days.

Assessing the CO2 Exchange of Wetlands: Padul Site a Case Study

Written by: DeShawn Samad

This research project was meant to determine the different relationships between CO_2 and photosynthetic effluxes, versus other abiotic factors in a *Phragmites Australis* dominated wetlands during its growing season. The amount of CO_2 exchange was measured alongside air temperature, soil temperature, and water level. Each of these factors was graphed against the CO_2 exchange measurements to determine if there was a function relationship between the two. It was found that there was no relationship between air or soil temperature and CO_2, however there was an inverse relationship between the CO_2 exchange and the water level. Due to some difficulties beyond the control of the researchers, the data for Photosynthesis was not usable for analysis. These findings may be useful for other researchers who are interested in determining sources of CO_2 and other greenhouse gases being released into the atmosphere in order to mediate and control climate change around the world.

The Study of Esterification of Free Fatty Acids (FFA) in Waste Oil

Written by: Marlissa Stalling

The purpose of this research paper is to study biodiesel production using waste oil. The raw material utilized in this work was used frying oil afflicted from University of Cordoba's cafeteria. All the properties of the samples were analyzed according to the European Standard Methods. Water content was determined by the Karl Fisher method (ISO 12937, 2000) and acid value was determined by titration with KOH in accordance with EN 14104 Standard (Determination of acid value,2003). In order to recover biodiesel, EN 14104 Standard (Determination of acid value, 2003) limits the acid index value to 0.5 mgKOH/goil.

Results of this work shows that the greater the molecular ratios of MetOH and H_2SO_4 are, the lower the acid index value of waste oil was. In the oil recovery process, the efficiency of the biodiesel production was measured by the acid index value. The lower the index the better the efficiency of the process. Results of this research showed that there is a correlation between the molecular weight of the waste materials mixture of waste materials with H_2SO_4. Lower molecular ratio indicated a higher and undesirable index value. Mixtures with higher ratios of solvent to waste such as 60:1, and 45:1, seems to give better results and lower acid values

During beginning stage of esterification, methanol and sulfuric acid were at its lowest yield and cannot act against FFA and water. There is high content of FFA and water at this point. As the process of esterification progressed, the yields of methanol and sulfuric acid yields are much higher which allows them to act with more force against FFA and water. There is now low FFA and water content at this point.

There must be further research of biodiesel production in order to use low feedstocks. Low feedstocks can be useful for biodiesel but it is prone to processing problems due to biodiesel's sensitivity of water, free fatty acids and other impurities from low feedstocks.

The Role of FSTL3 in the Placenta

Written by: Chelsea Straughn

The placenta is a highly vascularized organ attached to the lining of the uterus during pregnancy. The glycoprotein FSTL3 (Follistatin-like 3), along with EphB4 and its ligand Ephrin B2 are highly expressed in the mouse placenta. The hypothesis for this project was that in the absence of FSTL3, the expression and signaling of EphB4 would be altered. Immunofluorescence and immunohistochemistry revealed that the expression of EphB4 and Ephrin B2 were evenly expressed throughout the placenta in the wild type and FSTL3 deleted tissue (knock out). Through a western blot analysis, a higher expression of EphB4 and Ephrin B2 was found in the knock out placenta tissue. Immunoprecipitation revealed that phosphotyrosine was expressed at a higher level in the knock out tissue. This verified a functioning signaling pathway. These experiments suggest that EphB4 and its ligand Ephrin B2 are evenly expressed throughout the placenta and are highly expressed in FSTL3 deleted tissue. The proteins EphB4 and its ligand Ephrin B2 are necessary for the formation of blood vessels and capillaries. When there is a reduction of blood flow, the signaling of these proteins will increase angiogenesis.

Maternal Choices for Children's Health; the use of biomedicine or alternative methods in Tshianzwane village

Written by: Dominique White

The purpose of this study was to determine if mothers in the HaMakuya area still prefer traditional or alternative medicine over public healthcare systems that have recently been implemented. Ten mothers living in the village of Tshianzwane in the HaMakuya area of Limpopo province were interviewed. A set of seven demographic questions and thirteen questions on mothers' first responses to various health issues with their children were used. The results showed that the majority of the participants preferred the free clinics over their own traditional medical practices. When asked about children catching the flu or breaking a bone, the majority of participants chose to use clinics or hospitals. On the other hand, for injuries such as wounds, stings, and bites, the participants chose their own methods. Some of these methods included medicinal plants, ginseng violet, and saltwater. Based on the results, it could be concluded that over the past two decades, health choices in the HaMakuya area have changed. Clinics have become more prominent in rural areas that originally could only depend on traditional medicine for healing. With this change of infrastructure in South Africa, mothers in Tshianzwane village are becoming more dependent on biomedical methods to treat their children.

Child Health: Treatment Seeking Behaviors of Mothers in Mutshitkilini Village

Written by: Ticara Wicks

This interview-based research project explored mothers' treatment options for their ill or injured children in rural South Africa. The purpose of the study was to determine which methods were more preferred by these mothers when given the choice of biomedical options (clinic/hospital) and traditional or home remedies. It was hypothesized that mothers would prefer traditional or home remedies to biomedical methods. Eleven mothers in Mutshitkilini Village participated in the research. Depending on their age, mothers reported different illnesses and treatment options for their children. Most importantly, results demonstrated that mothers used clinics more than home remedies showing a new trend towards western medicine. This generational change is important because it opens up room for both cultures to learn from each other, helping medicine to reach new heights and possibilities.

Exposure of Planktonic Cells of Escherichia coli to Atmospheric- Pressure Non-thermal Plasmas

Written by: Brianna Stewart

The effects of atmospheric pressure non-temperature plasmas (APNTP) on planktonic cells of *Escherichia coli (E. coli)* were studied to understand how this interaction would influence any biological processes. Spectroscopic tools were used to characterize the products of the plasma jet. Spectral data averaged over three trials for specific amounts of oxygen warranted the use of filter to enhance the intensity of the plasma. Certain unique gases were found in the analysis of the data, which in turn affected the cell growth. Bacterial cells prepared using Luria broth (LB) were exposed to the plasma, the growth of which was studied at regular time intervals of exposure. The results from the experiment also showed that the longer the species is exposed to the plasma the smaller the amount of growth is to the point where after so long it becomes totally eradicated by 1.5 minutes. From these results it is concluded that there may be two mechanistic pathways affecting the growth rate: one affecting the bacterial growth initially, and the other affecting the bacteria later in different ways. In exposing the species *E. coli*, as planktonic cells, to APNTP, the amount of bacteria growth was found to be significantly less as the exposure time increased. From this it can be seen that atmospheric pressure non-temperature plasmas can be used in eradicating bacteria, such as *E. coli*, and therefore can be used to *in vitro* to kill the bacteria.