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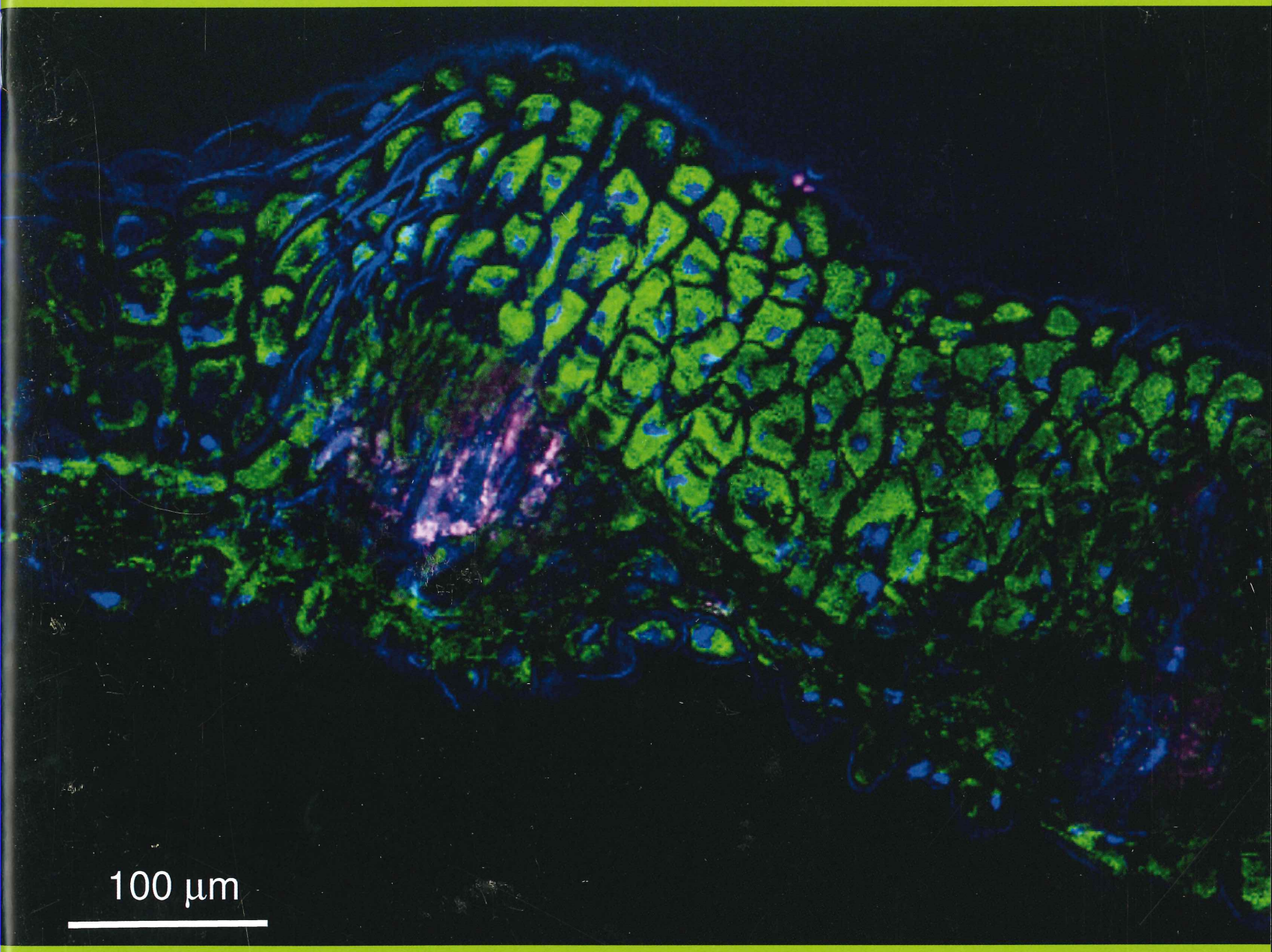

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Contents

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ON THE COVER: *Helianthus annuus*, Asteraceae ray floret cross section treated with DAPI nuclear stain. Confocal Laser Scanning Microscope image- Nikon A1 Confocal System. Laser information: DAPI (blue, 400 nm)/FITC (green, 588 nm)/Texas Red (red, 561 nm)/Cy5 (pink, 626 nm). Photo supplied by Jennie Wojtaszek, Texas Women's University, Department of Biology, Denton, TX 76204-5799.

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PRESIDENT'S MESSAGE

Last year's meeting on the TCU campus was a success. Grateful thanks go to Ernest Couch, Past Program Chair, who worked very diligently in providing the Society with an excellent venue for the 2012 meeting and to all participants to that meeting. I would like to thank all Executive Council members, listed below, for working with me for two years in managing the Society's business: Robert Droleskey, Secretary, Sandra Westmoreland, Treasurer, Ernest Couch and Pam Neill, Program Chairs, Jiechao Jiang and Laura Hanson, members of the Program Chair Committee, Kevin Cronyn, Corporate Member Representative, Jennie Wojtaszek, Student Representative and Facebook helper, E. Ann Ellis and Michael Pendleton, Co-editors for the *Texas Journal of Microscopy*, Becky Holdford, Webmaster, and Nabarun Ghosh, Facebook master. The corporate sponsors deserve special thanks for their commitment to TSM materialized through participation to the vendor exhibit and financial support of our Society through registration, donations, and advertisements in our journal.

The 2013 meeting is shaping up well. We have a total of twenty-two presentations in biological sciences, materials science and education, among which thirteen are poster presentations, many more than ever before. We also have three excellent invited presentations by Drs. Robert Keller, Dave Piston, and Boris Kharisov. Kevin and Pam along with her Program Chair Committee need to be commended for great jobs in organizing the FIB and HR-SEM workshops and working on the meeting's program, respectively. The Society has a newly designed website which follows the MSA website template at www.texasmicroscopy.org (make sure you click 'renew' in your window browser to get the new website). Thanks are going to Ezekiel Bierschank who together with Becky Holdford have made successful transition between the old and new websites. The TSEM Executive Council is very appreciative of Becky's dedication and sustained work as Webmaster for the last 13 years. Becky is pursuing other professional service endeavors and we wish her high achievements along that path.

For a few years now, the TSM membership and meeting participation are low. Some of you may not know that our Society is almost 50 years old. In the early 1960s, a group of enthusiastic electron microscopists got together and organized the Texas Society for Electron Microscopy whose first meeting was held in 1964. During this half of century of activity, the Society had its ups and downs. I remember those times, not that far ago, in early 1990s, when we used to have two meetings per year with so many presentations that we had to have two parallel sessions, one for biological sciences and the other for materials science. We used to load slides on carousels and project them on a screen for our presentations. That was before the Power Point and digital era. To keep up with the changes in the real life of microscopy and to attract new members, the Society changed its name to Texas Society for Microscopy, and its mission to embrace and serve all types of microscopy not only electron microscopy. Other significant changes occurred in meantime; one, to be mentioned in particular, was going from two meeting per year to only one when participation dwindled. But positive changes happened as well. We had great meetings with excellent invited presentations, an excellent journal, a competitive student presentation award, a very informative website and more recently we have made our way into Facebook. This year I will bring a proposal for a Small Grants Policy to the Executive Council meeting in an effort to attract both students and their mentors to actively participate in out Society's activities.

Our Society has had its ups and downs during the fifty years of activity indeed. However, the new bottleneck situation we are currently in is of a different kind than those before. If several years ago our treasury was almost empty, now we have money but not enough people to serve in the Executive Council. At this point in time, we practically need to elect all TSM officers: President, President Elect, Secretary, Secretary Elect, Treasurer, Treasurer Elect, Program Chair, Program Chair Elect. We also need to appoint the Corporate Member Representative, Student Representative, and Webmaster. I am appealing to all TSM members to please run for office and volunteer for the appointed positions. Our Society showed to be very resilient during its lengthy activity and rebounded every time after a crisis. I have big hopes

that the TSM will pass successfully over the current crisis and become even stronger. We need new generations of microscopists to successfully take the TSM into the next fifty years of activity. The field of microscopy is more diverse and more integrated with other sciences and technologies than ever before. I envision another group of young microscopists getting together and re-shaping the Texas Society for Microscopy into a vibrant and engaging society. Please, please, please, offer a bit of your time and energy to the great Texas Society for Microscopy!

Sincerely,

Camelia Maier
TSM President 2011-2012

Correction:

In the President's Message contained in the last issue of the Texas Journal of Microscopy, Volume Number 43, page 3, the entire fourth paragraph was duplicated in error in other sections of the Presidents Message. The editors wish to apologize for this error and hope that any misinterpretation of the information or concepts contained in the President's Message are now eliminated with the publication of this correction statement.

Sincerely,

E. Ann Ellis, co-editor TJM, Vols. 42-44.
Michael Pendleton, co-editor TJM, Vols. 42-44.

Howard J. Arnott Student Competition Award Winners Chris Evans (Left), Department of Biology, Texas Christian University; Mirza Hasan (Right), Department of Chemistry, Texas Christian University, and Texas Society for Microscopy President Camelia Meier at the 2012 Texas Society for Microscopy Meeting at Texas Christian University in Fort Worth, Texas. (Photo supplied by Nabarun Ghosh, West Texas A&M University).

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ABSTRACTS OF THE 2013 TEXAS SOCIETY FOR MICROSCOPY MEETING

BIOLOGICAL SCIENCE

PLATFORM PAPERS

PHYLOGEOGRAPHIC STUDY OF *PINUS PONDEROSA* & *P. JEFFREYI*

KERI BARFIELD[1], ERNEST COUCH[2], GLENN KROH[2], DEAN WILLIAMS[2], and PATTY MARKSTEINER[1], [1]Botanical Research Institute of Texas, Fort Worth, TX 76107 and [2]Dept. of Biology, Texas Christian University, Fort Worth, TX 76129.

In this study we have used both genetic and morphological measures to delineate two very similar species of pine trees, *Pinus ponderosa* and *Pinus jeffreyi. Pinus* is the largest genus in the Pinaceae, with more than 100 species. Data support the identification and separation of the species. Needles of both species were collected from the study site established in 1992 by Dr. Glenn Kroh, Dr. John Pinder and their graduate students. The site included fifty-three permanent 200-m^2 plots in the Chaos Jumbles disturbed area of Lassen Volcanic National Park. In 2003, continued documentation of conifer establishment and rates of forest development were documented. Both *P. jeffreyi* and *P. ponderosa* were present at the site. They are very similar morphologically (leaves in bundles of three, needle length for both species range from 12–27 cm, leaves are yellow green to blue-green in color). *Pinus jeffreyi* is distributed throughout California and the 8 lower southwestern most counties of Oregon. In contrast, *Pinus ponderosa* is widely distributed across western North America. We are now using scanning electron microscopy to study potential stomatal differences between these two species. In addition, we are using molecular markers to determine if there are diagnostic genetic differences between the two species.

THE EFFECT OF MEDIA CONDITIONS ON CELL WALL THICKNESS AND CELL DIVISION OF *BACILLUS ANTHRACIS* LACKING CLPX

C. EVANS, E. COUCH and S. MCGILLIVRAY, Dept of Biology, Texas Christian University, Fort Worth, TX 76129.

ClpXP is an intracellular protease that regulates the life span of multiple bacterial proteins such as transcriptional regulators, rate-limiting enzymes and damaged proteins. ClpXP consists of two proteins, ClpP, the proteolytic core, and ClpX, a regulatory ATPase. ClpXP is conserved across many bacterial species and is often associated with cellular stresses such as heat shock, nutrient deprivation and oxidative stress. ClpX/P has also been implicated in the virulence of several pathogens. We recently demonstrated that ClpX is critical for the pathogenesis of *Bacillus anthracis*, a Gram-positive bacterium that is the causative agent of anthrax, and that the loss of ClpX leads to susceptibility to multiple cell wall-acting antimicrobials. We hypothesize that the misregulation of cell wall genes due to the loss of ClpX leads to a change in cell wall structure, making it susceptible to cell-wall targeting antimicrobial agents.

Cell wall thickness has been shown to affect bacterial susceptibility to cell wall-acting antimicrobials in multiple gram-positive bacterial pathogens. We have used transmission electron microscopy to analyze the cell wall thickness of ΔClpX *B. anthracis* in the standard culture broth Brain Heart Infusion (BHI), and in a more stringent media, RPMI. We quantitatively show that ΔClpX has a decreased cell wall thickness compared to wild type after growth in RPMI, and to a lesser extent in BHI. We also show that ΔClpX undergoes abnormal cell division in RPMI, resulting in the formation of minicells. We are currently continuing to use transmission electron microscopy and phase-contrast microscopy to determine why minicells form in RPMI and in the future will determine if this has any effect on antimicrobial sensitivity.

ANDROGENS MAY INDIRECTLY REGULATE SPERMATOGENESIS BY DIRECTLY REGULATING PROTEINS OF THE BLOOD-TESTIS BARRIER TIGHT JUNCTIONAL-COMPLEX

SAMUEL SANG, BARKHA SINGHAL, ARPITA TALIPTRA, DIBYENDU DUTTA, IN PARK, and DIBYENDU DUTTA, Dept. of Biology, Texas Woman's University, Denton, TX.

Genes regulated by testosterone (T) are needed for male fertility and virility. Testosterone is thought to act in the Sertoli and /or peritubular cells to create an enabling environment for normal progression of germ cells during spermatogenesis. Using ethylene dimethane sulfonate (EDS) we selectively destroy Leydig cells - the source of androgen in adult rat testis. Seven days post-EDS, we examined the changes in gene expression of TJ proteins with and without testosterone replacement. Using RT-qPCR, we examined the tissue mRNA levels for the tricellulin (*Marvld2, Tric*) claudin 11 (*Cldn11*) and aquaporin-9 (*Aqp9*), major AJ and TJ proteins. A decrease in *Marvld2* and *Cldn11* and an increase in *Aqp9* occurred in the absence of testosterone and with exogenous testosterone replacement, values returned to near control levels. This suggests that testosterone may regulate paracellular diffusion of water, electrolytes, nutrients, and biomolecules and transcellular transport of water, glycerol, electrolytes and other small solutes needed by Sertoli cells and developing germ cells in the seminiferous tubules by modulating components of the cellular junctions.

ENHANCING VOCABULARY AND LANGUAGE ACQUISITION SKILLS OF ENGLISH LANGUAGE LEARNERS IN THE RURAL MIDDLE SCHOOL SCIENCE CLASSROOM THROUGH THE USE OF WORD WALLS, INQUIRY-BASED SCIENCE METHODS, AND THE SCANNING ELECTRON MICROSCOPE

CHERYL L. SIEBER and SANDRA L. WESTMORELAND, Department of Biology, Texas Woman's University, Denton, Texas 76204.

This study incorporated hands-on science learning through the use of inquiry-based science methods, a word wall, and a discovery wall for micrographs taken with a scanning electron microscope. In this nine month, action research study, an experimental research design was used to collect and analyze data from criterion referenced pre-tests and post-tests. Descriptive statistics were used to analyze data from the 2010-2011 school year baseline tests and the 2011-2012 school year pre-tests and post-tests. A t-test was used to analyze data from the baseline test, the pre-test, and the post-test of the study group. Additionally, artifacts were collected through photographs of the word wall, photographs of the discovery wall, and electron micrographs of student selected items. The specific research question that the researcher attempted to answer was, "Can vocabulary acquisition of ELLs increase over a nine month study period by using a science word wall, a discovery wall with micrographs from a scanning electron microscope, and the process of scientific inquiry"? The researcher predicted that by incorporating these specific techniques into his curriculum, the scores of the ELLs in the sixth grade science classroom would increase significantly more on the end of year vocabulary post-test assessment than the native English speaking students and that the overall gain of the ELLs on the post-test would show an increase of at least 10% over the pre-test. The data showed that the ELLs gained an average of 12% from the pre-test to the post-test compared to the non-English Language Learners (non-ELLs) who gained an average of 9% from the pre-test to the post-test. These data indicated that the use of inquiry-based science methods, the word wall, and the discovery wall of micrographs taken with the scanning electron microscope did indeed help to increase the vocabulary acquisition skills of the ELLs in a sixth grade science class in rural North Texas.

MATERIAL SCIENCE

PLATFORM PAPERS

SYNTHESIS OF TEM CHARACTERIZATION OF HOLLOW GOLD NANOSPHERES

M. CHIVARAT, RUIQIAN JIANG, and Y. H. HAO, Dept. of Materials Science and Engineering, College of Engineering, The University of Texas at Arlington, Arlington, TX 76019.

A large quantity of hollow gold nanoparticle with well-controlled size, hollow dimension, and shell structures has been synthesized by bubble template

synthesis method. In this method, electrochemically generated nanobubbles are used as templates. The size and shell structure of hollow gold nanospheres can be tuned by varying gold electrolyte composition and electrodeposition conditions. Synthesized gold nanoparticles have a size range of 100 – 200 nanometers and the hollow dimension can be controlled in the size range of 30 – 50 nanometers. TEM micrographs showed that hollow gold nanosphere were polycrystalline and porous.

In addition, core/shell structures of silver/gold hollow nanospheres have been fabricated. A thin layer of silver was successfully coated onto the gold hollow nanospheres by using galvanic replacement methods. These nanostructures have also been characterized by TEM, SEM, HRTEM, and Energy-Dispersive X-ray analysis.

AU/PD CORE-SHELL NANOPARTICLES WITH VARIED HOLLOW AU CORES FOR ENHANCED FORMIC ACID OXIDATION

CHIAJEN HSU, CHIENWEN HUANG, YAOWU HAO and FUQIANG LIU, Department of Materials Science and Engineering, University of Texas at Arlington, Arlington, TX, 76019, USA.

A facile method has been developed to synthesize Au/Pd core-shell nanoparticles via galvanic replacement of Cu by Pd on hollow Au nano-spheres. The unique nanoparticles were characterized by X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), Ultraviolet–visible spectroscopy (UV-VIS), and electrochemical measurements. When decreasing concentration of the Au solution, grain size of the polycrystalline hollow Au nano-spheres reduced and the structures became highly porous. After the Pd shell formed on these Au nano-spheres, the morphology and structure of the Au/Pd nanoparticles varied and hence significantly affected the catalytic properties. The Au/Pd nanoparticles synthesized with reduced Au concentrations showed higher formic acid oxidation activity (0.93 mA cm^{-2} @ 0.3V) than the commercial Pd black (0.85 mA cm^{-2} @ 0.3 V), suggesting a promising candidate as fuel cell catalysts. In addition, the Au/Pd nanoparticles displayed lower CO-stripping potential, improved stability, and higher durability compared to the Pd black due to their unique core-shell structures tuned by Au core morphologies.

HIGH-RESOLUTION TRANSMISSION ELECTRON MICROSCOPY OF OXIDE HETEROSTRUCTURES

JIECHAO JIANG, Department of Materials Science and Engineering, University of Texas at Arlington, Arlington, TX, 76019, USA.

Transition metal oxide heterostructures are of enormous fundamental interest and technological importance due to their intriguing properties such as high T_c superconductivity, ferroelectric and ferromagnetic properties. Such heterostructures possess remarkable properties which are different from their bulk counterparts and present exciting opportunities for applications in electronic and optoelectronic engineering and therefore have attracted much interest. In this talk, an overview of our recent research works on high-resolution transmission electron microscopy of a wide variety of multifunctional oxides will be presented. Specifically, four topics will be addressed: (1) microstructure of ferromagnetic and ferroelectric epitaxial oxide thin films; (2) Epitaxial self-patterned nano structures and self-assembled one-dimensional nanorod/nanofinger structures in ferroelectric and ferromagnetic oxides; (3) interface engineered ferroelectric superlattices and multilayered structures and (4) interfacial structure of the heterostructures.

MICROSTRUCTURAL ANALYSIS OF TISIN NANOCOMPOSITE THIN FILMS BY HRTEM

JESSICA MOONEY[1], JIECHAO JIANG[1], JIE HE[1], YUHANG CHENG[2], and EFSTATHIOS I. MELETIS[1], [1]Department of Materials Science and Engineering, University of Texas at Arlington, [2]American Eagle Industries, Inc.

Materials for use in extreme environments have attracted significant scientific attention for decades. Of particular interest are coating materials displaying high hardness. While the nc-TiN/a-Si_3N_4 model is frequently cited in the literature in the discussion of high hardness films, a lack of detailed

TEM analysis exists. Such analysis is imperative to gain a clear understanding of the relationship between hardness and detailed microstructural characteristics. In this study, TiSiN coatings deposited using large area filtered cathodic arc deposition technique were studied using X-Ray Diffraction (XRD) and High Resolution Transition Electron Microscopy (HRTEM) to gain a detailed understanding of the microstructure as a function of Si content as well as an understanding of the relationship between microstructure and mechanical properties of the coatings. Cross-sectional and plan-view TEM show that the two films are composed of a columnar structure with column diameter ranging from 100-200 nm in the low Si film and 40-100 nm in the high Si film. Within the columnar structure, subgrains with 5-10 nm diameter were formed. XRD and electron diffraction analysis show that phases present in the films include an FCC TiN phase, as well as an FCC TiSiN phase formed by the substitution of Si into the TiN crystal lattice. Increasing the Si content in the films resulted in a decrease of the observed lattice constant by about 3.5%. The relationship between processing and microstructure is discussed.

SYNTHESIS AND CHARACTERIZATION OF SIZE DEPENDENT PROPERTIES OF SILICON CARBIDE QUANTUM DOTS

MUNUVE MWANIA[1], PETER KROLL[1] and CSABA JANAKY[1], Department of Chemistry and Biochemistry, College of Science, The University of Texas at Arlington, Arlington, TX 76019-0065.

Silicon carbide (SiC) quantum dots (QDs: particles with a size of 1-5 nm, where 1 nm = 1 x 10^{-9} meters), exhibit useful optical properties in the visible range and their surface can be functionalized with various chemical groups. Consequently, these particles can be used for fabricating electronic devices, disease detection (cell imaging) and drug delivery and are better suited than any other inorganic compound for applications in life science.

Here, we report the synthesis and optical properties of β-SiC QDs with diameters smaller than 5 nm, via a novel and inexpensive method, providing an effective and high-yield route. We synthesize β-SiC quantum dots (QDs) through a two-step route - electrochemical etching of commercially available bulk SiC powder followed by ultrasonic dispersion in polar and non-polar solvents. The colloidal suspensions contain β-SiC QDs with diameters ranging from 2 nm to 10 nm. We separate fractions through ultra-centrifugation and determine size distributions using transmission electron microscopy (TEM). Small (< 5 nm) β-SiC QDs without an oxide shell can be imaged. TEM results also reveal lattice fringes with spacing of 0.25 nm, which corresponds to (111) planes of bulk β-SiC. Optical properties are characterized using absorption spectroscopy (UV-VIS) and fluorescence spectroscopy.

Our results confirm quantum confinement in β-SiC quantum dots. We observe a correlation between particle size and absorption edge, as well as between particle size and maximum of the emission spectrum. UV-VIS spectroscopy of ultra-small β-SiC QDs with diameters of less than 5 nm exhibit discrete and sharp absorption features characteristic of the discrete energy levels. Our results expand the fundamental understanding of β-SiC QDs, necessary to exploit their applications.

"GREENER" SYNTHESIS OF ULTRASMALL NANOPARTICLES ON THE BASIS OF IRON BY MICROWAVE-HYDROTHERMAL TECHNIQUE

BETSABEE OLVERA PÉREZ,[1] EDGAR G. DE CASAS ORTIZ,[1] OXANA V. KHARISSOVA,[1] MARÍA DEL RAYO CAMACHO CORONA,[1] VICTOR M. JIMÉNEZ PÉREZ,[1] RASIKA DIAS,[2] and BORIS I. KHARISOV[1], [1]Universidad Autónoma de Nuevo León, Monterrey, México. [2]The University of Texas at Arlington, TX, USA.

In this work, we used a series of polyphenol-containing plant extracts for obtaining nanoparticles on the iron basis in the conditions of microwave-hydrothermal method in friendly conditions. Dependence of possibility of nanoparticle formation on reaction conditions (pressure, temperature, metal salt / extract ratio) was established. The formed products were studied by SEM and HR-TEM techniques, confirming formation of ultrasmall

many science teachers have not had authentic inquiry experiences in their pre-service training programs, they are not well-prepared to help their K-12 students plan their own inquiries. In this study, three pre-service teachers, who were enrolled in a Master of Arts in Teaching degree program for initial science certification, were engaged in a 17-week course to use scanning electron microscopy (SEM) for inquiry investigations. Students in this program were taught to use the Hatachi-1000 Tabletop SEM and then allowed to ask questions, design investigations, collect data, and present their findings at professional meetings. The students' investigations represented a variety of inquiry types. Students reported that they encountered both challenges and benefits from the course experience. Challenges included their lack of preparation to engage in asking questions and planning investigations, which they had not previously experienced. Benefits that the students reported included the development of pride and confidence in their ability to design and execute an inquiry study. A future study may track whether pre-service teachers who have experienced inquiry using this SEM research tool are more likely to use this teaching method in their future science classrooms.

BIOLOGICAL SCIENCE

POSTER PRESENTATIONS

INCREASED MINERAL CONTENT AND THE OSTEOCYTE LACUNAR NETWORK OF TRABECULAR BONE FOLLOWING OSTEONECROSIS OF THE FEMORAL HEAD

OLUMIDE O. ARUWAJOYE[1,2], HARRY K.W. KIM[2], and PRANESH B. ASWATH[1], [1]Department of Materials Science and Engineering, University of Texas at Arlington, Arlington, TX 76019 and [2]Center of Excellence in Hip Disorders, Texas Scottish Rite Hospital, Dallas, TX 75219.

Loss of blood flow to the femoral head leads to ischemic osteonecrosis. Ischemic osteonecrosis can lead to subchondral fracture and subsequent femoral head flattening or collapse. The subchondral fracture is one of the earliest signs of this disease. The preceding events that lead to fracture have yet to be elucidated. We used a well-established piglet model of ischemic osteonecrosis to measure the mineral content and map the osteocyte-lacunar network of trabecular bone. We hypothesized that ischemic osteonecrosis leads to changes in the mineral content and the osteocyte-lacunar network, due to extensive cell death. We prepared portions of bone from the femoral head at 2 and 8 weeks post onset of ischemia of the femoral head. The subchondral and calcified cartilage regions were imaged with a calibrated back scattered electron microscope for calcium concentration. In addition, samples were acid-etched to image the resin cast osteocyte-lacunar network. There was significantly increased calcium distribution in the calcified and subchondral regions of the necrotic bone. In some instances, resin-casting revealed the osteocyte-lacunar network to be fragmented in the necrotic bone. And finally, mineralized osteocytes were present in trabecular bone of necrotic tissue. Taken together, the results suggest that mineralizing cells may take a possible role in the increased mineral content of necrotic bone.

PRESENCE OF *SITOPHILUS* IN PRESUMED BILGE SEDIMENTS OF AN ANCIENT SHIPWRECK

MEKO KOFAHL, Nautical Archaeology Program, Department of Anthropology, MS4352,Texas A&M University, College Station, TX 77843-4352.

During screening of sediments retrieved from a 9th century AD shipwreck off the coast of Dor/Tantura, Israel, a number of insect parts were recovered. Imaging of the most complete of these was conducted under dissecting microscope as a member of *Sitophilus*, the weevil family. This specimen includes the prothorax, head and rostrum. The antennae are incomplete and the legs are broken close to the prothorax. While an initial identification as *Sitophilus granarius*, or wheat weevil, seemed likely for a number of reasons, additional SEM imaging was undertaken in the hopes of confirming that initial identification and for comparison with weevils found at other archaeological sites.

The additional detail of the SEM images allowed for a confirmation of *Sitophilus granarius*, in large part due to the detail of the compound eye, leg attachment points, and rostrum shape.

MICROSCOPIC ASSESSMENT OF GENOTOXICITY OF HERBICIDE MIXTURES AT ENVIRONMENTALLY RELEVANT CONCENTRATIONS FOR THE TEXAS HIGH PLAINS

WILLIAM H. MIMBS, NABARUN GHOSH, Ph.D., GARY C. BARBEE, Ph.D., Department of Life, Earth, and Environmental Sciences, West Texas A&M University, Canyon, TX 79016.

The possible genotoxic effects of pesticides commonly applied in the Texas High Plains have not been characterized at environmentally relevant levels, nor have their genotoxic mixtures been well assessed. A commonly used sentinel species, the freshwater crustacean *Ceriodaphnia dubia*, was utilized for the assay. *C. dubia* were exposed to each pesticide separately at environmentally relevant concentrations found in playa lakes around the Texas High Plains. In addition to the individual pesticides, the mixture genotoxicity of the herbicides was also investigated. In many cases, wildlife exposures to environmental pesticides occur not to single, isolated chemicals but rather to pesticide mixtures. Thus to assess the toxic impact of pesticides, it is important to focus on both environmentally relevant concentrations, and environmentally relevant mixtures. In this study we investigated the genotoxic effects of the cotton herbicides Diuron and Metolachlor as individual chemicals as well as various pre-determined mixtures using the Micronucleus and Comet assays. Whole body cells from the aquatic invertebrate *Ceriodaphnia dubia* were used in the Comet assay, and peripheral erythrocytes from the vertebrate fish *Gambusia affinis* were utilized in the Micronucleus test. Micrographs were captured using a BX40 Olympus microscope equipped with FITC and TRITC fluorescent filters, a mercury lamp source, an Olympus DP-70 digital camera attached to the computer. The results suggest synergistic genotoxicity from the pesticide mixtures in these diverse invertebrate and vertebrate species.

ANALYSIS OF PERMISSIVITY OF RAT B-35 NEUROBLASTOMA CELLS FOR MURINE CYTOMEGALOVIRUS

PRAPTI MODY, DR. LAURA HANSON and DR. DIANNA HYNDS, Department of Biology, Texas Woman's University, Denton, TX 76209.

Cytomegalovirus is a virus belonging to the Herpesvirideae family and is species specific. Human cytomegalovirus (HCMV) is present in 60-100% adults worldwide. It is often latent and causes no harm to the host but can cause serious birth defects. HCMV is a common cause of diseases in immunocompromised individuals. Over the last ten years, researchers have demonstrated localization of HCMV inside brain tumor cells. Thus HCMV is believed to play an active role in tumor growth. Its exact role and mechanisms are unknown and are hard to determine since HCMV only infects humans. Thus we need animal model systems to try and understand the infection process. Murine cytomegalovirus (MCMV) is a good model system to characterize the infection and viral gene expression process since it has been extensively studied and provides us with a number of tools such as viruses with mutations in each gene. The rat B-35 cell line provides us with a well-known non-differentiated tumor cell model. We first demonstrated the ability of MCMV to infect the rat neuronal cells. We used microscopic studies to visually test whether the virus induced changes in cell morphology in rat cells as it does in completely permissive mouse cells. To characterize viral gene expression times and its localization, we did western blots for viral protein kinetics and immunofluorescence studies.

IMPROVED SPECIMEN PREPARATION AND SEM IMAGING REVEAL THE MORPHOLOGY OF A WEST AFRICAN SORGHUM RESISTANT TO STORAGE INSECTS

MICHAEL W. PENDLETON[1], E. ANN ELLIS[1], BONNIE B. PENDLETON[2], and NIAMOYE YARO DIARISSO[3], [1]Microscopy & Imaging Center, Texas A&M University 2257, College Station, TX 77843-2257, [2]Agricultural Sciences, Box 60998, West Texas A&M University, Canyon, TX 79016-0001, and [3]Institut D'Economie Rurale, B.P. 258, Bamako, Mali.

The insect pest which causes the most damage to stored grain is the maize weevil, *Sitophilus zeamais* Motschulsky. The resistance to 8 varieties of sorghum, *Sorghum bicolor* (L.) Moench to maize weevils was determined by the percentage of kernel weight loss and damage score (1 to 5, 5 being greatest damage) of kernels following weevil exposure. Shallow cuts (1mm depth) were made with a razor on kernels of 7 genotypes of sorghum (except Seguifa), fixed in 2.5% glutaraldehyde - 1% acrolein in HEPES buffer (pH 7.3), followed by 1% osmium in HEPES buffer (pH 7.3). Methanol dehydration was done at 5% steps, followed by 3 changes of Hexamethyl-disilazane (HMDS). Dried kernels were polymerized in low-viscosity resin (6 changes), cut using a MicroStar diamond knife in cross section with a Porter Blum MT-1 ultramicrotome, and coated with iodine (IKI) vapor. Block faces were coated with 35-50 nm of carbon by using Cressington 308R evaporative coater. Kernels were imaged in both secondary and backscatter modes in a JEOL JSM-6400 SEM (15 kV and 15 mm working distance). Seguifa kernels were initially cut using a razor in cross-section, then fixed, dehydrated, and polymerized in the same way as the other 7 varieties. Sectioning was done using a MicroStar diamond knife with a Porter-Blum MT-1 ultramicrotome just below the initial razor cut and the sectioned blocks were coated with iodine (IKI) vapor and also carbon coated (50 nm) using a Cressington 308R coater. The improved sectioning and embedding technique using to produce the blockface of the Seguifa kernels allowed SEM backscatter signals to penetrate the resin within the voids in the blockface so details of void structure were visible. The voids of the blockfaces of the other 7 varieties of sorghum showed little detail. Compositional contrast (bright areas) shown under SEM backscatter mode was used to locate the layer of starch on the blockface of each of the 8 varieties of sorghum tested in this study. These well-defined bright areas on the blockface facilitated the measurement from the starch layer to the outer edge of the kernel seed coat to be made for all 8 varieties of sorghum. An increase in depth of the starch layer to the seed coat was determined to be highly correlated to a decrease in the percentage of kernel weight loss and to a decrease in damage score for the varieties of sorghum tested. [Supported in part by the Sorghum, Millet & Other Grains Collaborative Research Support Program (INTSORMIL CRSP), USAID Grant No. LAG-G-00-96-900009-00].

NANO DRUG DELIVERY SYSTEMS SPECIFICALLY TARGET SUBSETS OF NEURONS AND EFFECTIVELY PROMOTE AXON GROWTH (NEURITE OUTGROWTH)

SUMOD SEBASTIAN[1], REMYA A VEETTIL[1], SANTANEEL GHOSH[2] and DIANNA. HYNDS[1], [1]Texas Woman's University, Denton, TX 76204, [2]Southeast Missouri State University, Cape Girardeau, MO 63701.

Traumatic injury to the central nervous system causes acute neuronal death and surviving injured neurons do not readily regenerate their axons, leading to permanent functional loss. Nanomaterial-based drug delivery systems provide potential for encouraging axon regrowth from specific neurons. In the present study, we analyze both time-dependent and dose-dependent effects of magnetic nanoparticles (MNPs) on neurite outgrowth, specifically, neurite branching from PC12 and B35 cells. We expect non-toxic MNP levels will not affect neurite extension and branching. In future work, we will investigate the mechanisms of cellular uptake of surface functionalized nanospheres and assess the ability of derivitized MNPs to enhance axon growth in corticospinal tract neurons. Together, these results will identify potential nanocarriers for targeted drug delivery to encourage axon following nervous system damage. [Supported by TWU Department of Biology, The Southeast Missouri State University Department of Physics and Engineering Physics, and grants from the TWU Research Enhancement Program]

TESTICULAR MACROPHAGE MAY BE INVOLVED IN LEYDIG CELL APOPTOSIS FOLLOWING EDS TREATMENT

BARKHA SINGHAL, SAMUEL SANG, ARPITA TALAPATRA, DIBYENDU DUTTA, IN PARK and NATHANIEL MILLS, Department of Biology, Texas Woman's University, Denton, Texas 76204.

Tumor necrosis factor alpha (Tnfα), a cytokine secreted by macrophages is known to initiate cell apoptosis. Direct associations of macrophage with Leydig cells in testicular interstitium have been reported previously. RT and qPCR products were quantified using (-) ΔΔCt method. Leydig cell loss

was tracked by quantifying the mRNA expression of 3 beta hydroxysteroid dehydrogenase (*3βHsd*), luteinizing hormone receptor (*Lhr*) and Insulin like peptide 3 (*Insl3*). We have found >80% loss of *Lhr* and *Insl3* mRNA along with a 100% loss in *3 beta HSD* mRNA at 24 hr post-EDS. Using TUNEL, a DNA fragmentation detection assay we have observed a time dependent loss of Leydig cells by apoptosis. We find a high percentage loss of Leydig cell markers suggesting that the Leydig cell is depleted. We next measured genes that are associated with inflammation. A significant increase in *Tnfα* mRNA, a six-fold increase Tnfα receptor superfamily member 1A mRNA (*Tnfrsf1a/ CD120a*) was found. However, no change was observed in tumor necrosis factor receptor superfamily member 1B mRNA (*Tnfrsf1b/CD120b*). *CD68* mRNA (a macrophage/monocyte/dendritic cell marker) was elevated at 24 hr post-EDS. Thus, not only are macrophages present in testes but macrophages may also be increasing in cell number/testis following EDS treatment. We suggest a potential paracrine action of macrophages through secretion of TNFα to induce Leydig cell apoptosis. However, we need to look at earlier time points for changes in Leydig cells. Altenately, we need to look for the increase in receptors for TNFα in other testicular cell populations in response to EDS.
[Research Support: Research Enhancement Program (REP 2011-2012).]

TESTOSTERONE MODULATES THE ROLE OF THE Bcl2 FAMILY OF APOPTOTIC GENES IN TESTES

ARPITA TALAPATRA, SAMUEL SANG, BARKHA SINGHAL, DIBYENDU DUTTA, IN PARK, HIWOT GUILILAT, and NATHANIEL MILLS, Department of Biology, Texas Woman's University, Denton, TX 76209.

Apoptosis, characterized by shrinkage of total cell volume, increased cell densities and compaction of cell organelles, is an important regulatory process during spermatogenesis. Male rats were injected with ethane dimethane sulfonate (EDS) (75mg/kg body weight) to selectively eliminate mature Leydig cells thereby ablating testosterone. Tissues were collected after 7-days of treatment for the TUNEL assay and analysis of gene expression modulated by testosterone. To substantiate testosterone's role, separate groups received exogenous testosterone for either supplementation or replacement of testosterone following EDS. Significant germ cell apoptosis in EDS-treated rats was demonstrated by the TUNEL assay and testosterone replacement prevented the germ cell apoptosis. The levels of pro- and anti-apoptotic genes of the Bcl2 family in testes were determined by RT PCR. The results showed a significant increase in pro-apoptotic *Bak1*, and anti-apoptotic *BclW*, *Bcl2* and *Mcl1* genes in testes of EDS-treated rats. We suggest that the Bcl2 family of genes is involved in germ cell apoptosis and is modulated by testosterone.

MICROSCOPIC EVALUATION OF GENOTOXICITY INDUCED BY ELIMINATOR, A WEEDICIDE TO ALLIUM CEPA TEST SYSTEM

MICHELE VELOZ, GRISELDA ESTRADA and NABARUN GHOSH, Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79015.

The last two centuries have witnessed the indiscriminate development and overexploitation of natural resources by man causing alterations and impairment of our own environment. The *Allium cepa* assay is an efficient test for chemical screening and *in situ* monitoring for genotoxicity of environmental contaminants. The test has been used widely to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberrations in root meristems1 of *A. cepa*. Weedicide residues can be present in fruit and vegetables and represent a risk for human health. *Allium cepa* has been established historically as the standard test system for plants when studying the effects of chemicals on cell processes, namely mitotic index and chromosomal abnormalities. It is preferred by scientists for its high root tip yield, large, well-dispersed chromosomes, low cost, and ease of cultivation (Sharma, 1986). *A. cepa* exhibits prolific root growth. We established a hydroponic culture in the culture room with controlled photoperiod and temperature. We exposed the fully growing roots of the plant to 1%, 0.1% and 0.01% of Weed and Grass Killer: Eliminator. This weedicide contains 41% glyphosate and isopropylamine salt. The ingredients are known for

toxicity to humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity to aquatic organisms. This weedicide is widely used in lawn and gardening practices. We after pretreatment with pDB solution, fixing with 3:1 Aceto-Ethanol and staining with 2% Aceto-Orcein we observed the squashed root tips from Control and treated sets under BX-40 Olympus microscope attached to a DP-70 digital camera to the computer. The visual examination revealed the gradual decay of the root tip cells and discoloration of the root tips after the treatments with the weedicide. There was a gradual decrease of the Mitotic Indices from the control with 7.9% to 4.6% and to 0.3% in course of time. We observed pycnosis all over the spreaded root tip smear slide with formation of micronuclei at 24 hours with 1% treatment. We also recorded multipolarity, anaphase bridges, laggard and chromosomal abnormalities. Our investigation revealed that the said weedicide could be potentially genotoxic.

AEROBIOLOGY AND MICROSCOPIC ASSESSMENT OF SAFETY MEASURES OF AHPCO NANOTECHNOLOGY IN ERADICATION OF AEROALLERGENS

MICHELE VELOZ[1], RACHEL PALADINO[1], JONATAN SANCHEZ GAMA[1], JEFF BENNERT[2] C. SAADEH[3] AND NABARUN GHOSH[1], [1]Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, Texas 79015. [2]Air Oasis, 3401 Airway Blvd. Amarillo, Texas 79118. [3]Allergy ARTS, Amarillo, Texas 79124.

Global warming and climate change exert immense impact on biotic systems. There are increased cases of allergy and asthma cases with the increased aeroallergen index, early flowering and shift in peak allergy season. We analyzed 12 year's aeroallergen data of Texas Panhandle using a Burkard Volumetric Spore Trap. Exposed, stained Melinex tapes were analyzed with a BX-40 Olympus microscope attached to DP-70 Digital camera with Image Pro Plus. We observed aeroallergens including pollens, fungal spores, dusts, plant fibers, burnt residues and plant products like gums and resins are often the cause of serious allergic and asthmatic reactions, affecting millions of people each year. A decade research developed an air purification system that uses Advanced Hydrated Photo Catalytic Oxidation Nanotechnology to reduce indoor aeroallergen to improve air quality and better food preservation. We cut uniform sections of tissue from fruits and vegetables using a standardized cork-borer and exposed the tissue samples to the AHPCO system and room air (Control). Tissue samples with AHPCO system showed no sign of infection. The fungal and bacterial colonies were analyzed from the tissues in room air after 72 and 120 hours. We evaluated the safety measures of the air purification system by exposing human epithelial cell, blood and plant cell cultures in a fiber glass (AO) chamber with the air purification system. The cultured cells showed no cytological anomaly or instability when exposed in the AO chamber. A brief exposure of UV ray was administered in UV chamber, RBCs and WBCs got distorted showing cytological instability and coagulation. Human cells in culture exposed to AO chamber, images captured with FITC Filter, at 40x, BX 40 Olympus Microscope. With a brief exposure to UV rays the cells lysed in culture leaving residues that could be detected with TRITC imaging. The Air Oasis air purifiers reduced MRSA during transfer in the BSA Hospital and also reduced the symptoms of allergy in dogs as evidenced in projects ran at the BSA Hospital and Coulter Animal Hospital, Amarillo, Texas.

MUTATION OF ARP3 SERINE 226 ALTERS PROTEIN CONFORMATION

ALEXANDRA WRIGHT[1], AMRUTA C. MAHADIK[1], S. HALDAR[1], D. L. HYNDS[1], BRIAN W. BECK[1,2,3], [1]Departments of Biology, [2]Math. & Comp. Science, and [3]Chemistry, Texas Woman's University, Denton, TX 76204-5799.

In developing neurons, the branching of the actin filaments is the main force spurring the formation of lamellipodia, which play an important role in the progression of axonal growth cone. The Arp2/3 complex is responsible for the actin filament branching. Arp2 and Arp3, the major subunits of the Arp2/3 complex, nucleate actin branches by forming the first short pitch dimer of the daughter actin filament.

Using computational analysis, Haldar and colleagues have demonstrated that R161A Arp3 mutant destabilizes the inactive conformation of Arp 2/3 complex, leading to increased actin branching in the B35 neuroblastoma cells. We hypothesize that a point mutantion at the 226 position to glutamine would also destabilize the inactive conformation of Arp 2/3 and may lead to increased actin branching. We used confocal microscopy to analyze the levels of actin filaments in untransfected cells or B35 cells transfected with the empty vector (just GFP), GFP-tagged wild type Arp3, or Arp3 mutated at serine 226.

We believe that this study will help to understand the cytoskeleton arrangements in the lamellipodia during axonal growth cone progression. This may help in the developing treatments that promote the axon regeneration following the spinal cord injury.
Supported by the TWU Department of Biology and grants from the TWU Research Enhancement and Multidisciplinary programs.

MATERIAL SCIENCE

POSTER PRESENTATIONS

STUDY OF ANTI-WEAR BEHAVIOR OF NEW ASH-LESS ANTI-WEAR ADDITIVES IN BLENDS WITH ZDDP IN LUBRICATING OILS.

VIBHU SHARMA†, ALI ERDEMIR†† and PRANESH B. ASWATH†, †Materials Science and Engineering Department, University of Texas at Arlington, Arlington, TX 76109, †† Argonne National Laboratory, Argonne, IL.

New ashless antiwear additives have been examined as possible supplements to ZDDP to enhance wear resistance and formation of stable tribofilms while reducing the sludge formation and deposits in automotives. The present study reports the anti-wear behavior of two new ashless anti-wear chemistries. Four different blends of lubricating oil containing two ashless addtives in base oil with ZDDP were examined using a pin on flat type high frequency reciprocating rig (HFRR) tribometrical test set-up at two different load conditions i.e 54N and 350N. The incubation times for the formation of tribofilms were recorded from ECR results. The morphology of tribofilms was studies using high resolution SEM images and 3D profile of tribo films were obtained using scanning probe microscopy (SPM), which indicates a nice patchy film formation on the substrates materials. Mechanical properties were also measured using nano-indentation technique.

FABRICATION AND CHARACTERIZATION OF Au/Fe MULTILAYERED NANODISKS

THUMTHAN ORATHAI, HUANG CHEN-WEN, and HAO YAOWU, Materials Science and Engineering Department, University of Texas at Arlington, Arlington, TX 76019.

Au/Fe multilayered nanodisks were fabricated by pulse electrodeposition into anodic aluminum oxide (AAO) template. Using an electrolyte containing both Au and Fe ions, the alternative segments of Au and Fe can be formed by controlling applied potential and pulse duration. Au and Fe were deposited by applying potential of -0.45V and -1.2V (VS. Ag/AgCl(sat.KCl)) respectively. Pulse durations were varied to tune the thickness of the disks. Each layer was limited under 100 nm thick and the diameter was in range of 80-100 nm. The morphology of Au/Fe multilayered nanodisks was studied by SEM and TEM. The magnetic properties of Au/Fe multilayered nanodisks of different segmental length of Au and Fe were studied by vibrating sample magnetometer (VSM) at room temperature.

UTA CCMB: STATE-OF-THE-ART RESEARCH FACILITY FOR ADVANCED MATERIALS AND BIOLOGY CHARACTERIZATION

D. J. YAN, J. C. JIANG and E. I. MELETIS, UTA Characterization Center for Materials and Biology, Department of Materials Science and Engineering, University of Texas at Arlington, Arlington, TX, 76019, USA.

One of the key issues for developing advanced materials with desired properties is the characterization of their chemistry, microstructure,

crystallography, and defect structure (i.e., interfaces, grain boundaries, dislocations, etc.) even at the atomic scale level. UTA Characterization Center for Materials and Biology (c^2mb, http://ccmb.uta.edu) is a recently established centralized research facility to provide state-of-the-art instrumentation for a variety of materials characterization and property measurements such as chemical composition, micro/nano-structure, defects, morphology, and related thermal, electrical, magnetic and mechanical properties. The facility houses multi-user, major materials research instrumentation in a 5500 sq. ft. space. The facility is available to researchers from UTA, other institutions and industry. Instrumentation in C^2MB includes X-ray photoelectron and Auger spectroscopy, scanning electron microscopy, X-ray energy-dispersive spectrometry, analytical transmission electron microscopy (TEM), high-resolution TEM, Atomic Force Microscopy, X-ray diffractometry, Nanoindentation/Scratch, FTIR Spectrometry, Raman, Thermal Analysis Systems, hardness and microhardness testing and facilities for sample preparation. Applications of these pieces of instrumentation in materials research will be presented.

IN MEMORANDUM

Texas Society for Microscopy member and former Secretary Tina Halupnik lost her battle with cancer on October 7, 2012. Even while she was battling the disease she managed to attend the 2012 TSM meeting in Fort Worth, Texas. Tina is shown in this photo sharing a meal with her husband Steven at the Texas Society for Microscopy meeting in 2007. Tina will be remembered for her energy, sense of humor and devotion to those around her and to our society. (Photo supplied by Nabarun Ghosh, West Texas A&M University).

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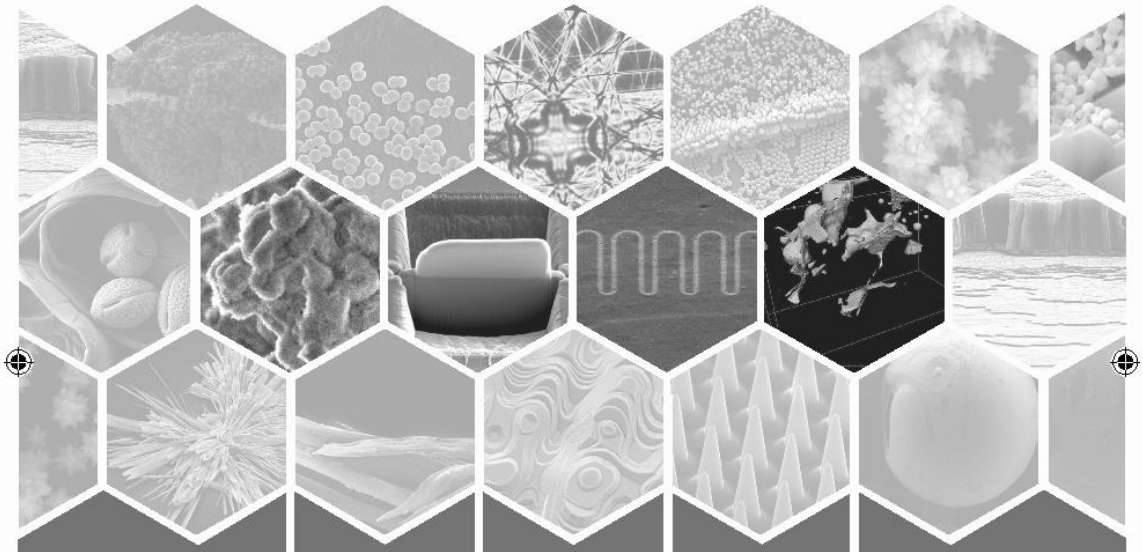

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Enhancing Vocabulary Acquisition Skills of English Language Learners in the Middle School Science Classroom Through the Use of Inquiry-based Science Methods, Word Walls, and the Scanning Electron Microscope

C. L. Sieber and S. L. Westmoreland

Texas Woman's University, Department of Biology

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Abstract

A nine-month study was conducted in a sixth grade science classroom in rural North Texas to determine if hands-on science learning through the use of inquiry-based science methods, a word wall, and a discovery wall with micrographs from a scanning electron microscope would increase the vocabulary acquisition of English Language Learners (ELLs). The researcher predicted that by incorporating these specific techniques into his curriculum, the scores of the ELLs in the sixth grade science classroom would increase significantly more on the end of year vocabulary post-test assessment than the native English speaking students. Furthermore, he predicted that the overall gain of the ELLs on the post-test would show an increase of at least 10% over the pre-test. The data showed that the ELLs gained an average of 12% from the pre-test to the post-test compared to the non-English Language Learners (non-ELLs) who gained an average of 9% from the pre-test to the post-test. These data revealed that the ELLs demonstrated a significantly greater gain ($p<0.001$) on the end of year vocabulary post-test than the non-ELLs.

Introduction

The population of individuals in Texas who identify themselves as Hispanic has increased by 41.8% in the last ten years. This increase ties Texas and California at 37.6% with the most number of people who speak Spanish as their primary language in the United States (United States Census, 2010). Across the state of Texas, school districts have seen this increase as well. The student population of Pilot Point Independent School District was 32.66% Hispanic at the end of the 2010-2011 school year (Pilot Point ISD, 2011). The large Hispanic subgroup coupled with "consistently lowest-achieving" students qualifies Pilot Point ISD as a Title I school as defined by the United States Department of Education (Texas Education Agency, 2011).With the increase of students who speak English as a Second Language (ESL), the demands placed on teachers to meet the needs of these students also increases. Additionally, with the implementation of the No Child Left Behind Act (NCLB) in 2001, the pressure on students and educators alike to meet the state and federal requirements has also increased. Lincoln and Beller (2004) found that due to increased pressure to improve student performance on standardized tests, teachers find themselves required to meet the needs of students with diverse language skills (p. 28).

With this increase in student diversity, it is imperative that educators adjust their teaching strategies. The same strategies that were used ten years ago do not address the dynamic needs of the diverse population of students entering today's modern classroom. More specifically, in the science classroom, the ELL is a not only struggling to understand conversational English, he or she is struggling to comprehend scientific language as well. ELLs often lack the developed English literacy necessary to acquire the academic language that is essential for learning scientific language (Lee and Fradd 1996, 1998). Wellington and Osborne (2001) believe that "teachers must take into account the nature of scientific concepts, scientific language, scientific reasoning, and scientific value" and that "one of the major difficulties in learning science is learning the language of science" (p. iv, 1). Educators must strive to meet the needs of all the

learners in their classrooms whether they are predisposed towards science or not. As a veteran educator with twelve years of science teaching experience, the researcher has become increasingly aware of the struggles that non-English speaking students face in the science classroom on a daily basis. Settlage et al. (2005) found that:

> The science education community seems unable to come to terms with the disparities in science learning of students from different cultural groups. The lingering divide between white student achievement and all other groups would imply we are only able to teach science to those students who are favorably predisposed towards the subject (p.39).

In order to meet the specific needs of the changing diverse population of learners, the modern educator must find new avenues to accommodate the ELL in a way that does not dilute the content. Hansen, 2006 believes that "English language learners have special needs that must be met with pinpointed strategies . . . so they can understand the content being taught" (p. 22).

Scientific Inquiry

In the diverse middle school classroom, the process of scientific inquiry offers many benefits. The instructor builds a strong rapport with his students as he guides them through the learning experience. Also, by being the facilitator, instead of the lecturer, the teacher creates a classroom climate that fosters student leadership. Students become more responsible as they take control of their individual learning experience. Polman (2001) suggests that "inquiry-based science instruction offers great promise as a means of actively engaging students in authentic scientific problem solving" (p. 223). When student are actively engaged in their learning experience, then they are invested in the scientific process which allows for greater understanding of real-world science.

Word Walls

Often students learn the definition of a word but do not know how to demonstrate what the word means. Scientific language is even more difficult for the learner to comprehend than academic language which causes students to struggle with the vocabulary even more. The National Center for Educational Statistics (2002) reported, "Although 92.7% of students could understand basic scientific principles, only 57.9% could apply them. The use of word walls in the science classroom in this study allowed students to review and revisit difficult scientific terminology repeatedly until they fully understood the meaning of the word and were able to retain and apply that knowledge. Harmon et al. (2009) believe that "the use of interactive word walls holds instructional potential for enhancing vocabulary learning as students engage in activities centered around the word wall-activities in which students explore, evaluate, reflect, and apply word meanings in meaningful context" (p. 399).

The Scanning Electron Microscope

In the classroom setting, the SEM allows students to experience "real world" science. When student are actively engaged in their learning experience, then they are invested in the scientific process which allows for greater understanding of real-world science. Newmann (1986) argued that "engaged students care about their work and commit themselves to it because their work seems valuable beyond the confines of the classroom" (p. 240). Additionally, higher-level thinking skills are used as the students synthesize the information gained in the process of inquiry by collaborating with their student colleagues.

Although the use of a SEM in the middle school classroom has not been well documented, the use of microscopes in the process of scientific inquiry offers students the opportunity to view their world from a different perspective. The majority of the Hispanic student population in this study may never have the opportunity to learn about a SEM or participate in "real world" science. Therefore, by providing students with the opportunity to learn about the SEM and participate in the process of scientific inquiry the researcher is nurturing future scientists. Jarrett (1997) suggests that "students develop critical thinking skills by learning through inquiry activities" (p. 8). Also, students who address questions that arise from student experiences are more invested in the process of scientific inquiry

and therefore have a richer learning experience (National Research Council, 1996).

Materials and Methods

Participants

The participants in this study were 194 (52.1% Caucasian, 42.3% Hispanic, and 3.6% African American 1.55% Native American, .52% Asian) sixth grade students. These students were divided into five sections of sixth grade science. All sections were heterogeneously mixed with special education, section 504, regular education, gifted and talented, dyslexic, and ELL students that overall represented a range of learning abilities. Students in all sections of science participated in regular classroom activities. Students worked together in cooperative groups of approximately four to six students. During this study, the Texas Essential Knowledge and Skills (TEKS) were used as a guideline to design inquiry-based science lessons. TEKS are the Texas state standards which designate concepts to be learned in K-12 public classrooms. The inquiry-based science method of teaching was used to engage students in the learning process. Through this process the students were able to control their own learning experiences.

Word Wall

At the beginning of the 2011-2012 school year, a word wall was constructed in the classroom for all students to view and use throughout the year (figure 1). As an introduction to the word wall concept, the researcher included information describing what a word wall was and the purpose of having a word wall in the science classroom. Kieff (2003) said that, "word walls are organized collections of frequently used words, written in large bold letters and displayed in the classroom" (p. 84I). Instead of dictating how the word wall was to be used, the researcher allowed the students to decide how the identified words would be displayed on the wall. After discussing several options, it was decided that the students would identify any word that was unfamiliar in the class, textbook, or other source of media. The students were encouraged to select words to add to the wall anytime they identified a word as new or unfamiliar. Occasionally, words from previous science classes were identified and added. Eventually, the students felt that a definition of the word would be a beneficial addition to the word wall. Then, as new learning strands and concepts were introduced, the students defined and discussed the words and added the words identified as hard to comprehend to the wall. Words that needed to be re-emphasized were left on the word wall until all students felt that they understood the meaning. Students were encouraged to create their own personal dictionaries for extra points. After each unit was completed, the students played bingo with the words from the word wall to reinforce what they had already learned.

Scanning Electron Microscope and Inquiry

During the spring semester, the students were introduced to the idea of using the SEM to learn about the natural world. To extend the inquiry learning experience, the Hitachi TM-1000 SEM was used to take the micrographs of the items that the students brought to class. The items were taken to Texas Woman's University and imaged using the SEM. The micrographs were displayed on a bulletin board for the students to view and make predictions as to what the items were (figure 2). The different items were changed on a weekly basis so that students from each class were able to contribute items to the SEM discovery wall. After the specimens were collected, then the students prepared each specimen by mounting it on a stub. Within the group, the students worked together and decided which specimens to have imaged (figures 3, 4, and 5). By working cooperatively throughout the inquiry process, the students gained ownership of their experience and became more interested in the scientific study of their natural world. Finally, the portfolio that the students created with the micrographs that were taken throughout the year and displayed on the discovery wall enriched the learning experience of the students in this study and gave them a sense of accomplishment.

Assessment

A quantitative research design was used to collect and analyze data from criterion referenced pre-tests and post-tests. The baseline test, pre-test, and the post-test consisted of 100 vocabulary questions and

were designed using the Texas Essential Knowledge and Skills (TEKS) guidelines. All three of the tests were identical. Descriptive statistics were used to analyze data from the 2010-2011 baseline tests.

These data were used to compare to the results of the 2011-2012 post-test in order to show the difference between the end-of-year scores of the students who had no intervention to the end of year scores of the students who received the intervention. A student's t-test was used to analyze the data from the baseline test compared to the post-test, and also from the pre-test to the post-test of the study group. Additionally, artifacts were collected through photographs of the word wall, and micrographs of the specimens that students chose to have taken with the Hitachi-TM 1000 SEM to provide supporting evidence of the success of the intervention.

At the end of the 2010-2011 school year, a baseline test was given to 102 (50.98% Caucasian, 41.18% Hispanic, 2.94% African American, .98% Native American, and 1.96% Asian) sixth grade students. The baseline test was administered in May 2011. The students to whom the baseline test was administered did not receive any intervention above the normal classroom lessons created from the CSCOPE curriculum that the school district mandated.

The pre-test that was given at the beginning of the 2011-2012 school year was given to 92 (53.26% Caucasian, 4.35% African American, 1.09% Native American, and 41.30% Hispanic) sixth grade students. All students were given the test two weeks after school started. Prior to the pre-test, the students were not given any preparation or vocabulary list. All results were recorded on a spreadsheet to compare with the baseline test results and the post-test results.

A post-test was given at the end of the 2011-2012 school year to 96 (54.7% Caucasian, 4.2% African American, 1.05% Native American, and 40% Hispanic). All of the students were given the post-test in May 2012, three weeks before the end of the 2011-2012 school year. Prior to the post-test, the students were not given any time to review the material in their science journals or given any type of study materials. All results were recorded on a spreadsheet to compare with the baseline test results from 2011 and the pre-test results from the beginning of the 2011-2012 school year.

Results

Observational Results

From the onset of this intervention, the non-ELL students spoke out often if there was a word that they did not know. This was observed for approximately six weeks. Then, as the ELL students became more comfortable in the classroom and began to realize that they were not the only students who did not understand words, the ELL students began to slowly request specific words be added to the science word wall. The researcher observed that the ELL students began to relax in the science classroom and participate more freely in the activities and lessons. Frequently, the ELLs were observed looking at the word wall as they worked independently.

Additionally, the ELL students had a similar response to the inquiry-based learning methods that were used in the classroom. When the first inquiry-based lesson was introduced many of the ELLs seem confused and unsure of what to do. They would pair up with another ELL student and try very hard to be invisible as the non-ELLs would dominate the classroom activities and discussions with questions, ideas, and feedback. However, by the end of the first six week time period the ELLs were beginning to understand the concept of inquiry-based learning. Although it took time for the students to transition from teacher-led to student-led activities, the ELLs began to realize that the process of asking questions and seeking answers could be accomplished in any language.

The use of the SEM was not implemented until the second semester of the 2011-2012 school year. After the students became familiar with the science word wall and the process of scientific inquiry, then the SEM was introduced to the students. The researcher presented micrographs that were taken with the SEM to the students along with video, pictures, and an explanation of how the SEM

worked. All students were allowed to use the internet to explore additional micrographs and information regarding the SEM. Next, the students were encouraged to bring any item that would fit on a dime to school. The students were very serious when they placed their specimens on the SEM mounts. At this point in the school year, the students were comfortable with the concept of inquiry and eager to explore their home as well as their school environment. As the researcher began posting the micrographs on the discovery wall the students' excitement grew and they enjoyed trying to guess what the micrographs were. The experience with the scanning electron microscope cumulated in a visit from a Hitachi representative to the sixth grade classroom. The gentleman brought a TM-2000 Tabletop scanning electron microscope for the students to use. As the gentleman spoke about the uses of the scanning electron microscope in the field of science, the students prepared their slides by placing each item on the small, round, metal stub used to insert items into the SEM. From the collecting of the specimens to the focusing of the image, the students were involved and eager to demonstrate their knowledge of the scanning electron microscope. By the end of the day, each student had their own micrograph of an object that was discovered through inquiry; they exhibited their understanding of the SEM, and were able to take home evidence of their scientific discoveries to share with their families.

Statistical Results

At the beginning of the study, the students were administered a 100-question vocabulary pre-test. The results were recorded on a spreadsheet and segregated according to the student's ethnicity. Then, at the end of the 2011-2012 school year the sixth grades students took the summative vocabulary post-test. Data from the baseline test were compared to the data from the post-test to determine if there were any significant differences between the baseline test results and the post-test results of the students who received the intervention. Next, the study group pre-test data were compared to the data from the study group post-test to determine the mean of the pre and post-test. Then, the students were separated into an ELL group and a non-ELL group and data were further analyzed. As the researcher was segregating the groups, students who left the school district, were new to the school district and/or did not take the pre-test, or were unable to take the post-test due to other extenuating circumstances were excluded from the final groups. The difference between the 2011-2012 pre- and post-test scores was then analyzed.

When comparing the 2010-2011 baseline test and the 2011-2012 post-test there was no significant difference. However, the 2011-2012 sixth graders as a whole showed an increase of 11.34% ($p<0.001$) on the post-test. The non-ELLS showed a total gain of 8.99% ($p<0.001$) when comparing the pre- and the post-test. The ELLs gained 12.17% ($p<0.001$) from the pre-test to the post-test (table 1). Therefore, the data show that while both the ELLs and the non-ELLs made significant gains, the ELLs gained 3.18% more on the 2011-2012 post-test (Figure 6).

Discussion and Conclusion

With the rapid increase of the Hispanic population in the state of Texas over the last ten years, the face of the 21st century classroom has changed. The changing demographics of the modern classroom have created new challenges for educators. No longer can teachers expect their students to speak "their" language. The modern educator must develop new strategies to meet the needs of the diverse learners in their classroom. As a veteran science teacher, the researcher became increasingly aware of the changing demographics in the Texas classroom. This awareness, along with the rising demands of standardized test scores placed on educators, led the researcher to question traditional methods of teaching science to middle school students. The idea of hands-on, discovery-based learning has been around for years, nevertheless, many educators balked at the idea of teaching in a student-led environment. However, as the needs of the student population change, so must the methods of teaching. With this change, the researcher created a visually stimulating science classroom and laboratory, a cooperative learning environment where students sat in collaborative groups instead of rows, and a vocabulary rich classroom where displays and activities promoted understanding of scientific language.

The inquiry-based science lessons gave students the opportunity to be in charge of their learning experience. At the beginning of the year many students were confused and even a little afraid to make decisions. The researcher observed that the ELLs specifically were very hesitant when it came to participating in class activities. Despite their original trepidation, the students began to enjoy the learning experience and look forward to the next lesson. Eventually, students would approach the researcher with new ideas of projects and laboratory investigations that would enhance the lesson being taught. By the end of the school year some of the ELLs were the first students to step forward and volunteer to share their work with the rest of the class. These observations of the newfound confidence of the ELLs and the enthusiasm displayed by all students led the researcher to conclude that teaching through inquiry-based learning allowed students to use their strengths and work collaboratively with their peers to successfully gain understanding of the subject matter taught.

Before the intervention began, the researcher believed that by using inquiry-based learning, a word wall, and the SEM, ELLs would become more interested in the scientific learning process. Additionally, he believed that the ELLs would show an increase of 10% over the formative pre-test assessment. The data from this experiment showed a significant increase by the ELLs and the non-ELLs on the vocabulary post-test. These three interventions increased the scores of the students on the 2011-2012 end of year vocabulary assessment by 8.99% (Non-ELL), 12.17% (ELLs), and greater than 11% overall for the whole 2011-2012 study group which was greater than the predicted hypothesis.

Future enhancement of this experiment could include a baseline pre- and post-test. The word wall could facilitate the integration of Spanish vocabulary into the science curriculum by placing the words on the word wall in English and Spanish. Also, the word wall could include images of the vocabulary words and concepts placed on the wall. Additionally, student input and comments on the pre- and post-test could help the researcher understand each individual's thoughts and comprehension of the subject-matter taught. Finally, possible open-ended questions instead of multiple-choice questions would allow students to use higher-order thinking skills as they described the science concepts.

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Tests Compared	Group Tested	Significance	Percent Change
Baseline vs. Post-Test	All 2010-2011 Students compared to All 2011-2012 Post-test Students	P=0.47 No significance	0.13%
Pre-test vs. Post-test	All 2011-2012 Students	P<0.001 Highly Significant	11.34%
Pre-test vs. Post-test	2011-2012 Non-ELL Students	P<0.001 Highly Significant	8.99%
Pre-test vs. Post-test	2011-2012 ELL Students	P<0.001 Highly Significant	12.17%

Table 1. Percent change of all tests analyzed.

Note: The results of the analysis show that the comparison between the 2010-2012 baseline test and the 2011-2012 post-test showed no significance in the percent change. Additionally, the other three groups tested showed highly significant results with a greater than 8% change.

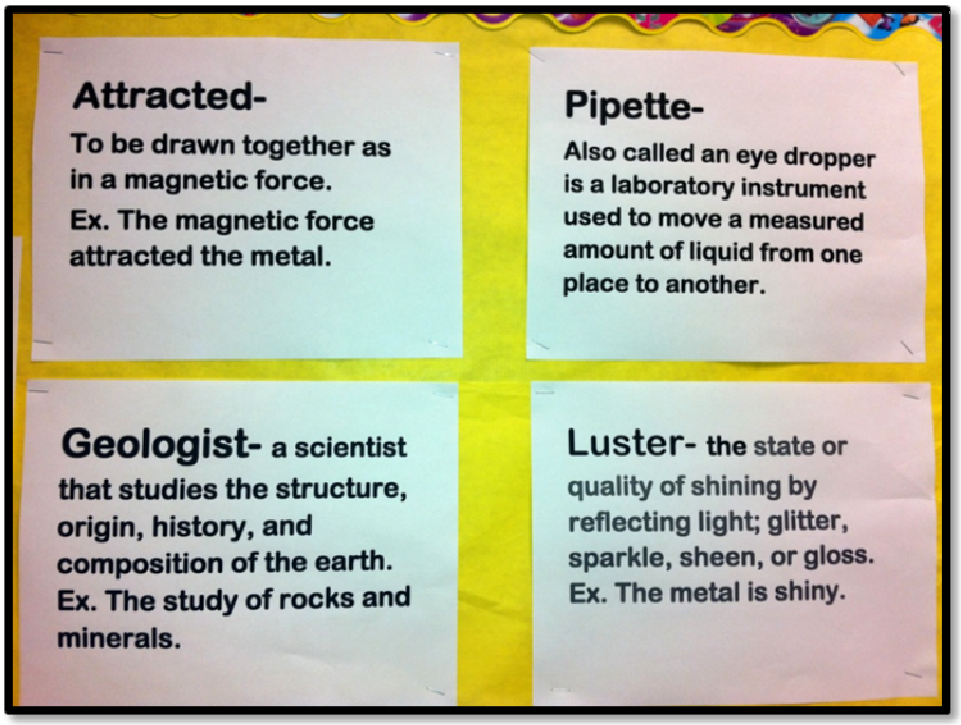

Figure 1: A photograph showing several words which were identified as difficult to understand or remember by students during the first six weeks of the 2011-2012 school year.

Figure 2: This photograph is of the discovery wall with micrographs of student selected items imaged with the TM-1000 Scanning Electron Microscope at Texas Woman's University.

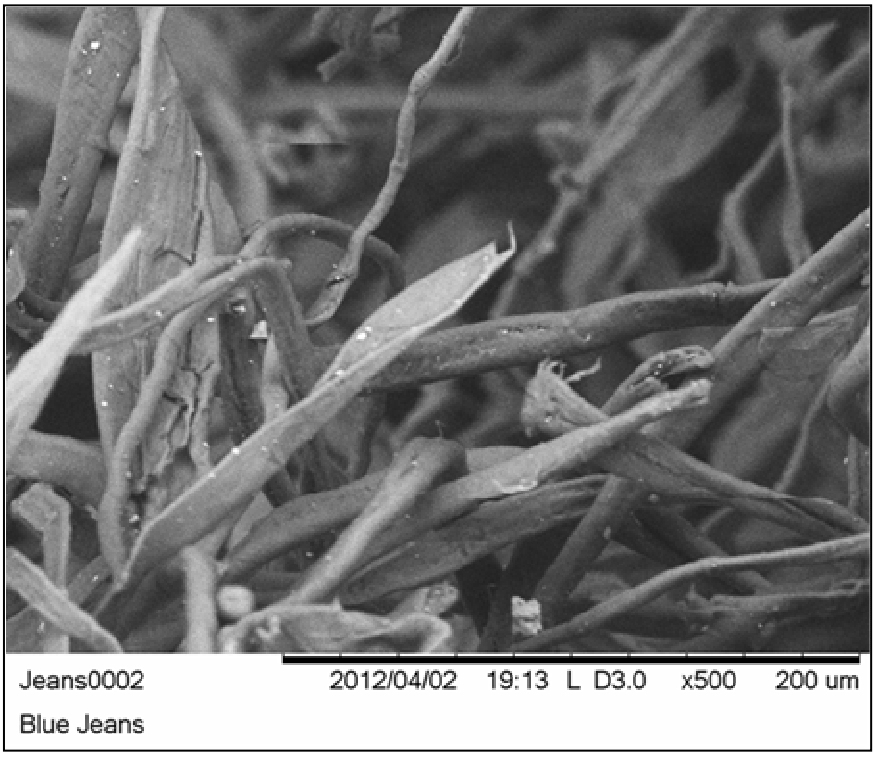

Figure 3: This image is of a piece of blue jean fabric that a student found during trash pick-up for Earth Day. The magnification on this image is 500X.

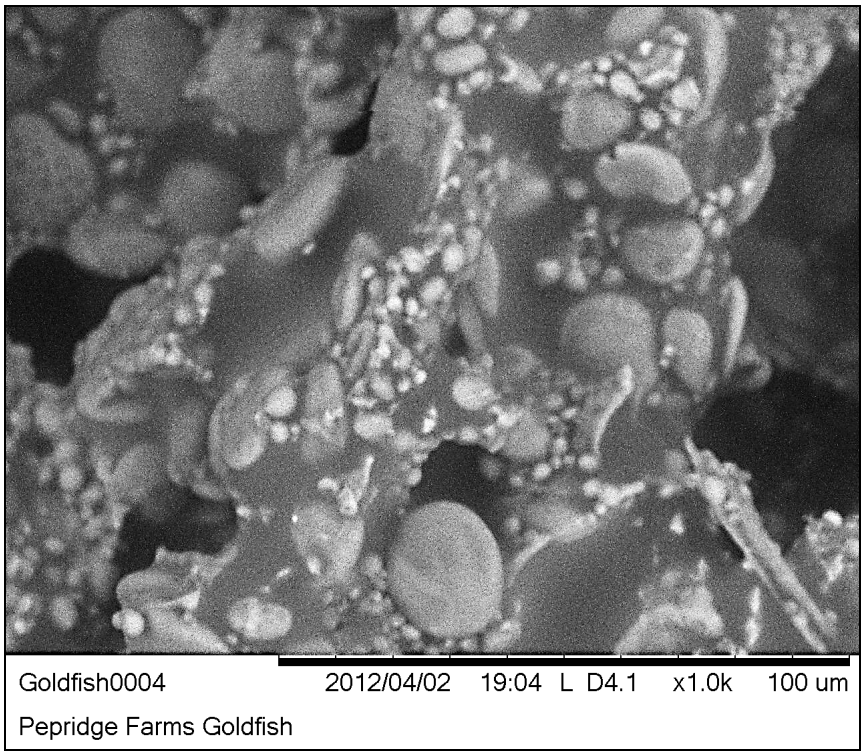

Figure 4: This image is of a piece of Goldfish cracker from a student's lunch. Magnification 1000X.

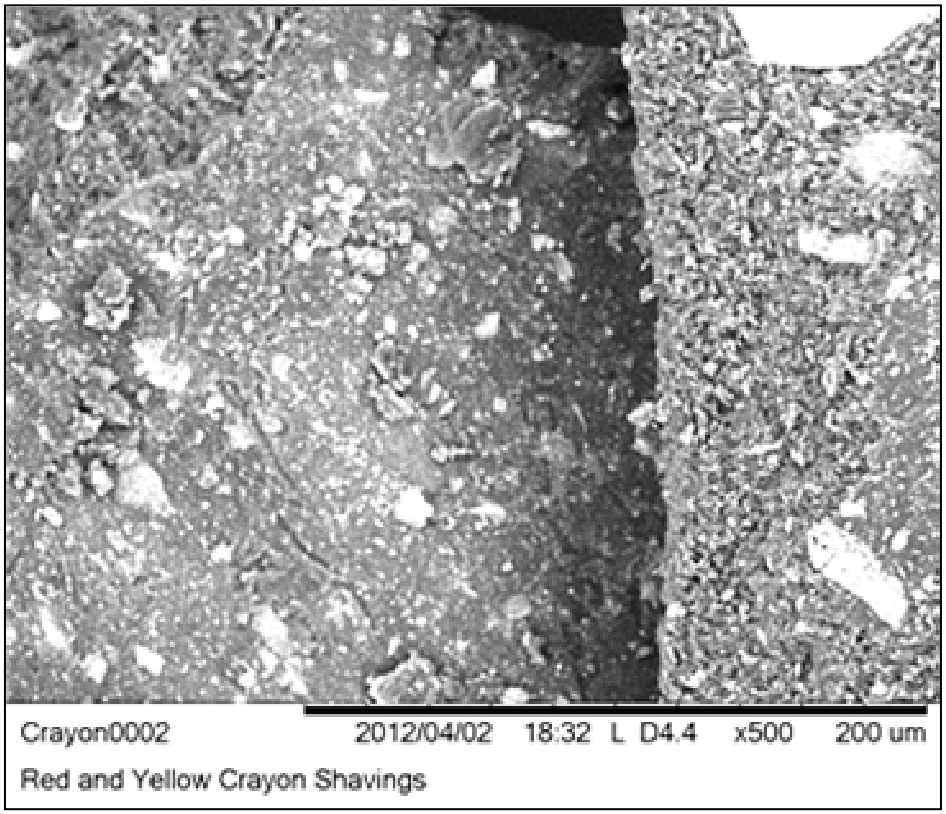

Figure 5: This image is of red and yellow crayon shavings from a student's school supplies. Magnification 500X.

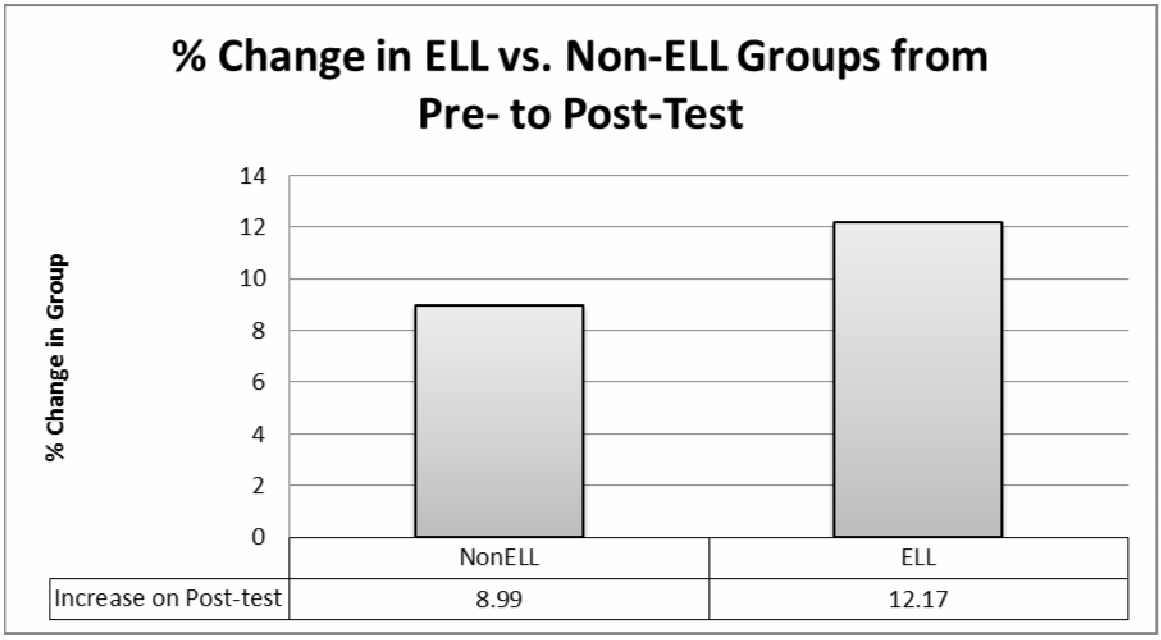

Figure 6: This graph illustrates the % change by the ELLs and non-ELL students on the post-test at the end of the 2011-2012 school year. ELL students gained 3.18% more than non-ELL students.

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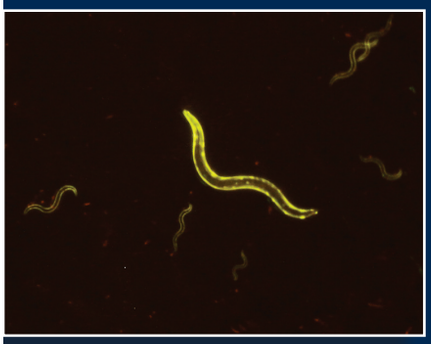
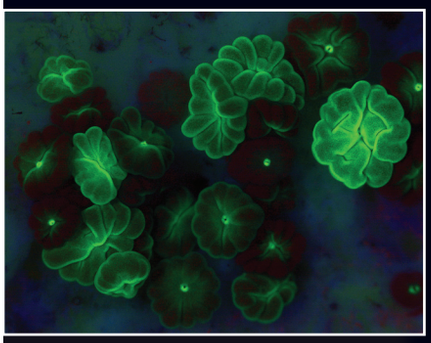


Fluorescing YFP-transgenic *C. elegans* photographed using the NIGHTSEA Stereo Microscope Fluorescence Adapter. Photograph © NIGHTSEA/Charles Mazel

GFP-tagged *Drosophila* larva. Photograph © NIGHTSEA/ Charles Mazel



Coral Polyps — Fluorescence. © Wade Cooper

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Effects of Storage Conditions on the Morphology and Titer of Lentiviral Vectors

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Abstract

Lentiviral vectors are commonly used in laboratory experiments to stably integrate transgenes into host genomes. It has long been observed that storage of virus stocks leads to a decrease in viral titer, but the mechanisms driving this decrease have yet to be identified. To that end, lentiviral vector stocks were generated and stored as follows: room temperature for less than one hour, -80ºC for 24 hours, 4ºC for three days and 4ºC for 7 days. These stocks were subsequently evaluated with regard to their transducing ability and their morphology, specifically particle diameter. The vector that was stored at room temperature served as the control with viral morphology similar to other VSV-G pseudotyped viruses. These stocks were able to transduce ~100% of HEK 293T cells. Particles were unstable under the storage conditions tested, as evidenced by the fact that all stocks stored at -80°C and 4°C required concentration with an ultracentrifuge to generate a preparation suitable for visualization with TEM. The vector stored at -80°C for 24 hours exhibited some morphological changes, but only a slight decrease in titer. The morphology of vectors stored at 4°C for 3 and 7 days was not significantly different from the room temperature control, although titer was reduced to 60% and 30-40%, respectively. Thus, the decrease in titer observed in the lentiviral stocks generated and stored during this investigation appears to be the result of viral particle instability rather than morphological changes to individual particles.

Introduction

Lentiviral vectors are a common tool for many scientists who are interested in manipulating the genomic content in a laboratory setting. Lentiviruses are widely known for their ability to infect both dividing and non-dividing cells which allows them to target a wide range of cells, including neurons, hepatocytes, monocytes, macrophages and hematopoietic stem cells (Segura *et al*., 2006). Transgenes expressed from lentiviruses are not silenced during development; thus, they are often used to make transgenic animals by directly infecting early stage embryos or through cloning transgenic cells (Rubinson *et al*., 2003). Lentiviral vectors can stably integrate up to 10 Kilobases of foreign DNA into the host genome without transferring viral genes (Zuffery *et al*., 1998), allowing for long term expression of the transgene which can lead to long term therapeutic effects (Segura *et al*., 2006). They are self-inactivating which renders them safer for clinical use. These vectors are very useful to those investigating treatments for genetic disorders, cancer, cardiovascular, neurological and ocular diseases (Segura *et al*., 2006).

Lentiviral vectors are constructed in a tissue culture setting by simultaneously transfecting three expression constructs into Human Embryonic Kidney (HEK) 293T cells. Lentivirus particles are produced when all three constructs are simultaneously transfected into a single HEK 293T cell. The transfected cell produces viral particles and releases them into the culture media. This media is then collected and used to transduce other cell lines.

The production of lentiviral vectors is extensively time consuming, labor intensive and costly. It requires a minimum of five days to generate a single vector stock for transferring a specific gene. In addition, because every transfection may not be successful, HEK 293T cells must be maintained in culture throughout the process and repetition of the transfection experiments requires an additional investment of time and resources.

Viral titer refers to the concentration of vector particles capable of transducing target cells (Sastry *et al*., 2002). In a clinical or laboratory research setting it would be advantageous to have a stock of lentiviral vector already produced, with a known titer, to use in experiments. Thus it is necessary to evaluate viral stocks maintained under various storage conditions and determine the cause of any subsequent loss of titer. This will allow future studies to evaluate protocols for storing these

At 72 hours post-transfection the cover slip from each 35mm plates was placed on a slide and fixed in place with clear enamel. These slides were observed under ultraviolet epiillumination (excitation 330-385 nm, mirror 400 nm, barrier 420 nm; Olympus) to determine the transfection efficiency as cells that had taken up the transfer construct expressed GFP. Cultures with at least 80% of the cells expressing GFP were considered successful transfections.

Virus was harvested from cultures with successful transfections as follows. First, the media was collected from the 100 mm plates, put into 15 mL conical tubes and centrifuged at 300 × g for 3 minutes to precipitate cellular debris. The supernatant was then filtered through a pre-wet 0.45 micron filter into a fresh 15 mL conical tube.

Lentivirus Storage Conditions:
Upon collection, the viral media was distributed into 2 mL aliquots and these were stored at the following conditions: (1) room temperature for less than one hour, (2) -80ºC for 24 hours, (3) 4ºC for 3 days and (4) 4ºC for 7 days. These stocks were subsequently evaluated with regard to their transducing ability and morphology.

Transduction of HEK 293T Cells:
HEK 293T cells were grown in complete media in 35 mm plates with glass cover slips prior to transduction with the lentiviral vectors. With the cells at ~80% confluency, the lentiviral-containing media collected previously was prepared for use by the addition of polybrene to a final concentration of 0.2 mM. To each 35 mm plate, the complete media was replaced with 0.5 mL of viral media with polybrene. The cells were incubated overnight at 37°C and 5% CO_2. The same procedure was followed for viral vector stocks at each of the storage conditions and each experiment was run in triplicate.

Lentivirus Titer Estimate by Fluorescence Microscopy:
At 48 hours post-transduction, cover slips were recovered from each well, fixed on a glass slide with enamel, and observed under ultraviolet epiillumination as described previously. The percentage of cells which expressed Green Fluorescent Protein (GFP) was estimated for each storage condition.

Observation of Morphology by Transmission Electron Microscopy:
Negative staining with uranyl acetate was used to visualize the virus following previously published protocols (Vale *et al*., 2010; Goldsmith *et al*., 2009). A 5 µL droplet of virus in complete media was placed on a Formvar-coated, carbon stabilized 200 mesh copper grid, and after five minutes the excess was wicked away with filter paper. Then 5 µl of a 4% (w/v) aqueous uranyl acetate solution was placed on the Formvar grid and wicked away immediately. The grid was examined with a Hitachi H-7000 TEM operating at 100 KeV. Images were obtained and diameter measured of at least 10 virus particles for each of the storage conditions with the exception of the stocks stored at 4°C for 7 days, of which only 7 viral particles were photographed and measured. The micrographs were digitized using an Epson Perfection 4870 Photo flatbed scanner (16 bit grayscale; 600 dpi).

Concentration of Lentiviral Stocks with Ultracentrifugation
In order to observe virus particles which had been stored at -80°C or 4°C, ultracentrifugation to concentrate the samples was required. A volume of 1000 µL of each of the three stocks stored at these conditions was placed into thin-walled centrifuge tubes with a 15% sucrose cushion. Samples were centrifuged in a Beckman L8 80M ultracentrifuge at 19,000 × g for 90 minutes. The supernatant was removed and the virus pellet was resuspended in 30 µL of complete media for a 33 fold increase in concentration of each sample.

Statistical Analysis
A permutation procedure was used to test the null hypothesis that the mean particle diameter of viruses stored at the various conditions did not differ. A total of 9999 permutations of the data were generated and the absolute difference between means for each permutation trial was calculated. In order to refute the null hypothesis, differences between means in the original data must be greater than that seen in the reshuffled data ($p < 0.01$).

Results:

Lentivirus Titer Estimation by Fluorescence Microscopy:
The virus stored at room temperature for less than one hour exhibited the highest functional titer and was able to transfect ~100% of the HEK 293T cells (Table 1). Titer was reduced under all of the storage conditions tested, declining to ~80% when stocks were stored at -80º C for 24 hours, and to ~60% and ~30%-40% when virus was stored at 4º C for three and seven days, respectively (Table 1).

Observation of Particle Morphology Using TEM:
Negatively stained viral particles were circular to ellipsoidal in shape, frequently clustered, and varied in size from 80 to 410 nm across all samples (Figures 1 & 2; Table 2). For virus that was stored at room temperature, the concentration of particles was sufficient to observe without ultracentrifugation (Figure 1). Mean particle size for the room temperature stock was 208 nm (Table 2).

Viral stocks stored at -80°C and 4°C required concentration with an ultracentrifuge to generate a preparation suitable for visualization with TEM. In stocks that were stored at -80ºC, particles ranged in diameter from 80-200 nm with a mean diameter of 128 nm (Table 2). Mean diameter of particles stored at 4ºC for three days was 188 nm (Figure 2; Table 2). Particles stored at 4ºC for seven days had the highest mean diameter and the largest range in size, 277 and 140 – 410 nm, respectively (Table 2).

Statistical Analysis
The mean particle size of virus stored at -80º C was significantly lower than that of the other treatment groups, but all other comparisons were not statistically significant (Table 3).

Discussion

Vector expression in transduced cells using transgenes such as GFP is a technically simple and accurate method to assess functional titer (Sastry *et al*. 2002), and is less likely than other methods (ELISA assays, PCR analysis) to overestimate titer (Logan, *et al*., 2004). As expected, the fresh lentiviral vector stock had the highest titer. Negative staining revealed that particles in the fresh preparation were consistent with the general description of an enveloped virus provided by Goldsmith and Miller (2009). These viruses may take any shape (i.e. are pleomorphic), depending on how they land on the grid and the surface tension of drying forces. The short, fragile surface projections of retroviruses, of which lentiviruses are one type, are rarely seen in negatively stained preparations, and the nucleocapsid is morphologically nondescript (Goldsmith and Miller, 2009). The variation in particle size observed in this investigation is supported by the work of Fuller *et al*. (1997), who used cryo-electron microscopy to examine HIV-1 virus-like particles and found intact particles to be heterogeneous in size, varying in diameter from 120 - 260 nm. Vogt and Simon (1999) observed populations of retrovirus virions to be non-homogeneous in mass, with up to two-thirds of the Rous sarcoma virus (RSV) particles that they analyzed deviating from the mean mass by more than 10%.

The unstable nature of the virus under all storage conditions is evidence by the fact that ultracentrifugation was required to concentrate the samples so that TEM examination could be conducted. Segura *et al*. (2006) reported that retrovirus instability translates into low overall recoveries of infective viral particles. Ultracentrifugation is a common method to concentrate viruses in fluid samples to a level that they can be characterized microscopically (Goldsmith and Miller, 2009). Viruses pseudotyped with the VSV-G envelope usually withstand the force of ultracentrifugation better than most other viral envelopes (Burns *et al*., 1993; Logan *et al*., 2004), thus it is unlikely that concentration using this method affected viral morphology.

The virus stored at -80º C for 24 hours had the second highest functional titer. Although these particles were significantly smaller than the other three stocks, they retained their ability to transduce HEK 293T cells. Storage at 4ºC had a more negative effect on titer, with particles stored for 7 days exhibiting the lowest titer of all conditions tested. While the decreases in viral titer observed here may seem minimal, the highly transfectable nature of 293T cells should be noted (Pear *et al*.,

1993; Sastry *et al*., 2002). Even the minimal decrease in titer of stocks stored at -80°C would eliminate the ability of this preparation to effectively transduce a primary cell line (Ichim and Wells, 2011).

These results are consistent with previously published studies including the work Ichim and Wells (2011), who observed a decrease in viral titer of nearly ten-fold in magnitude when VSV-G pseudotyped retrovirus was stored at 4°C, concluding that this type of vector is highly unstable under these conditions. Kutner *et al*. (2009) reported that the low titers of lentivector preparations bearing glycoproteins other than VSV-G is the result of poor survival of these pseudotypes upon freezing. Xu *et al.* (2005) investigated the effect of storage conditions on the transduction efficiency of adeno-associated virus (AAV), a non-pathogenic defective parvovirus with a broad host range that is known for its extreme resistance to environmental extremes. The virus stored at -80°C remained stable and retained high transduction efficiency throughout a one-month monitoring period as assayed by measuring luciferase activity in transduced 293 T cells. Transduction efficiency of virus stored at -20°C, 4°C, room temperature and 37°C decreased continuously over time. This drop was most precipitous with the room temperature and 37°C treatments, with a sharp fall observed at day 1. At 20°C, transduction efficiency remained relatively high for the first 5 days, whereas at 4°C sharp drops were observed at days 1 and 7 and efficiency had declined to 55% by the end of one month of storage.

Morphological difference between 4ºC and room temperature particles were not detectable with the techniques employed in this investigation, and thus could not be associated with the differences in titer between the two preparations. Such was not the case in other studies, including the work by Cheslock *et al*. (2003). These researchers compared wild-type virions of murine leukemia virus to noninfectious mutant virions in which 33 amino acid residues of their capsid were deleted. Particle sizes of the wild type virions were tightly clustered within a 90-130 nm range of diameters. Mutant particles exhibited a much wider range in size, with some measuring more than 400 nm in diameter. Zhao *et al*. (2008) observed differences between infectious and empty particles using size exclusion chromatography and electron microscopy. Complete particles ranged in size from 40-100 nm, with empty particles being larger in diameter (up to 180 nm). Yeager *et al*. (1998) compared the size and morphology of mature, wild type and immature, protease-deficient retroviral particles using electron cryo-microscopy. Although the lipid bilayer envelope, pleomorphic shape, and diameter (approximately 120 nm) were similar for both types of particles, they varied in the shape of their electron dense central core.

Conclusion

This study indicates that it is a decrease in the number of virus particles, not a change in morphology, which is responsible for the decrease in functional titer of lentiviral vectors pseudotyped with VSV-G. Although a decrease in titer did occur, storage at -80ºC was superior to 4ºC. Future research will focus on the development of more effective storage protocols in order to reduce the effects on viral titer observed in this investigation. Improved storage methods will allow for large quantities of these vectors to be generated and used repeatedly rather than making fresh stocks for every experiment.

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Storage condition	**Transduced HEK293T cells (%)**
Room Temperature	100 %
-80° C	80 %
4° C, 3 days	60 %
4° C, 7 days	30 – 40 %

Table 1. Assessment of functional titer of viral stocks at each of the storage conditions, as measured by the percentage of cells which expressed Green Fluorescent Protein (GFP).

Storage condition	Range in Particle Diameter (nm)	Mean Particle Diameter (nm)	Standard Deviation
Room Temperature	160 – 240	208	24
-80° C	80 – 200	128	34
4° C, 3 days	160 – 220	188	16
4° C, 7 days	140 – 410	277	98

Table 2. Viral particle diameter at each of the storage conditions. n=10 for all treatments with the exception of 4º C, 7 days for which n=7.

	Room Temp.	-80° C	4° C, 3 days	4° C, 7 days
Room Temp.	x	**0.0002**	0.0845	0.0689
-80° C	x	x	**0.0008**	**0.0006**
4° C, 3 days	x	x	x	0.0166
4° C, 7 days	x	x	x	x

Table 3. p values for comparisons of the mean particle size of viral stocks maintained at different storage conditions. Statistically significant differences are shown in bold.

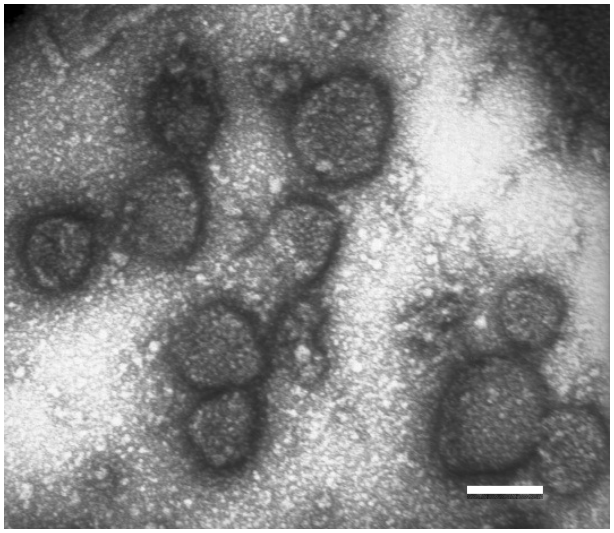

Figure 1. Transmission electron micrograph of negatively stained viral particles stored at room temperature for less than one hour. Final magnification 50,000X; Scale bar = 200 nm.

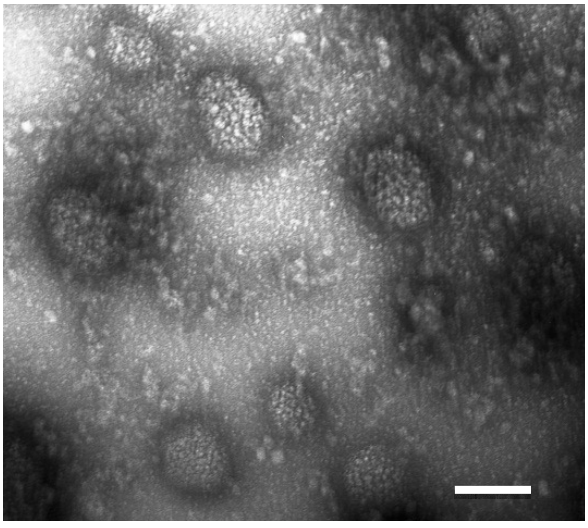

Figure 2. Transmission electron micrograph of negatively stained viral particles stored at 4º C for three days. Final magnification 50,000X; Scale bar = 200 nm.

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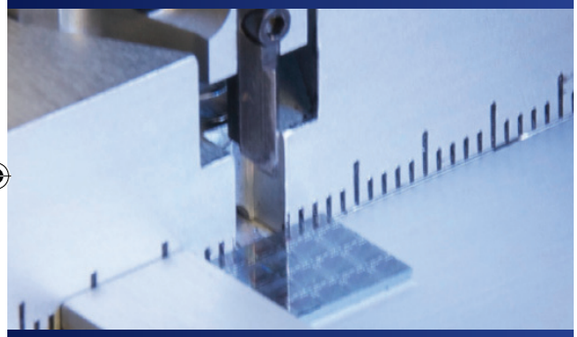

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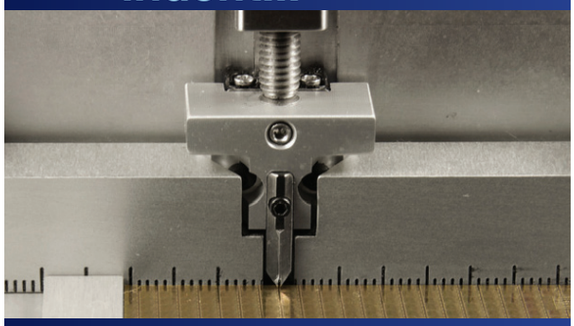

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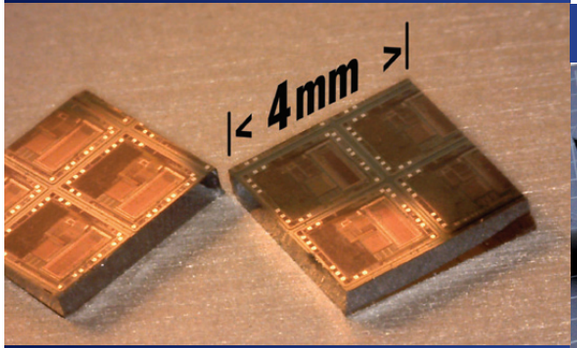

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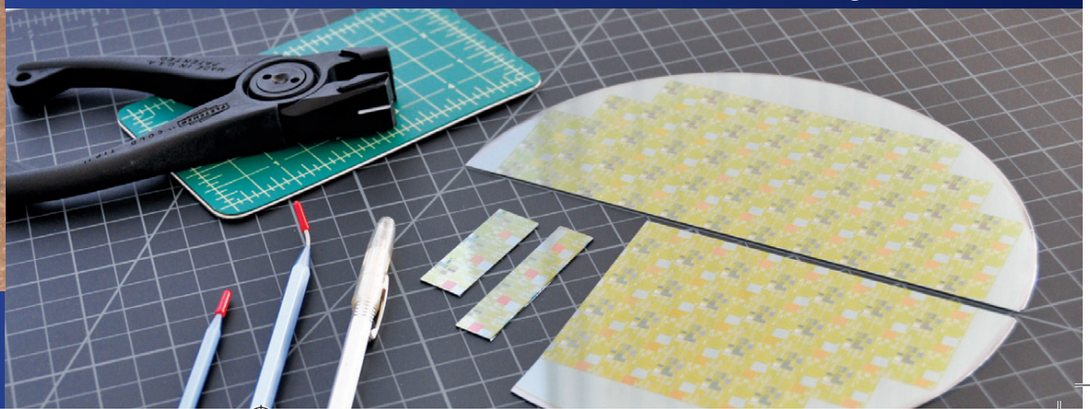

Facilitating Inquiry Investigations by Pre-Service Teachers Using Scanning Electron Microscopy

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Abstract

Scientific inquiry is promoted in state and national standards as a necessary part of K-12 science education. Scientific inquiry may involve a variety of kinds of investigations, including descriptive, comparative, and experimental. However, since many science teachers have not had authentic inquiry experiences in their pre-service training programs, they are not well-prepared to help their K-12 students plan their own inquiries. In this study, three pre-service teachers, who were enrolled in a Master of Arts in Teaching degree program for initial science certification, were engaged in a 17-week course to use scanning electron microscopy (SEM) for inquiry investigations. Students in this program were taught to use the Hatachi-1000 Tabletop SEM and then allowed to ask questions, design investigations, collect data, and present their findings at professional meetings. The students' investigations represented a variety of inquiry types. Students reported that they encountered both challenges and benefits from the course experience. Challenges included their lack of preparation to engage in asking questions and planning investigations, which they had not previously experienced. Benefits that the students reported included the development of pride and confidence in their ability to design and execute an inquiry study. A future study may track whether pre-service teachers who have experienced inquiry using this SEM research tool are more likely to use this teaching method in their future science classrooms.

Introduction

Scientific inquiry is a way of thinking in which people seek to ask and answer questions in order to understand their world (Martin-Hansen 2002, Narayan 2010). Scientific Inquiry is currently emphasized in United States national and state education standards as a necessary part of science education for students in kindergarten through 12th grade. An understanding of what scientific inquiry is and how it is conducted is necessary for teachers to be able to plan appropriate experiences for students' engagement in the inquiry process. According to the National Science Education Council (1996), inquiry should emphasize more than the skills of the scientific process, such as observing, inferring, and experimenting. Students should also be encouraged to think as scientists do, using critical thinking and reasoning.

> The *Standards* call for more than "science as process," in which students learn such skills as observing, inferring, and experimenting. Inquiry is central to science learning. When engaging in inquiry, students describe objects and events, ask questions, construct explanations, test those explanations against current scientific knowledge, and communicate their ideas to others. They identify their assumptions, use critical and logical thinking, and consider alternative explanations. In this way, students actively develop their understanding of science by combining scientific knowledge with reasoning and thinking skills.

The state of Texas education standards, the Texas Essential Knowledge and Skills (TEKS), further describe the way in which science inquiry should be approached. In the Introduction to High School Science courses, the TEKS describe scientific inquiry and three methods of scientific investigation:

> Scientific inquiry is the planned and deliberate investigation of the natural world. Scientific methods of investigation are experimental, descriptive, or comparative. The method chosen should be appropriate to the question being asked.

Thus, the way in which inquiry is conducted, both by scientists and by students, is not confined to the traditional "scientific method," in which researchers use the controlled experimental design for conducting investigations. As stated by West (2010), "experimental, descriptive, and comparative research designs are not the only ones that scientists use, but for K-12 students they serve as a useful framework to conduct scientific studies of natural world phenomena." The three means of conducting investigations listed in the TEKS, experimental, descriptive, or comparative, use different approaches to achieve the same end: conducting inquiry. The "scientific method" or experimental method is used to test a hypothesis, using a procedure to test a single manipulated variable and measuring a response variable, while controlling for other confounding variables. Descriptive research is a systematic description of the subject or phenomenon being studied. A comparative study involves "collecting data from two or more groups, times, or locations" and then making comparisons (Windschitl, et al. 2007). However, Rorie, *et al*. (2007), completed a survey of the science education standards of 50 states of the United States and found that the standards of most states emphasize the experimental model of inquiry.

One problem with implementing inquiry in K-12 science classrooms is that teachers may not have had an opportunity to experience inquiry in their teacher training programs by asking and answering their own science questions. The authors propose that pre-service teachers would benefit from an authentic inquiry experience which would allow them to better understand the methods of scientific investigation, having experienced them first-hand. The purpose of this paper is to illustrate the way in which a research tool, the scanning electron microscope (SEM), was used to facilitate inquiry in pre-service teachers, allowing them to design and execute research studies using three methods of scientific investigation: descriptive, comparative, and experimental.

Materials and Methods

Pre-service teachers enrolled in the Master of Arts in Education graduate program at Texas Woman's University took a course in Scanning Electron Microscopy as one of their graduate-level science courses. Students were taught to operate the Hitachi 1000 Tabletop SEM and then allowed to form a research question, design a study to collect appropriate data, and finally to present their findings at a professional meeting. As a research tool, the SEM provided focus and an entry point to inquiry. Students were encouraged to ask their own questions and find their own answers using the SEM as a research tool. Students were asked to find scientific literature that explained the three types of inquiry investigations: descriptive, comparative, and experimental, which they shared in a class discussion. In the spring semester of 2012, three students enrolled in the SEM course asked research questions that led to investigations that illustrated three different types of investigations: descriptive, comparative, and experimental. Results from the investigations were presented by the students at both local and state professional research meetings.

Results

One student chose to investigate the amount of the element chromium found in jewelry imported to the United States from China using the Swift ED-TM Energy Dispersive X-ray Spectrometer attachment on the SEM. The student's hypothesis was that cadmium, which has been reported to cause a variety of health problems, would be found in the imported jewelry. This study was chosen, in part, from her concern that cadmium-containing jewelry might be in her personal jewelry collection or that of her family, thus the study was personally relevant. Her study was descriptive in nature, as she compiled data to characterize the elements of the jewelry samples (figures 1, 2, and 3). The student researcher's conclusion was that no cadmium was found in the samples of jewelry.

Another student designed a comparative study to examine the morphology and silicon content of two plants used for livestock feed, alfalfa and Coastal Bermuda grass. Her research focus was chosen from a natural curiosity about the differences in two sources of forage for her livestock. As a professional horse trainer, she had been told that alfalfa was a superior feed for horses as compared to the less expensive Bermuda grass. The student

examined the external stem morphology (figures 4 and 5), the stem cross section (figures 6 and 7), and the mineral content of the leaves (figures 8 and 9). Her conclusion was that there were both structural and mineral content differences in the two grasses.

A third student designed an experimental study to test the effects of exposing natural African American hair to a chemical hair straightening product. The straightening process is referred to as Lanthionization. This student stated that her interest in chemical treatment of hair resulted from a personal interest in the hair treatment process that she had personally experienced and which was a part of her African American culture. Her experimental study involved a control (natural, untreated hair), and previously chemically untreated hair with variable lengths of exposure to the chemical Lanthionization process. Her conclusion was that extended time exposure to the straightening product resulted in "weathering" or damage of the hair strands (figure 11), as compared to the control (figure 10).

Discussion and Conclusions

Students involved in this study found that there were both challenges and benefits associated with the SEM investigation experience. One of the challenges cited by students was the "wide open" nature of this inquiry opportunity. Students were allowed the freedom to create their own question and then to design an appropriate investigation. As one student stated, "This was way outside our comfort zone! We were used to confirmation labs in which we were told what to do." In addition, students had to learn to "build a fence" around their projects, limiting investigations in size and scope to the tools, expertise level, and time available. On the other hand, students found that there were many benefits in this SEM investigative opportunity. Interestingly, the freedom to "really act as scientists" was noted by one student as the overall benefit. She stated that students in this study were able to pose their own questions, design their own studies, make mistakes, backtrack, correct, and continue the investigations. In addition, students noted that the ability to pursue a question related to a topic that had personal interest and relevance made the SEM inquiry opportunity very engaging and rewarding. Students developed a sense of pride and self-confidence in their ability to "do science." As a result of having had this personal inquiry experience the researchers propose that it is more likely that these pre-service teachers will engage their own future students in authentic inquiry, the asking and answering of questions. One of the students suggested this herself, "Wouldn't it be interesting to know if pre-service teachers who have been given this unique opportunity to engage in authentic, self-directed inquiry will later use it in their own classrooms?" This would indeed be an interesting future study.

Acknowledgments

Thanks to Kathy Foley, Ellen Martin, and Brittany Randolph for permission to discuss their research projects.

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Figure 1: Jewelry samples analyzed for elemental content (photo by Ellen Martin).

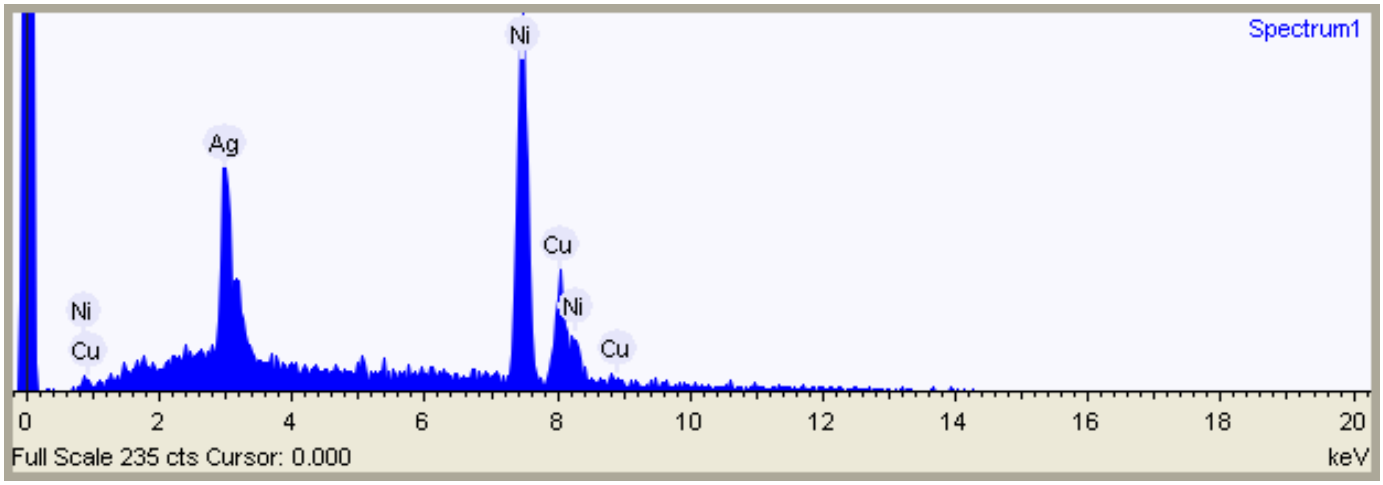

Figure 2: Elemental analysis of cat charm (analysis by Ellen Martin).

Element	Weight %
Nickel	80.9
Copper	1.6
Silver	17.5

Figure 3: Summary of elements for cat charm (analysis by Ellen Martin).

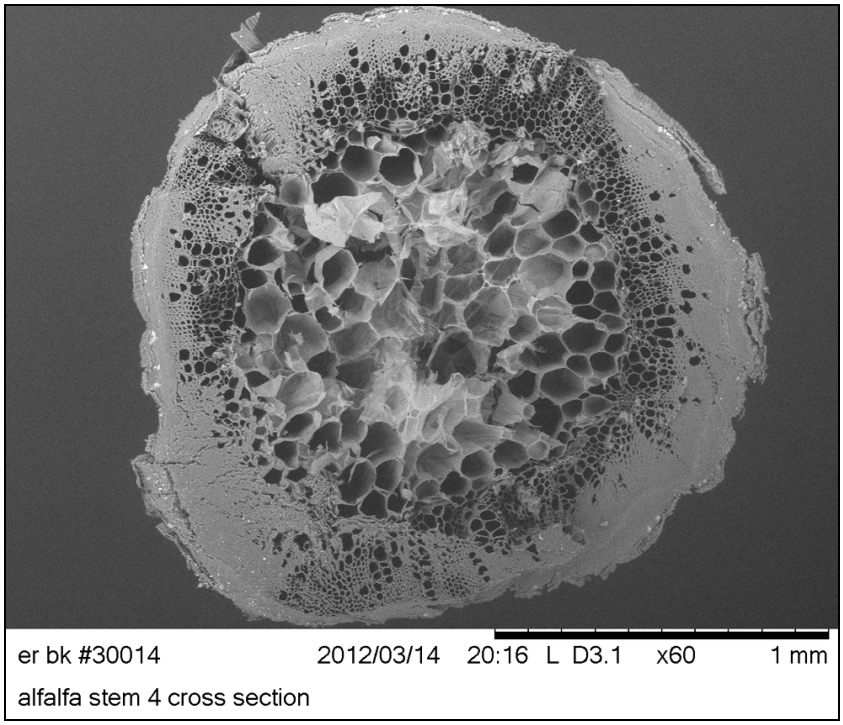

Figure 4: Cross section of alfalfa stem showing vascular bundles (micrograph by Kathy Foley).

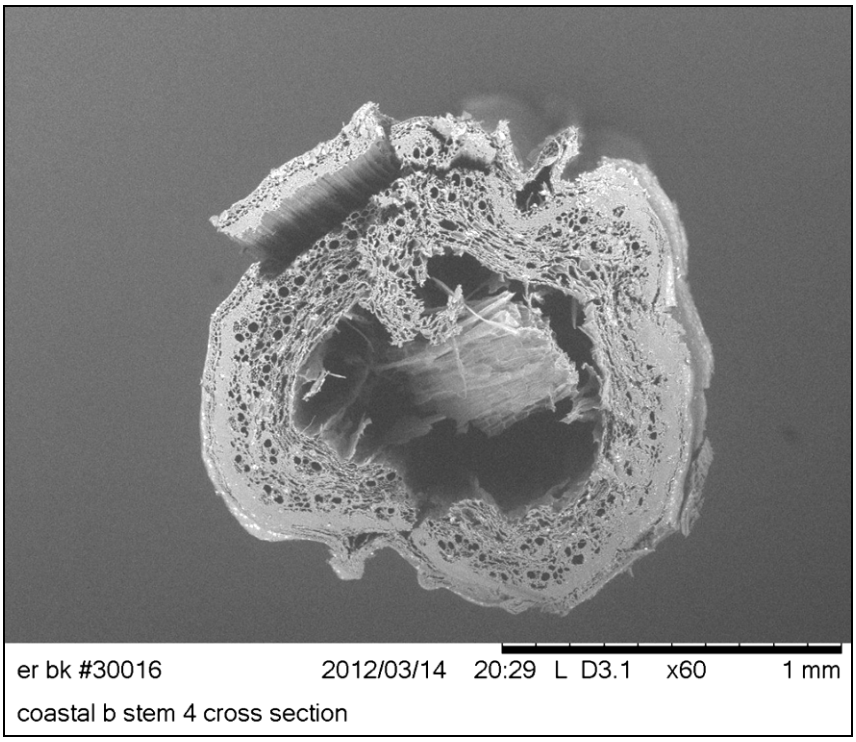

Figure 5: Cross section of Coastal Bermuda grass stem showing vascular bundles (micrograph by Kathy Foley).

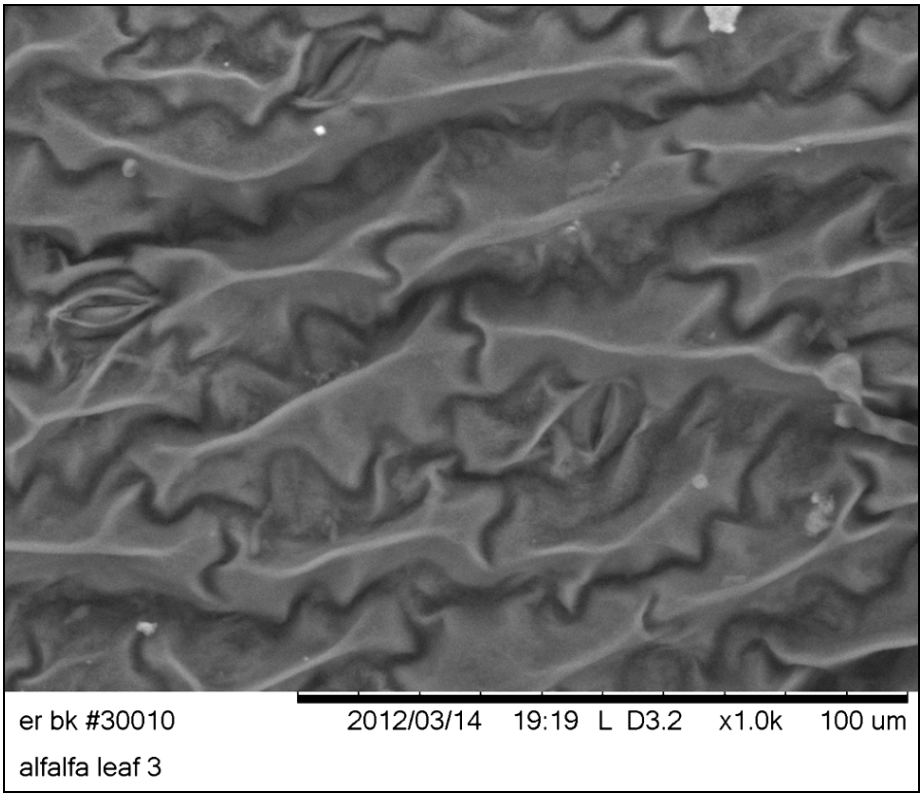

Figure 6: Alfalfa leaf showing cell morphology (micrograph by Kathy Foley).

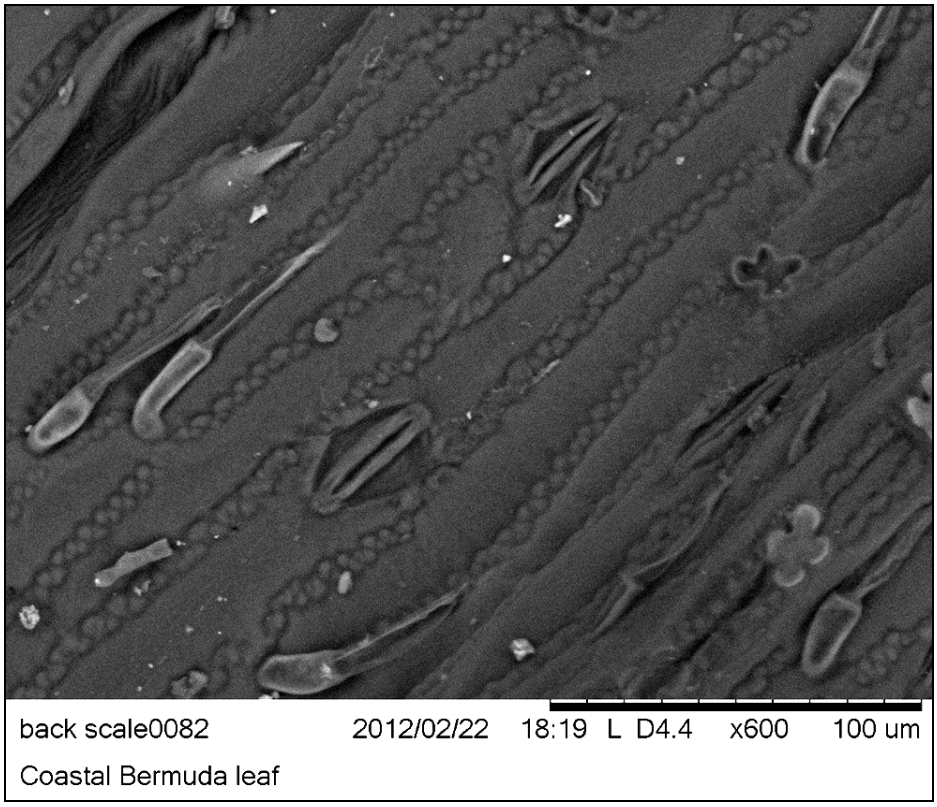

Figure 7: Coastal Bermuda grass leaf showing cell morphology (micrograph by Kathy Foley).

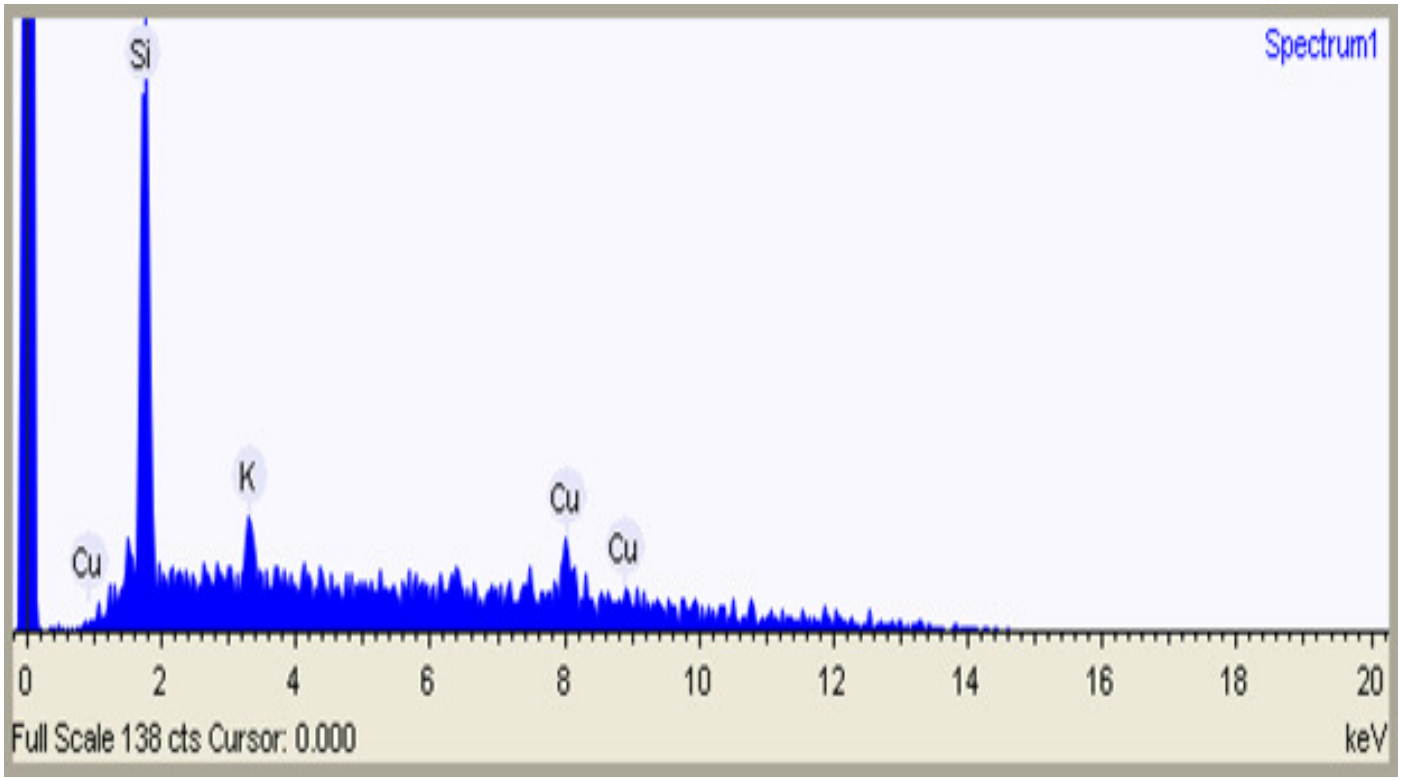

Figure 8: X-ray analysis, outer surface of Alfalfa stem (analysis by Kathy Foley).

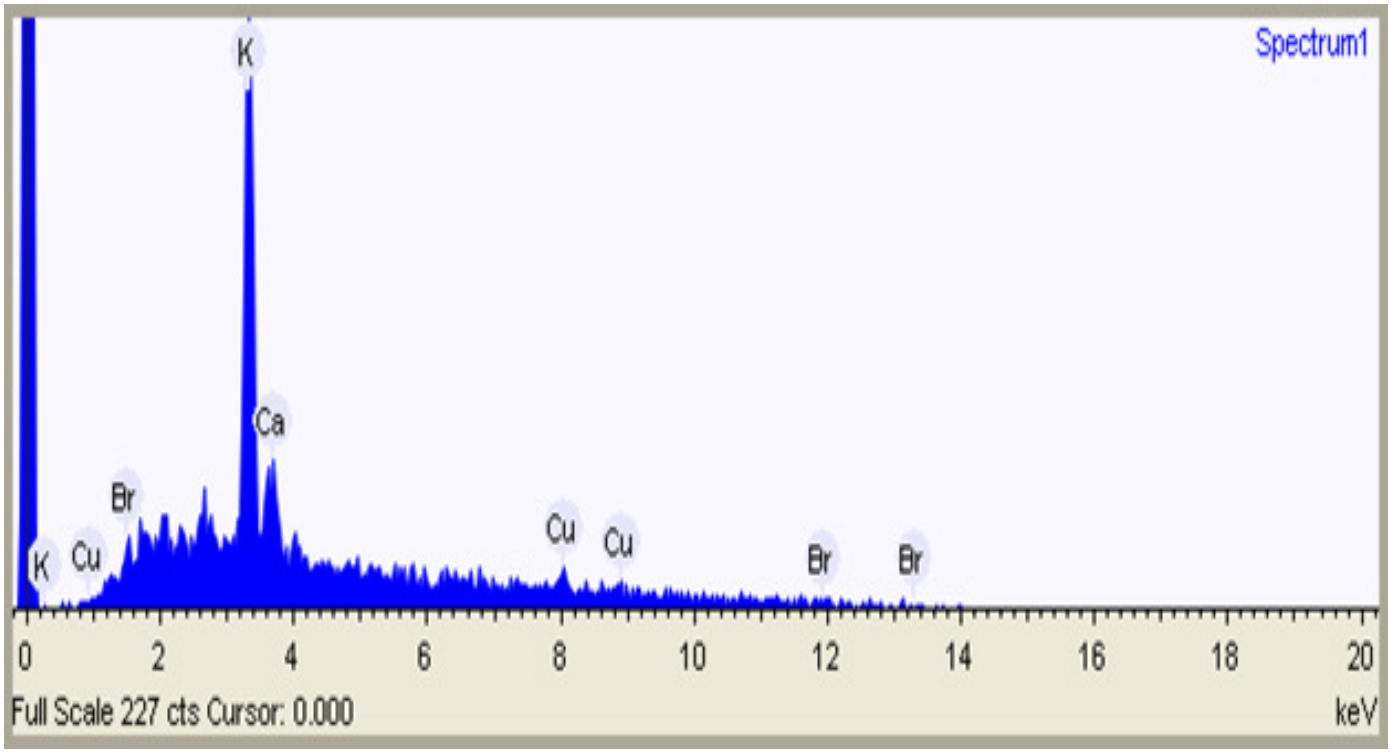

Figure 9: X-ray analysis, outer surface of Coastal Bermuda grass stem (analysis by Kathy Foley).

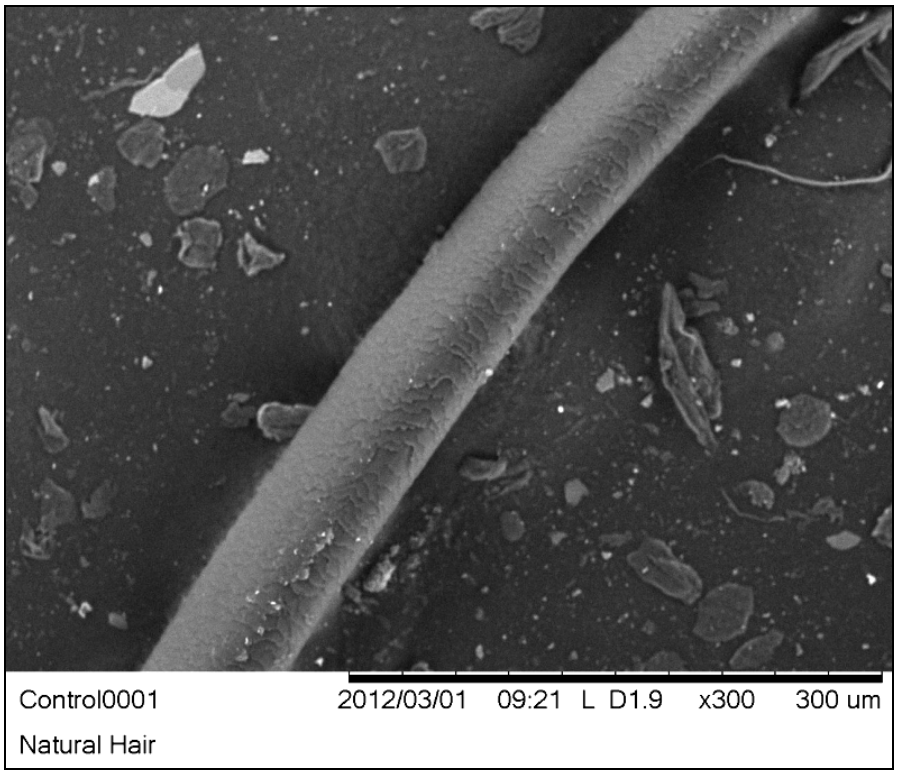

Figure 10: Control hair sample (micrograph by Brittany Randolph).

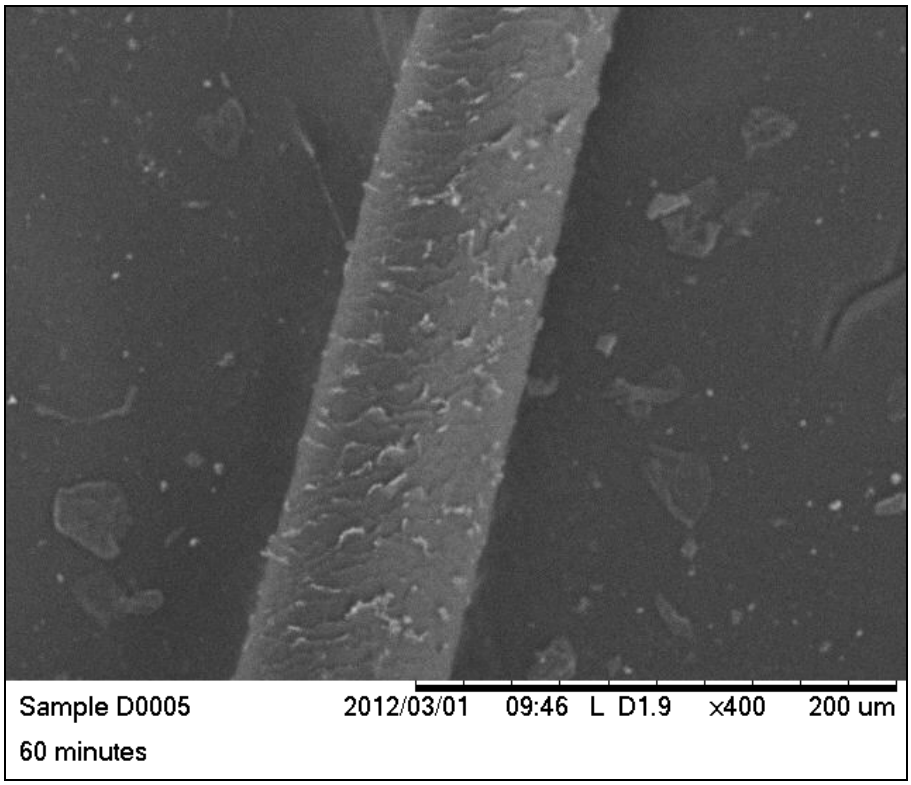

Figure 11: Experimental hair sample with 60 minutes of Lanthionization exposure. (micrograph by Brittany Randolph).

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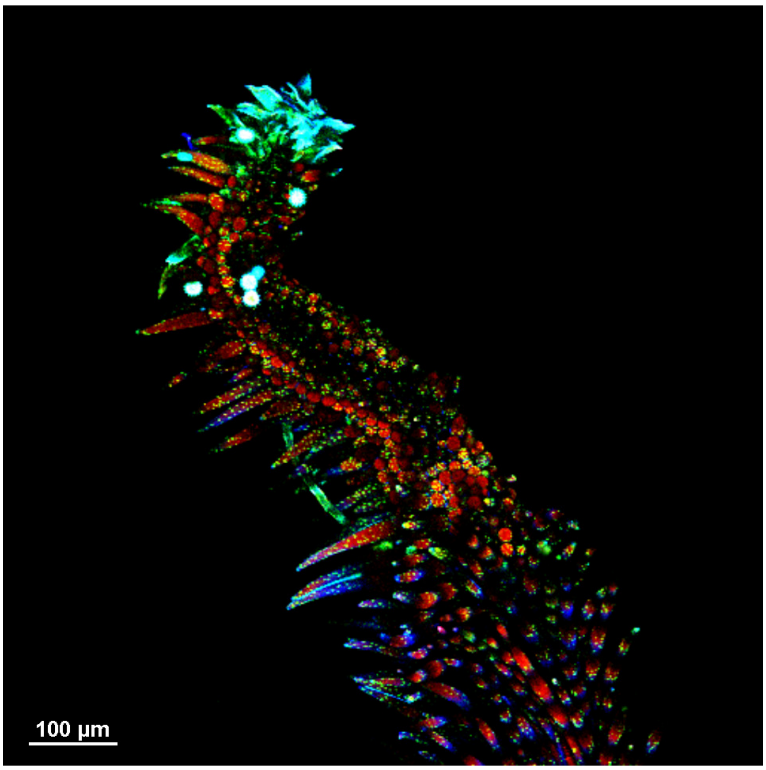

Helianthus annuus, Asteraceae disk floret stigma. Confocal Laser Scanning Microscope image- Nikon A1 Confocal System. Laser information: DAPI (blue, 400 nm)/ FITC (green, 588 nm)/TexasRed (red, 561 nm)/Cy5 (pink, 626 nm).

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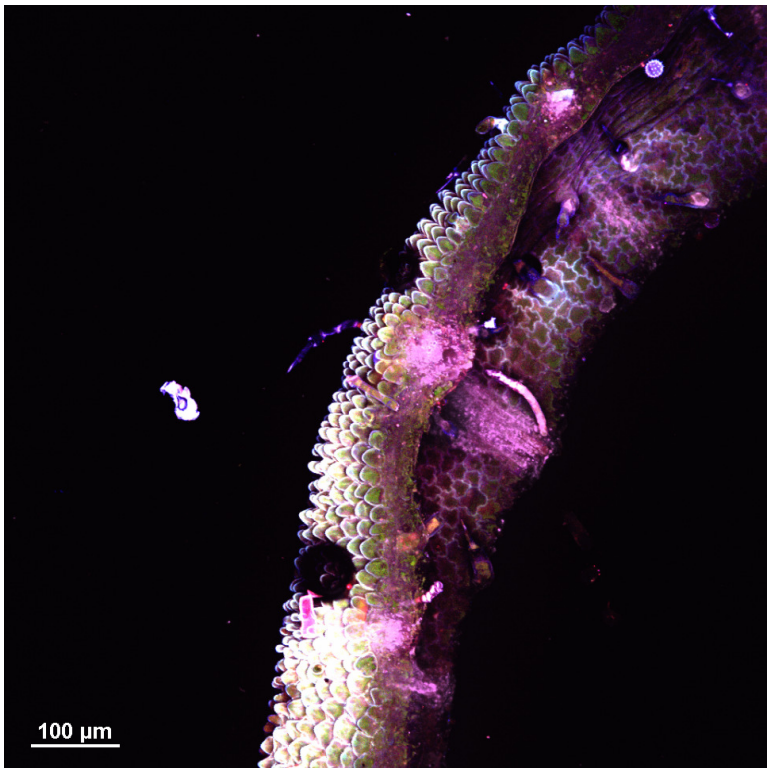

Helianthus annuus, Asteraceae ray floret cross section. Confocal Laser Scanning Microscope image- Nikon A1 Confocal System. Laser information: DAPI (blue, 400 nm)/FITC (green, 588 nm)/TexasRed (red, 561nm)/Cy5 (pink, 626 nm).

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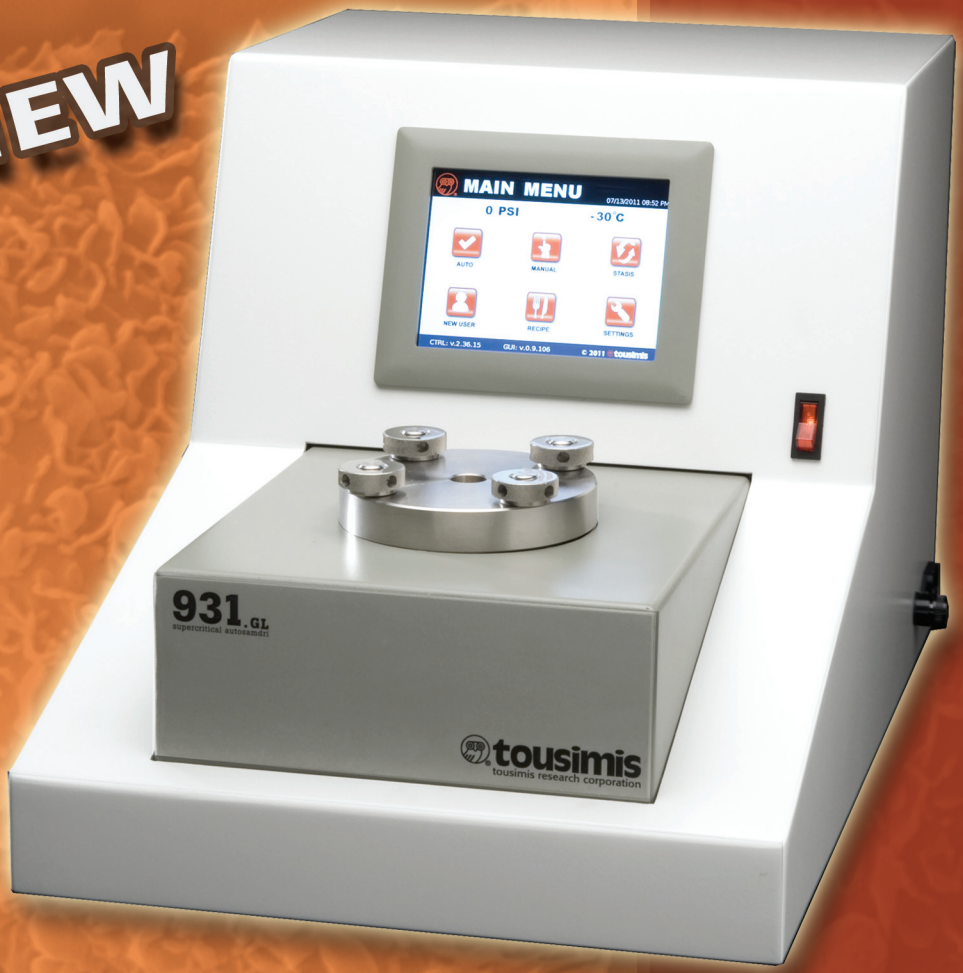

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