Award 1126080 - Annual Project Report

MRI: Acquisition of a Field-Emission Scanning Electron Microscope for a Primarily Undergraduate Consortium

Samples of Research Projects in 2013

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Harvey Mudd College Prof. Hal Van Ryswyk (seated) and Pomona College Prof. David Tanenbaum demonstrating the new NSF funded Hitachi SU-70 SEM facility during the open house held February 18, 2013 at Pomona College.
The Robert and Mary Jane Engman Fellowship Program focuses on interdisciplinary team-based projects in the field of bioengineering. The projects in our lab currently center around tissue engineering and medical device design and include corneal tissue engineering, neural tissue engineering, and the evaluation of the mechanical properties of nasal cartilage and lizard eggshells (in collaboration with Dr. Steven Adolph, HMC Biology Department). These projects involve cell culture, evaluation of cellular protein expression and differentiation, extracellular matrix design, mechanical analysis and modeling of soft tissues, and biomedical device design.

**Project: Corneal Tissue Engineering.**

**Students participating in this project.** Jennifer Zheng (HMC Engr 15) and Molly Kupfer (PO Molecular Bio 14). Molly will continue on this project for her thesis.

**Project Description.** The cornea is the tissue on the front of the eye that provides both structural and optical functions. The goal of this project is to create new corneal tissue using cells and biological structural materials using the native cornea as inspiration. A transparent artificial cornea derived from biological materials is a major challenge in corneal research. Over 10 million individuals worldwide experience bilateral corneal blindness, and corneal transplants are currently the only treatment for restoring vision. A transparent corneal model could also provide the basis for studying novel ophthalmic drugs and new gene delivery approaches, reducing the need for animal testing. Moreover, there is currently a clinical need for an improved understanding of corneal wound-healing mechanisms in order to solve corneal haze problems associated with LASIK procedures and outcomes in corneal transplants. In order to recreate the microstructure of the native cornea, we are electrospinning highly aligned, small diameter type I collagen fibers, which we have shown has a positive influence on cell behavior and overall optical properties of the tissue. We are using the SEM in this project to evaluate fiber alignment, density and diameter, as well as to assess proper collagen fibril formation.

Electrospun collagen nanofibers. We are culturing cells on unaligned (left) and aligned(right) collagen fibers.
Project: Neural Tissue Engineering.

Students participating in this project. Kate Crawford (HMC Engr 13), Meghan Jimenez (HMC Engr 14), Risa Egerter (HMC Engr 15) and Cleo Stannard (HMC Engr 15).

Project Description. This project focuses on harnessing regenerative medicine techniques and investigating novel combinations of cell source and scaffold materials to create a cell delivery system (or “brain patch”) for the treatment of traumatic brain injury. Traumatic Brain Injury (TBI) refers to any sudden physical damage to the brain, a condition that affects over 1.4 million people per year in the United States alone. In addition to the limited repair capacity of the damaged brain, secondary inflammatory mechanisms make the initial damage worse by inducing ischemia, hemorrhaging, excitotoxicity, free radical formation, and cell death. Due to these obstacles, it is difficult to restore function, and current treatments aim to minimize any further damage. Delivering an optimal combination of neural stem cells and stimulatory bioactive factors organized within a supportive extracellular matrix configuration is a promising strategy for neural tissue repair. The project brings competency in manipulation of adult stem cells and cutting edge tissue engineering techniques. The project involves design and testing of novel matrix materials, stem cell differentiation studies, and cutting edge imaging and analysis techniques. As part of this project, we are designing new composite collagen/chitosan matrices by gelation and testing them for use in our projects. We are interested in developing matrices with tunable mechanical properties that are also antibacterial. The SEM has been used in this project to investigate the structure of our different collagen gels to evaluate them for proper fiber formation as well as appropriateness for cell culture. Of particular note is the ability to image collagen banding (see figure below), which we could not do with the other SEM.

SEM Images. For this project, the SEM has been used to determine collagen fiber density, structure and diameter.

![SEM Images](image_url)

(Left) 0.5% collagen gel showing fibrous structure. (Right) Higher magnification image showing characteristic 67nm collagen banding pattern, indicative of proper collagen fibrillogenesis.

Publications.

Our abstract has been accepted for a poster presentation at the Biomedical Engineering Society Annual Meeting, September 2013:

*HMC student

**Project: Lizard Eggshell Characterization.**

**Students participating in this project.** Frances Su (HMC Engr 14).

**Project Description.** We are currently working on a project to characterize the properties of lizard eggshells. These eggs increase in volume by about 4 times over their incubation period. The larger project involves characterizing the mechanical properties of the eggshell material as well as the permeability over time. The ultimate goal is to create a mathematical model of this material over time in incubation. We have discovered that the material shows crystalline properties as well as viscoelastic behavior using mechanical analysis. We have used the SEM to help determine the structure of the eggshells that give rise to these properties. The shells appear to have 3 layers: an inner membrane, a fibrous layer, and an outer crystalline layer.

![SEM images of the outer surface (left) and cross-section (right) of lizard eggshells. Note the smooth outer surface and fibrous layers.](image)

**Publications.**

Our abstract has been accepted for a poster presentation at the Biomedical Engineering Society Annual Meeting, September 2013:


*HMC student
The research shown here was part of a study of the biogenic communities in geothermal systems of the Salton Sea geothermal field funded by Research Corporation to EJ Crane, Matt Sazinsky, and myself. Pomona College student Nick Sbardellati ('14) completed the initial phases of the research in summer 2013 using the Pomona SEM to characterize pyrite crystal morphology and distributions in mud volcano samples as a means of evaluating biogenic activity in these systems (Figs. 1 & 2). The pyrite contained within these samples was characterized texturally (Fig. 1), in order to identify sample textures for in situ stable isotope analyses. Additional SEM imaging was used to screen samples analytical spots for robustness and the student is currently synthesizing the results as part of a senior thesis project.

![Image of backscattered electron (BSE) images pyrite (FeS₂) grains from mud volcano samples from the Salton Sea geothermal areas. Samples show variable morphologies suggesting both biogenic and non-biogenic formation processes. BSE imaging was used to screen samples for sulfur isotope analyses (Fig. 2).]
This project fits into my overall study agenda by its linkages to geothermal activity and use of microscale isotopic zoning minerals as archives of pressure, temperature, and fluid evolution in geologic settings. The biogenic component of the research exemplifies they hybrid study configuration that allows me to work with colleagues in other fields to expand on my overall research agenda.

Figure 2. Topographic BSE images of pyrite (FeS₂) grains following in situ sulfur isotope analysis by secondary ion probe mass spectrometry (SIMS). More negative sulfur isotope ratios are indicative of biogenically mediated precipitation of pyrite by sulfide reducing bacteria. Topographic BSE images are used to screen SIMS spots for irregularities that may indicate compromised isotope analyses (e.g., third panel).

Additional use of the SEM over the next several years will focus on micro-mineralogical textural and compositional (by EDS) analyses of minerals in hydrothermal systems as well as igneous intrusions. The SEM analyses typically are used to demonstrate patterns of crystal growth under equilibrium or disequilibrium conditions and to establish growth irregularities that can be linked to fundamental changes in systems brought about by crustal deformation events, crustal melting, volcanic eruptions, changing fluid sources, or greater scale tectonic shifts.
**Prof. Robert Gaines**  
Geology Department, Pomona College

Gaines' research program involves understanding critical transitions in the history of life on earth in the context of the evolving chemistry of earth's surface environments. Geochemical analyses are only meaningful when the growth history and timing of mineral products that contain key chemical proxies are well-understood. SEM analyses are key to distinguishing early vs. late stage mineral growth and the sequence of events.

Microbially-precipitated mineral products from the Wheeler Formation (Cambrian; ~505 million years old): calcium carbonate (gray) displaces clay minerals (white) with cluster of pyrite crystals at center.

Characterization of morphology and physical relationship of mineral products in ancient marine sediments informs geochemical analyses and sheds light on the evolution of oceanic and atmospheric chemistry during critical transitions in the history of life.

Robert Gaines is the supervisor and PI

As of yet, I have no publications with the new instrument. I can supply many that use the previous facility.
Figure 1. Cross section of polyetherimide membrane with embedded silica nanoparticles. The above image shows the dispersion of the silica nanoparticles within the polymer matrix. The particles in this image display some visible agglomeration.
Motivations
The Lape Laboratory aims to increase both the effectiveness (selectivity) and speed of gas separations using polymer membranes by embedding nanoparticles in membranes. Typically polymer membranes exhibit a tradeoff between the selectivity and permeation performance causing limited range of effectiveness restricted by an upper bound. Previous studies have reported the addition of silica nanoparticles to ultra-high free volume polymers increase permeability without decreases in selectivity. It has been shown that these nanocomposite membranes surpass the upper bound through an increase in free volume. Multiple hypotheses exist for the increase in free volume: nanoparticles disrupt polymer chain packing, nanoparticles introduce void space at the interface with the polymer, nanoparticles introduce voids within their agglomerates. To determine whether free volume increase is due to void space within agglomerates, we synthesize uniform membranes free of agglomerates and test their gas permeation behavior. The scanning electron microscope is used characterize the size and physical dispersion of our incorporated silica particles within polymer membranes.

In addition to simply physically incorporating nanoparticles in the polymer membranes,
we have also begun synthesizing and chemically incorporating single nanometer-scale nanoparticles into the polymer matrix, allowing us to investigate an alternate method of modifying the free volume of the polymer by changing how the polymer cross-links. The SEM allows us to examine the morphology of the resulting membranes and look for regions where the nanoparticles may have formed a separate crystal phase, indicating either excess loading or incomplete chemical incorporation.

Members:
Professor Nancy Lape (faculty advisor)
Students: Anthony Chung, Jean-Claude de Sugny, Daniel Lee, Kaitlyn Dwelle, Jessica Szejer, Tuan Nguyen

Current Work
We are continuing investigations into evenly dispersed silica particles in polyetherimide, a glassy polymer. From our SEM image, the silica particles show visible agglomeration within the polyetherimide polymer matrix leading to non-uniform membrane morphology. We hypothesize that particle agglomerations could have formed while they were stored in liquid suspension prior to membrane addition. As a result, synthesis and direct incorporation of nanoparticles without storing them could lead to improved dispersions. We are also looking at including silica particles into polytetrafluoroethylene (Teflon), an ultra-high free volume glassy polymer.

Our work chemically bonding the nano-particles to the polymer matrix is still in its initial stages, and the SEM, and its ability to perform EDS as well, has been invaluable to study the morphologies and chemical distributions of our membranes produced so far, allowing us to confidently make procedure modifications to achieve better casting results.
Ryan Dodson (PO ’14) with Prof. Charles Taylor
Chemistry Department, Pomona College

This semester’s research was largely an extension of research that was performed during the summer of 2012. Throughout this update, I will reference information obtained during that time, and will neglect to include micrographs from that research.

During this semester, a total of 25 wafers were deposited on with vanadium oxytriisopropoxide. Temperatures ranged from 146°C to 650°C, with times varying between 30 to 90 minutes. (Table 1)

Prior to this semester, crystal morphologies created by deposition below 300°C were poorly understood. However, our heating apparatus was repaired, and the temperature controller used was recalibrated to stabilize at lower temperatures, and we were able to study them.

Between 150°C and 188°C, very little growth is observed, and crystals that can be seen are sparse and are seemingly amorphous. (Figure 1) Between 200°C and 250°C, the crystals cover the surface of the wafer, but do not seem to show much vertical growth. Roughness can be seen via electron microscopy, but no definitive structures can be elucidated. (Figure 2) More will be learned about these microstructures after cross sectional analysis can be performed. Crystals deposited at 275°C exhibited surprising amounts of deposition relative to lower temperatures – vertical growth of crystals to begin to occur at or before this temperature.

While many wafers were deposited at temperatures higher than 300°C, only several of them were analyzed via electron microscopy. (Figure 3) Among those were samples deposited at 400°C, 450°C, 500°C, and 571°C, temperatures which have all been studied previously. As would be expected based on earlier micrographs, wafers grown in the 400°C to 450°C range showed strong growth and showed complex crystal formation, while very little growth occurred at 500°C and 571°C. Growth that did occur in these ranges was very uniform. Interesting to note was the crystal shapes at these particularly high (>500°C) temperatures; 500°C deposition crystals are observed to be round, where at 571°C, crystals shape seems to be more variable, but octahedronal morphology is commonly observed.

Future research related to this project includes: determining the approximate temperature at which strong vertical growth begins to occur (between 250 and 275°C), studying higher temperatures (>450°C) in more detail to determine when strong vertical growth falls off, looking at temperatures between 500 and 571°C to see when crystal structure changes from round to varied, and examining deposition rates at all temperatures mentioned via varying time and examining cross sections of the wafers via electron microscopy.
Figure 1: Scanning electron micrographs of vanadium oxide thin films deposited on silicon oxide wafers via low pressure chemical vapor deposition of VOTiP at varying temperatures for 90 minutes. All micrographs are taken at 3,000x magnification.
Figure 2: Scanning electron micrographs of vanadium oxide thin films deposited on silicon oxide wafers via low pressure chemical vapor deposition of VOTIP at varying temperatures for 90 minutes. All micrographs are taken at 30,000x magnification. Little consistent deposition can be seen before 200°C.
Figure 3: Scanning electron micrographs of vanadium oxide thin films deposited on silicon oxide wafers via low pressure chemical vapor deposition of VOTIP at varying temperatures for 30 to 35 minutes. Micrographs seen right are taken at 30,000x magnification, while micrographs seen left are taken at 60,000x magnification.
Madeline McGaughey (PO ’16) with Prof. David Tanenbaum

Physics Department, Pomona College

Figure 1: Zinc oxide nanoparticles on a silicon wafer. The spacing of the particles (regarding both density and uniformity) is an indicator of the layer's effectiveness in an organic photovoltaic (OPV) cell. Ideally, the particles are dense enough that they create a smooth layer, but are also relatively evenly spaced (in particular, they avoid clumps). These parameters are controlled by the rotation speed and spin duration used to spincoat the ZnO onto the substrate.

The layer of ZnO nanoparticles in an OPV cell serves as a buffer layer to reconcile the energy level of the collecting electrode (here, indium tin oxide) with the active layer. ZnO is particularly well-suited for this task, as it exhibits high electron mobility while blocking electron holes. To optimize ZnO's effectiveness, it must be evenly spread and sufficiently thick to maximize conduction and hole-blocking. Observing the spatial properties of a ZnO film under the SEM gives insight into how the ZnO layer is structured under specific spincoating parameters.

Coworkers: Emily Yang, Charles Owens; Faculty Member: Professor David Tanenbaum

Our group is examining what affects the efficiency and stability of OPV cells. Being able to observe the physical characteristics of the various layers can therefore be quite useful in pinpointing degradation mechanisms and determining why our cells exhibit certain properties.
The following is an image of a graphene film deposited on copper foil by chemical vapor deposition (CVD), taken with the Hitachi SU-70 SEM at Pomona College. I have worked primarily to optimize a CVD graphene recipe for our lab. SEM imaging is one of the main techniques used to characterize the films we produce, so the Hitachi has been indispensable.

Image (above): Graphene on copper foil, deposited by chemical vapor deposition (CVD). The largest folds mark the copper domain boundaries, within which the finer copper stepping can be seen. The crispness and visibility of the copper structure indicate few layer graphene coverage across the sample.

The SEM will be involved heavily in future research as we continue to refine the growth process to accommodate the requirements of different graphene-based experiments.
Growth and Characterization of Graphene Films on Copper Foil

Eric Puma (PO ’14), David Torenbaum (PO), Paul McEuen (CU)

Abstract:
Graphene, a single-layer carbon nanostructure, has piqued the interest of the scientific community in recent years as a result of its intriguing mechanical, electronic, and optical properties. Here at Pomona, the successful growth of high-quality graphene would unlock a wide range of potential experiments and applications, including use as a transparent electrode for solar cells. We worked towards the growth of single-layer graphene on copper foil via chemical vapor deposition, characterizing our growth using SEM Imaging and Raman Spectroscopy. With a new Raman tool at our disposal, we determined growth attempts from prior years to be, in many cases, graphite (multi-layer) and have since been refining our growth process in hope of attaining single-layer films. We have achieved significantly improved understanding of the characterization techniques and are on the path to attaining single-layer results.

Growth Method:
A small square of copper foil, pressed flat and washed in acid, is heated to 1000°C in a quartz tube, as varying amounts of hydrogen and methane gas are flowed. The hydrogen serves to clean the copper surface, while the methane deposits carbon. The proper flow ratio of hydrogen to methane is key to high quality, single-layer growth. New digital flow controllers allow precise control over flow rates and settings to help us achieve our goal.

SEM Imaging:
There are two characteristic signs of graphene on copper in SEM images. The first sign is wrinkling, a result of the difference in the thermal expansion coefficients of the two materials. The second sign is visible copper stepping, a structural pattern of the copper protected against oxidation by graphene coverage. Image (A) shows two features: the deep copper grain boundaries and wrinkling of the graphene coverage. Image (B) demonstrates wrinkling of the film, in addition to linearity of copper stepping on the diagonal. The final image (C) depicts ideal, single-layer growth taken by Huifeng Li of the Ruoff graphene lab at UT Austin.

Raman Spectroscopy:
Raman spectra are obtained by examining the shift in wavelength of laser light reflected by a material. Peaks in the shifted wavelength indicates the bond structure of the material. The characteristic peaks of graphene Raman spectra are the (G) ~1500 cm⁻¹, (D) ~1350 cm⁻¹, and 2D ~2700 cm⁻¹, where the intensity of the 2D peak is ~10 times that of the G peak for single-layer graphenes. Background noise is a significant problem using a 514nm green laser, single-layer graphene on copper is near impossible to confirm as background removal is difficult. The spectra below illustrate the increase in background noise as the thickness of the sample decreases.

Conclusion:
Our work this past semester has been primarily in refining the growth methods. We have achieved significant improvements in the quality of the films and are finally approaching a standard growth procedure. The implementation of digital flow controllers and a higher-quality SEM imaging capabilities have made this progress possible. From here, we will be working towards the transfer of graphene from copper to other substrates for use in a wide range of experiments.
Aleksandra Ponomareva  
with Prof. Branwen Williams and Prof. Masha Prokopenko  
W.M. Keck Science Department Claremont McKenna, Pitzer, and Scripps Colleges

I am working with Professors Williams and Prokopenko on a deep-sea coral study, focusing on geochemistry and structural analysis of the coral. The broad goal of this research is to determine if the nitrogen isotopic composition of *Desmophyllum dianthus*, a globally distributed species of deep-sea coral, can be used as a proxy for changes in organic matter over time. The nitrogen cycle is very tightly linked to the carbon cycle, and as a result is very reflective of how life responds to changes in oceanic and atmospheric conditions. This study relies on calculating nitrogen values from nitrogen isotopes stored in the coral layers; however this does not account for diagenesis, or any additional changes that took place after initial conditions. Diagenesis would complicate nitrogen calculations and deem the samples unusable as climate proxies. This is where the SEM comes in: I am examining coral samples under the SEM to look for evidence of diagenesis. Initial investigation provides evidence of boreholes and burrows which were likely created after deposition by fungi or small marine invertebrates. It is not clear whether these alterations affect nitrogen isotopes in the sample; however, further studies will be conducted comparing isotopes in bored and non-bored samples. I will also use the EDS to see if “altered” areas reveal a different chemical composition than pristine areas.

This is an SEM image taken of a *D. dianthus* sample which was previously categorized as having “heavy boring”. A “burrow” indentation is visible at the bottom of the screen – this is a structural alteration that may have an effect on isotopic analysis, though it is unclear what exactly may have produced it. In addition the black holes in the image may be boreholes created by very small animals, which would also change nitrogen amounts over time.

I will also be using the SEM for a separate project focused on coralline algae. I will use it to better understand the skeletal crystal structure in the samples. In a methods-development process I will first acid etch sectioned samples in different concentrations and view them under the SEM to determine which acid concentration best reveals the algae skeleton. This project will also assess alteration in the skeleton: I will compare modern algae and fossil samples to see if significant differences in skeletal structure exist over time.
Figure 1. This is an image of the smaller pore surface of an anodized aluminum oxide (AAO) membrane filter that has 20 nm pores on this side and 200 nm pores on the other. It is coated with zinc oxide via atomic layer deposition (ALD) at the Stanford Nanofabrication Facility (SNF). The SEM image shows that there is zinc oxide deposited on this side, but that it may have pores in it.

I specifically work with the ZnO nanostructures used for the DSSCs. Taking SEM images allows us to determine whether the 20 nm-pore surface of the AAO filter is sealed off or not. This is crucial to our DSSCs because we do not want electrolyte leaking through the electrode. Without the SEM, our main method of determining this would be by constructing DSSCs and seeing if it works or not. Cross-sectional SEM images also allow us to determine how much ZnO is deposited inside of the pores.

None of my SEM images have specifically been used in any presentations, reports or publications. They are just used for verification purposes.

Other porphyrin DSSC project members: Emma Van Burns (graduated 2013), Guillermo Martinez (graduated 2013), Sejal Shah, Mo Zhao
1. Figure 1. A side-on view of ZnO nanotubes on anodized aluminum oxide. The nanotubes were grown by atomic layer deposition. From this image, we can see that the deposited nanotubes are polycrystalline in nature, and not single crystalline.

2. These nanotubes are the semiconductor substrate we use to build our dye-sensitized solar cells.

3. Coworkers: Mo Zhao

4. My current research involves trying to grow ZnO nanorods and nanotubes by a solution-based growth, rather than by atomic layer deposition. I use the SEM in order to verify that I've made these nanorods and nanotubes.
Ivan Wong with Prof. Adrian Hightower
Engineering Department, Harvey Mudd College

Figure 1: Observing 100nm TiO2 nanoparticle

Hightower lab uses the SEM in order to investigate whether our nanoparticle syntheses are successful. In the picture above, we zoomed in using a 400,000x magnification, high-current mode and 10kV accelerating voltage in order to get a rough image of the TiO2 nanoparticle. This understanding will help us determine properties of materials used in the construction of our fuel-cells, which are similar to dye-sensitized solar cells.

Coworkers on this project:
Ivan Wong, Ginah Han, Tiffany Liu, Sun Hwi Bang, Professor Adrian Hightower.

Project Summary and use of SEM:
We are investigating different types of electrode (anode) materials for fuel cells, and will be continuing to use the SEM in order to determine nanoparticle size with the objective of improving fuel cell performance.