



Garcia MRSEC



Polymers at Engineered Interfaces

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RESEARCH SCHOLARS PROGRAM 2010

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“The program has no set time limits. Research is a lifelong learning experience, and we hope to remain a resource to our students long after ‘graduation’.”



The Garcia Center for Polymers at Engineered Interfaces is a collaboration of eleven academic, industrial, and government laboratories. The Center was founded in 1996 and is named after the late Queens College professor, Narciso Garcia, a pioneer in the integration of education and research. The Garcia Center is funded by the **National Science Foundation as part of its Materials Research Science and Engineering Center (MRSEC)** program. The goal of the MRSEC is to combine the instrumentation and expertise of the participating institutions into a coordinated research program on polymer interface science. The principal focus areas include thin films, coatings, nano composites, self assembled structures, biomaterials, and tissue engineering.

These areas address both the fundamental and applied aspects that are relevant to the development of cutting-edge technologies in both engineering and medicine. In the community, the mission of the center is to serve as a valuable resource, providing easy access for technological assistance to educational and industrial institutions. For information on the numerous programs that are available, please see our web site at:

<http://polymer.matscieng.sunysb.edu>

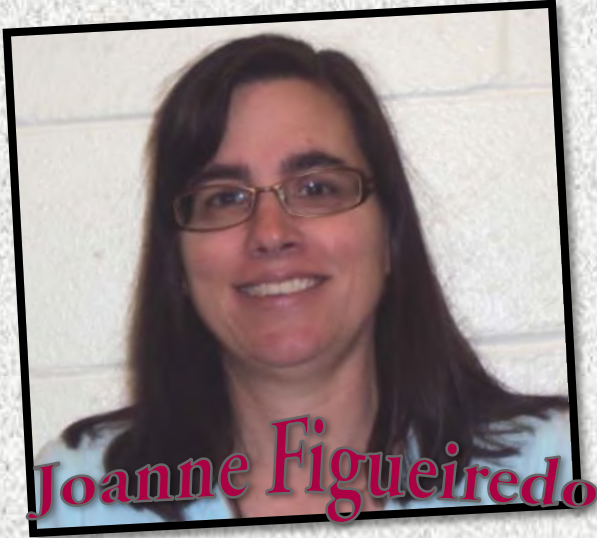
The Research Scholar Program offers the opportunity for high school teachers and students to perform research on the forefronts of polymer science and technology together with the Garcia faculty and staff. Students work as part of focused research teams and are taught to make original contributions of interest to the scientific community. In addition to entering national competitions, the students are encouraged to publish in revered scientific journals and present their results at national conferences.

Our goal is to convey to the students the excitement we enjoy daily in research. The program has no set time limits. Research is a lifelong learning experience, and we hope to remain a resource to our students long after “graduation”.



Miriam Rafailovich
Professor, Garcia MRSEC

Jonathan Sokolov
Professor, Garcia MRSEC



RESEARCH EXPERIENCE FOR TEACHERS

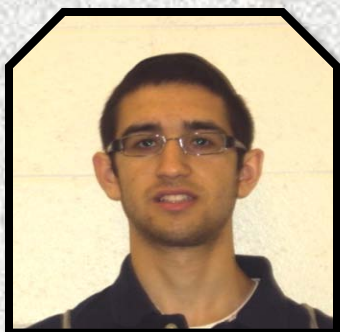


Research Experience For Undergrads

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AARON AKHAVAN



PAUL FOERTH



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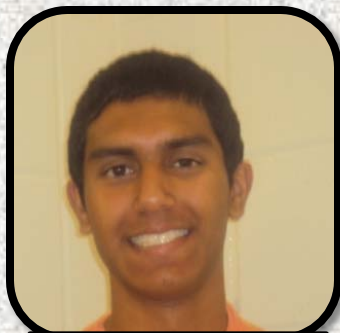
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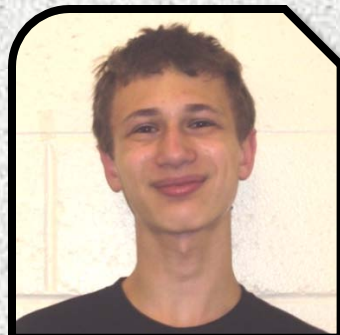
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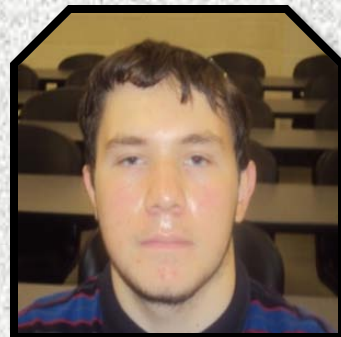
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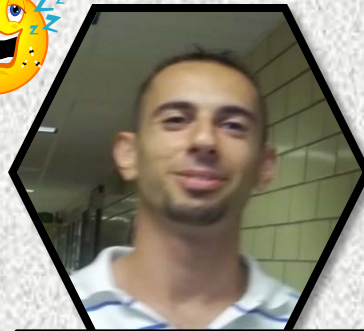
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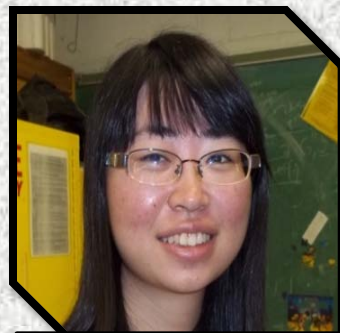
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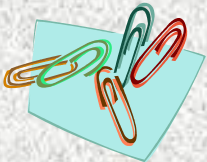
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LAUREN COLLAZO



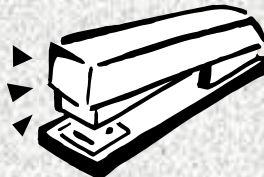
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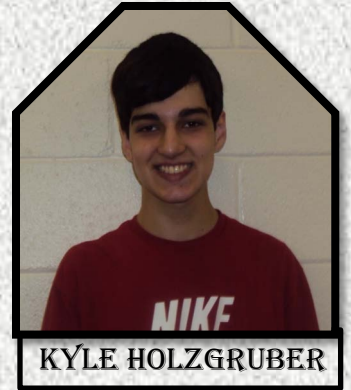


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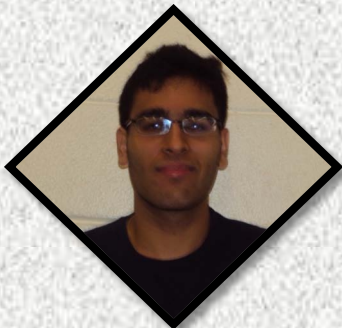
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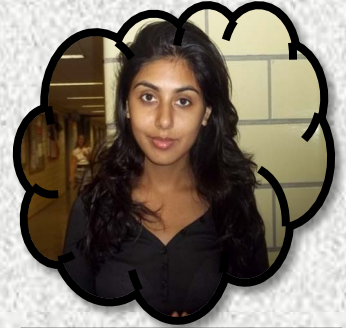


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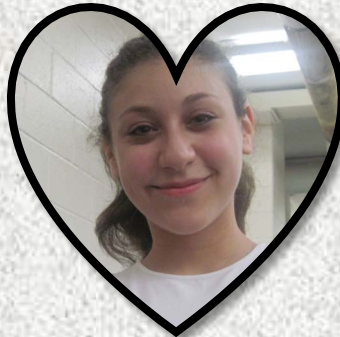
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SONYA PRASAD



BONNIE ROSE



ARPON RAKSIT



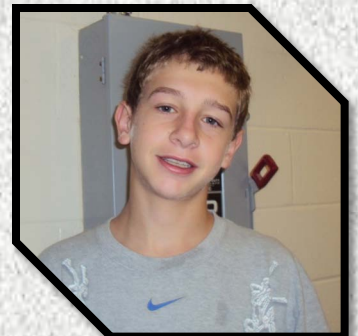
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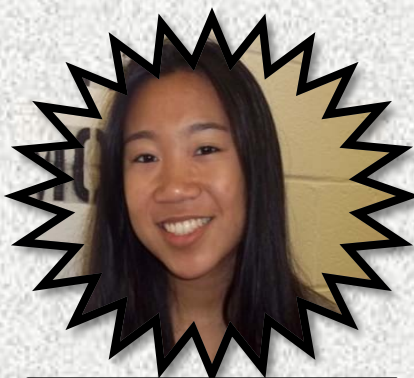
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KARAN SIKKA



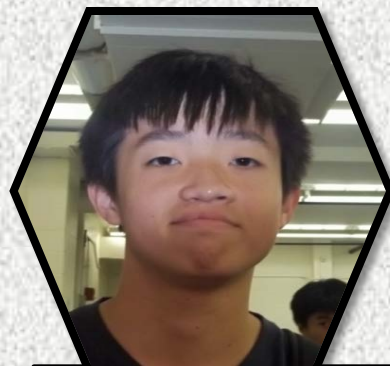
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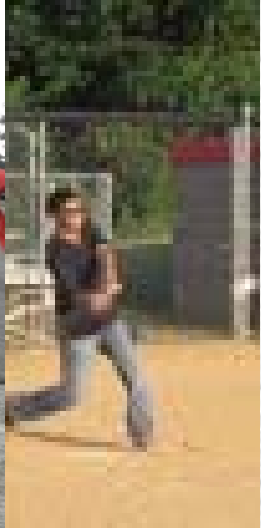
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SAI VANGALA



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Summer Fun





Learning





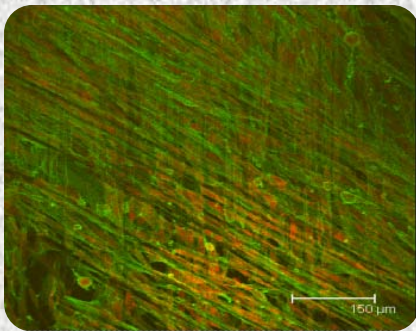
LAB LIFE

B B Q



Biological Devices

SESSION I



Chair: Adam Ossip

Cell Migration: Effects of Fiber Spacing and Diameter of Electrospun PMMA Fibers on the Migration Velocity of Dermal Fibroblasts

Elie Flatow, North Shore Hebrew Academy High School, Great Neck, NY
Alicia Franco, Chemistry Department, Stony Brook University
Sisi Qin, Material Science Department, Stony Brook University
Dr. Miriam Rafailovich, Department of Material Science & Engineering,
Stony Brook University

Cell Migration is a crucial aspect of one's every day life. It is a response to an injury and a bright area for research with its many biomedical applications. Electrospun polymer fibers can be used in wound healing and the treatment of burn victims. It can be deposited as a thin porous film onto a hard tissue prosthetic device designed to be implanted into the human body. Electrospun fibers can be used in the creation of reproducible and biocompatible three-dimensional scaffolds for cell ingrowth.¹

Previous research has shown that cells migrate along the fibers, provided that they are greater than one micron in diameter. If not, the cells will travel randomly through the open pores.²

In this study, a thin film of poly (methyl- methacrylate) (PMMA) was formed onto silicon wafers and glass cover slips by spincoating. Solutions were made from various concentrations of PMMA in different solvents, in order to create various diameters of the fibers (Figure 1). These solutions were then electrospun onto the thin films and annealed for 12 hours. On some samples, a second layer of fibers consisting of multiple angles were spun and tested. Dermal fibroblasts were cultured and prepared for the cell migration. Cells were packed into an agarose droplet and after 24 hours of incubation, migration patterns were watched and recorded for one hour (Figure 2). Preliminary results show that cells did migrate along the fibers and continued onto the second layer with the angles (Figure 3). The denser the fibers, the faster and farther the cells traveled.

Future work will include performing the cell migration using cancerous cells and comparing the velocity and migration distance to those of the healthy fibroblasts. Tests will also be run in order to help determine what substances within the serum cause the cells to migrate.

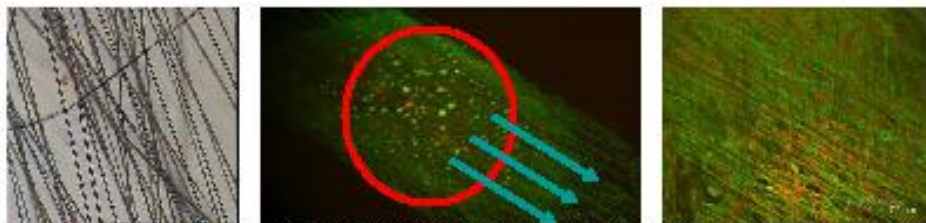


Figure 1: (a) Fibers of 30% PMMA in THF/DMF (1 micron) (b) agarose droplet- cells migrating out (c) cells lining up to migrate along fibers and switching onto residual angle of 55 degrees.

¹ Zheng-Ming Huang, Y.Z. Zhang, M. Kotakic, S. Ramakrishna; "A review on polymer nanofibers by electrospinning and their applications in nanocomposites"; *Journal of Composites Science and Technology* Nov 2003

² Liu, Ying, Yuan Ji, Kaustabh Ghosh, Richard A. F. Clark, Lei Huang, Miriam H. Rafailovich. "Effects of fiber orientation and diameter on the behavior of human dermal fibroblasts on Electrospun PMMA scaffolds." *Journal of Biomedical Materials Research Part A* May 2008 26 Sep 2008

The Re-Encasement and Redesign Of The Battery In The Tongue Touch Keypad To Improve Both Battery Life And Overall Effectiveness

Alexandria Lee¹, Dalia Leibowitz², Dr. Miriam Rafailovich³, Vincent Verderosa³,
Akshay Athalye³, Mihutin Stanacevic³, Dr. Aaron Segal³, Dr. Ronald Wender³,
Dr. Jonathan Sokolov³, Dr. Shmuel Einav³

¹Northern Valley Regional High School at Old Tappan, Old Tappan NJ,

²Yeshiva University High School for Girls, Queens, NY

³Stony Brook University, Stony Brook, NY

The TongueTouch Keypad (TTK), made from a now defunct company, is a wireless device with nine pressure sensitive keys that allows handicapped people to attain a high level of self sufficiency (see Figure 1). The system uses an intra-oral FM transmitter and allows the user to control various objects in his or her environment, such as bed position, room lights, computer, wheelchair, etc¹. For example, the Keypad has been the main mode of transportation for quadriplegic advocate of stem cell research, Brooke Ellison, but she has encountered problems. With the battery lasting only a few months, the TTK would stop functioning and cease to provide her with the freedom it once afforded.

Our research is directed towards the re-encasement of the battery. After the original battery is replaced, a new coating must be placed as to separate the battery from the dental embedding. After trying several different dental materials, we discovered that a Triad Tru-Tray Custom Tray Material worked best when draped naturally but solidified with a rough texture. We coated it with Triad Gel. This was then cured using visible light to harden, creating a hard, but smooth finish. This combination of substances alleviated the problem that occurred after the chemical battery was replaced.

The next goal of our work is to replace the chemical battery with a rechargeable one. This will eliminate the need to continually destroy and recreate the polymer coating. Using the technology of inductive wireless charging, we are attempting to devise a way for users of the TTK to charge the batteries of their devices. Inductive charging uses the electromagnetic field to transfer energy between two objects². A charging station sends energy through inductive coupling to the electrical device, which stores the energy in the batteries. With these two innovations, the TTK's effectiveness will dramatically improve.

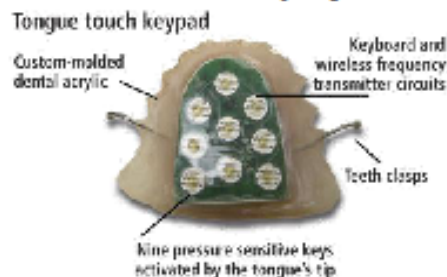


Figure 1: Sample picture of TTK and its components to illustrate function and appearance.³

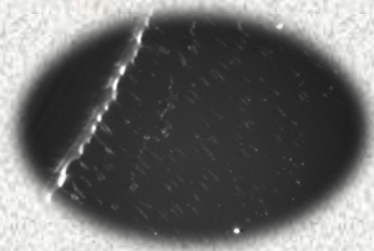
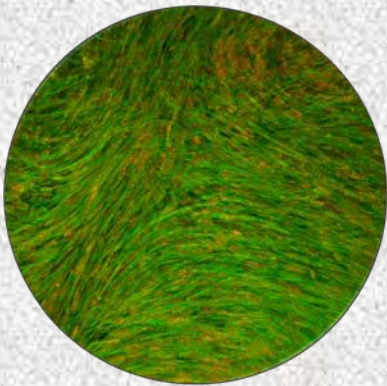
¹ Katz, et al. "Intra-oral control unit and system." Patent 4,605,927.

² Wells, Brannon P. "Series resonant inductive charging circuit." Patent 6,972,543.

³ <http://www.agd.org/support/articles/?ArtID=2451>

Biopolymers on Surfaces

SESSION II



Chair: Sandy Guerrero
Mariah Geritano

Studying the Effects of Static Magnetic Fields (SMFs) on the Biomineralization of Osteoblasts

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¹West Windsor – Plainsboro High School South, Princeton Junction NJ

²SUNY Stony Brook, Stony Brook NY

Osteoblasts are cells responsible for the formation of bone tissue by depositing a matrix that is then biomineralized, a critical part of the bone injury healing process (Y Meng, et al). Static magnetic fields (SMFs) could be applied therapeutically to accelerate or improve the healing of bone injuries (QC Yan, et al). The purpose of our experiment is to document, both quantitatively and qualitatively, the effect of SMFs (flux density 150mT) on the proliferation, differentiation, and biomineralization of osteoblasts.

To quantify the progression of proliferation, sulfonated polystyrene (SPS) thin films were spun on clean glass cover slips and annealed overnight in an oven. Cells were then cultured onto these and clean cover slips without the SPS surface. These samples were taken out at the appropriate time points, and the cells were fixed with formaldehyde and dyed with 4',6-diamidino-2-phenylindole (DAPI) for cell counting to generate a growth curve (see Fig. 1).

Our results show that cell density tends to increase more rapidly in the presence of a magnetic field than the control (without SMF exposure), suggesting that the SMF does enhance proliferation in osteoblasts. In order to measure the differentiation of the osteoblasts an alkaline phosphatase (ALP) assay was performed and normalized with the results from a bicinchoninic acid (BCA) assay to account for differences in cell number at specific time points. The resulting curves, however, were inconclusive in terms of creating a general trend for rates of

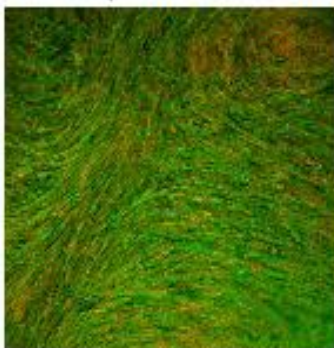


Fig. 2 10x confocal image of a 7 day magnetic sample

differentiation and may need to be repeated. The conformation of the cells was qualitatively analyzed with confocal microscopy (see Fig. 2). SPS thin films were spun on clean silicon wafers and annealed in an oven overnight. Cells were cultured on these and fixed with formaldehyde then dyed with alexa fluor 488 phalloidin and propidium iodide (PI) for confocal imaging. There are not yet sufficient images to draw any definite conclusions, but the cells seem to exhibit a rounded conformation until density prevents them from doing so.

Future experimentation includes further confocal imaging to see how conformation changes over time. The ALP assay will be repeated to verify or improve the existing data, and an osteocalcin assay can be done to quantify the biomineralization of the osteoblasts. Scanning electron microscopy (SEM) will be done as an indicator of biomineralization as well as a source of images. Meng, Yizhi, et al. "Biomineralization of a Self-Assembled Extracellular Matrix for Bone Tissue Engineering." *Tissue Engineering: Part A* 14.00 (2008): 1-12. PDF file.

Yan, Q.C., N. Tomita, and Y. Ikada. "Effects of static magnetic field on bone formation of rat femurs." *Medical Engineering & Physics* 20 (1998): 397-402. PDF file.

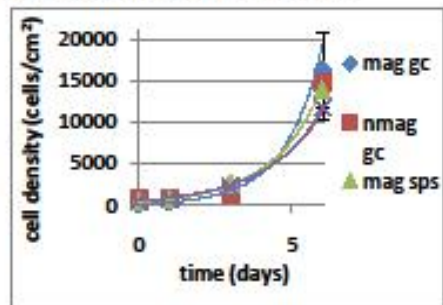


Fig. 1 growth curve for magnetic and nonmagnetic samples

Using Gel Electrophoresis to Separate DNA for Sequencing Applications

Sai Vangala, Brookfield Central High School, Brookfield, WI

Eli Hoory and Dr. Jonathon Sokolov, Department of Materials Science & Engineering, Stony Brook University, NY

Gel electrophoresis is a technique commonly used to separate DNA into single strands or sort them by size. Heat denaturation and subsequent gel electrophoresis ensures that ssDNA constitutes bands that can be extracted from the gel and characterized on polymer surfaces.

This study began with the testing of DNA travel speeds based on agarose gel concentrations, voltage, time, and use of pulsing (reversal of electric field). The DNA solutions tested for separation include a 4% lambda solution, a 5% lambda-monocut solution, and a 5% T4 solution; each was combined with 6x tracking dye for visibility and 8 M urea to keep complementary single strands separated¹. Single-stranded DNA solutions were prepared by heat denaturation at 95 degrees Celsius for ten minutes, and both single- and double-stranded DNA solutions were injected into prepared gels submerged in 1x TAE buffer, connected to an electrophoresis cell with a current, and allowed to run for a predetermined time.

After ssDNA was satisfactorily separated in the agarose gel, the DNA band is eluted in deionized water, and droplets are placed on various polymer surfaces for observation with confocal fluorescence microscopy. Of the surfaces tested, polystyrene (PS) and polymethylmetacrylate (PMMA) surfaces proved optimal for reliable observation of DNA strands, with PMMA surfaces allowing the widest range of observation.²

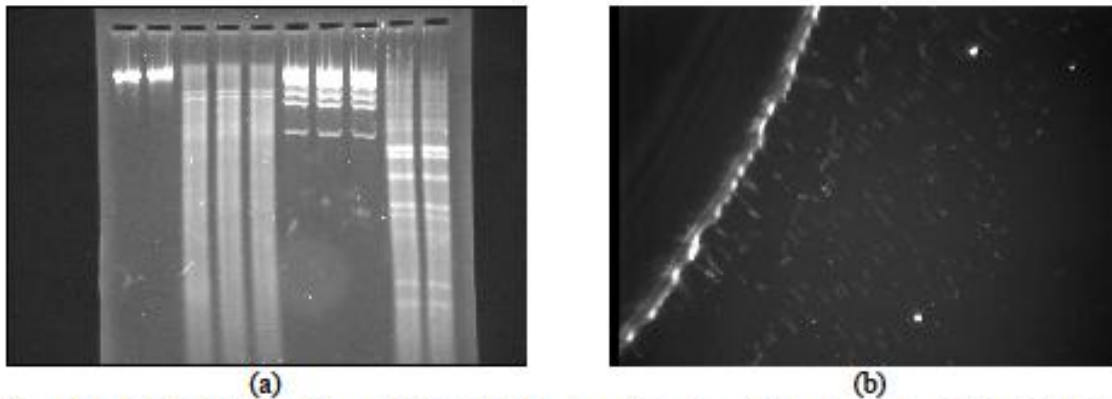


Figure 1: (a) A 1% gel run for 45 hrs at 20V with 1.5 s to .5 s pulsing. Lanes 1-2 are double-stranded lambda DNA, 3-5 are single stranded lambda DNA, 6-8 are double-stranded monocut DNA, and 9-10 are single-stranded monocut DNA. (b) A droplet of single-stranded lambda DNA placed on PMMA. The white dots represent 8 M urea used to keep ssDNA separated, and the 20 micron DNA strands are seen close to the edge of the droplet.

¹ Hegedüs, É., Kókai, E., Kotlyar, A., Dombrádi, V., Szabó, G. 2009. Separation of 1–23-kb complementary DNA strands by urea–agarose gel electrophoresis. *Nucleic Acids Research*. Vol. (37): pg e112

² Allermann, J. -F., Bensimon, D., Jullien, L., Bensimon, A., Croquette, V. 1997. pH-Dependent Specific Binding and Combing of DNA. *Biophysical Journal*. Vol. (73): pgs 2064-2070

Observing Single Stranded DNA on Polymer Coated Surfaces

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NaHyun Cho and Dr. Jonathon Sokolov, Department of Materials Science & Engineering,
Stony Brook University, NY

Observation of DNA molecules is a critical study due to DNA's role in many cellular functions. Recent observation of DNA has included mainly the double helix structure of the DNA molecule with fewer studies done on single strands of DNA.

It has been established that using surfaces of polystyrene (PS) and polymethylmetacrylate (PMMA) to drop molecules of DNA has allowed reliable observation of DNA strands, with PMMA surfaces allowing the widest range of observation.¹ Single molecules of DNA can be observed by using chromosome combing, allowing single molecules of DNA to stretch out by drying a drop of solution at a constant rate.²

In this study, different DNA solutions and surfaces were tested to achieve the best combination to observe single strands of DNA. Different surfaces including polystyrene (PS) and polymethylmetacrylate (PMMA) were used. DNA solutions included a less than 1% lambda DNA solution in sodium hydroxide (NaOH) and morpholinoethanesulfonic acid (MES) solutions and deionized water. The DNA solutions were heated at 95 degrees Celsius for ten minutes to allow denaturing and were dropped onto heated surfaces to allow the drop to dry. Single DNA molecules were observed using a TV camera and confocal fluorescence microscopy. Results indicate that PMMA and PS surfaces with NaOH=MES solutions produce the best results for observing DNA strands (Figure 1).

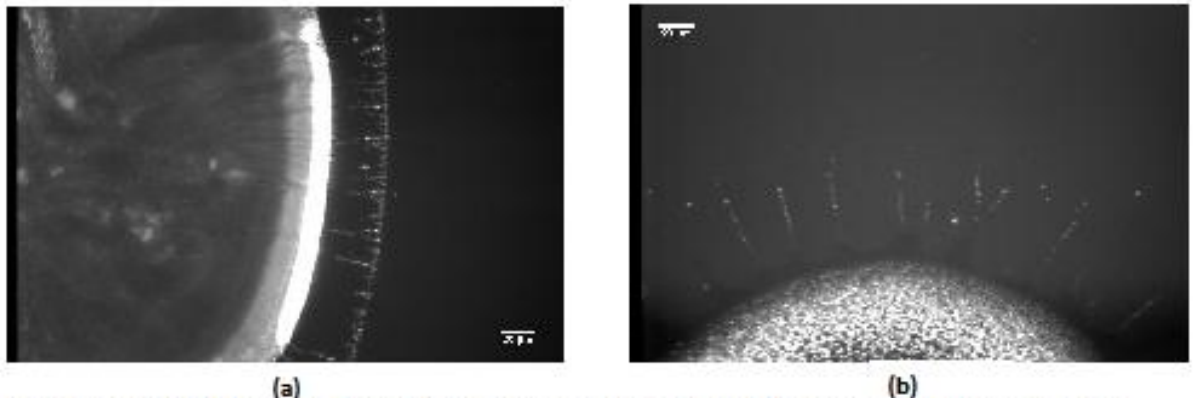


Figure 1: 0.025% DNA solution on PS surface viewed under x40 lens with (a) NaOH=MES (6=50) solution and (b) NaOH=DI (6=50) solution. Notice that the MES solution has more strands of DNA that are closer to 20 nanometers, the correct length of single strands of lambda DNA.

¹ Allermann, J. -F., Bensimon, D., Jullien, L., Bensimon, A., Croquette, V. 1997. pH-Dependent Specific Binding and Combing of DNA. *Biophysical Journal*. Vol. (73): pgs 2064-2070

² Yi, Z., Shengfu, C., Zhenqian, O., Jun, H., Qihua, X., Bin, L., Yibo, H., Minqian, H., Chengzhi, J. 2000. Stretching and imaging studies of single DNA molecules. *Chinese Science Bulletin*. Vol (45): pgs: 1365-1368

Green Micropatterning: Stamping *Candida antarctica* Lipase-B onto Poly(ϵ -caprolactone)

J. Nachman¹, S. Guerrero², M. Rafailovich³, M. Manoj⁴, R. Gross⁴

1. North Shore Hebrew Academy High School 2. Pace University
3. Garcia MRSEC SUNY Stony Brook 4. NYU Polytechnic University

Microlithography, the process which is used to manufacture microprocessors, currently requires use of toluene and yields monomers due to UV light. Toluene is deadly in even very small quantities in groundwater, and monomers have been shown to kill human cells. A new method for micropatterning is proposed where enzymatic degradation replaces toxic solvents and UV light. This was achieved by using Polycaprolactone (PCL), a biodegradable polymer, and *Candida antarctica* Lipase-B (CALB), an enzyme that can break its structure [1]. The enzyme was dissolved in water and swabbed onto a PDMS stamp, which was then placed onto a PCL thin film. A 200g weight was placed onto the stamp and then removed. Samples were incubated at 38°C and examined under the Atomic Force Microscope every other day. It was observed that the stamping was successful at 0 days (Figure 1), and that within 4 days, the enzyme had etched into the polymer. Within 11 days, the enzyme had degraded to the bottom of the film. The degraded film had the original pattern imprinted with minimal accidental degradation. This proves that stamping with the CALB was on par with the current industrial process, and could replace it.

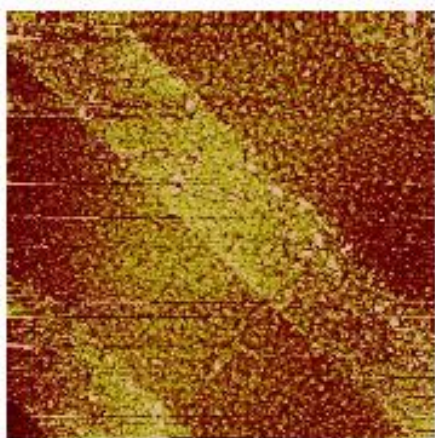
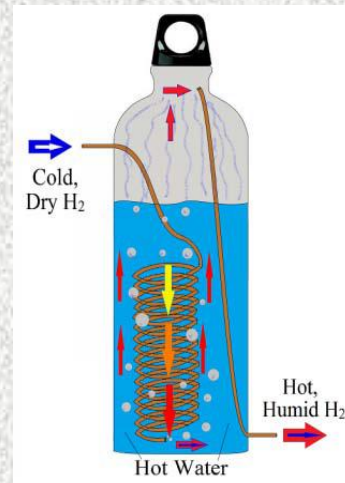
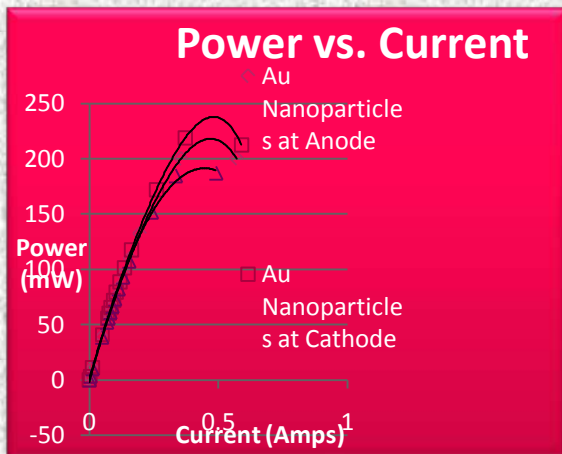


Figure 1: Stamped CALB on PCL at day 0.

1. Gross, Richard A., and Ajay Kumar. "Candida Antarctica Lipas B Catalyzed Polycaprolactone Synthesis: Effects of Organic Media and Temperature." *Biomacromolecules* 1 (2000): 133-38. *ACS Publications Online*.

Alternative Energy

SESSION III



Chair: Paul Masih Das
Paul Foerth

Improving the Efficiency of Dye Sensitized Solar Cells with Various Protein Coatings

Andrew Franco, Brentwood Ross High School, NY

Scott Dunaisky, Half Hollow Hills East, NY

Miriam Rafailovich, Cheng Pang, and Joanne Figueredo, Rose, Charles Fortmann

Dye sensitized solar cells are in the vanguard of innovation, contrasting rivals such as conventional silicon solar cells with benefits that no other solar technology possesses. It's cheaper to produce, more robust and has a smaller environmental footprint with materials that are easily available. It is based on a semiconductor formed between a photosensitized anode and an electrolyte. These nanocrystalline solar cells are made from a suspension of nanometer size particles of titanium dioxide distributed on a glass, which is then heated to form a porous, high surface area film. The counter electrode is coated with a thin catalytic layer of graphite.

Natural dyes, the most popular anthocyanin derived from blackberries is added to the TiO_2 to allow for greater absorption of electrons and allows for a wider range of wavelength absorption, especially those in the blue visible light spectrum. The dye must possess a chemical group that can attach to the TiO_2 surface and have energy levels on the right position necessary for electron injection and sensitization. The two plates are put together creating a sandwich with an iodine electrolyte inside.

Electric charges don't move easily within most organic layers, therefore a thin layer is active for charge injection. To further enhance light absorption protein layers were added to the TiO_2 plates. The experiments showed that the cell with the highest efficiency were the BSA cells with an average efficiency 20% higher than that of a control cell. Unfortunately the lysozyme cells previously thought to show an improvement had a negative effect on the solar cells, and the chitosan coating didn't have a significant effect. The current of the BSA cells were higher than the current of the control cells as the voltage (Fig. 1).

Future research will involve multiple tests to confirm the improvements made by the chitosan and BSA cells. Other test that will be conducted will be the use of proteins that bond well to Titanium Dioxide and the investigations on the effects of different dyes and mixtures of dyes on the cells.

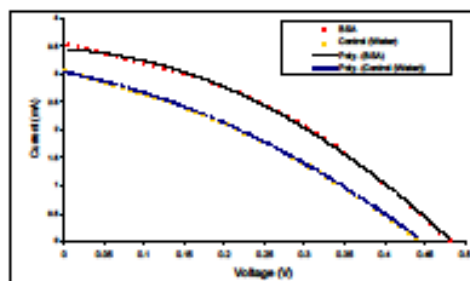


Figure 1- IV Curve of BSA cell (red) versus IV Curve of control cell (gold). The current is consistently higher for the BSA cell.

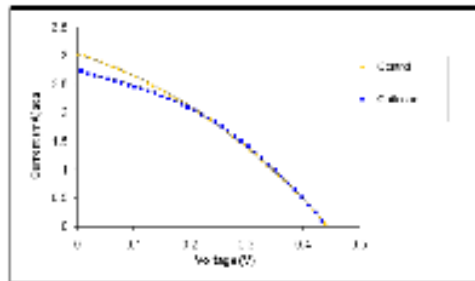


Figure 2- IV Curve of Chitosan Cell (Blue) versus Control Cell (gold). The current is basically the same for both.

1. O'Regan, B. and Graztel, M. "A Low Cost, High Efficiency Solar Cell Based on Dye Sensitized Colloidal TiO_2 Films." *Nature*, vol. 353
2. Smestad, G. P.; Bignozzi, C.; and Agrazzi, R. "Testing of Dye Sensitized TiO_2 Solar Cells I & II." *Solar Energy Materials and Solar Cells*, vol. 32, 1994

The Effects of Humidity and Temperature on the Performance of Gold and Palladium Nanoparticle Catalysts for Applications in a Hydrogen PEM Fuel Cell

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The Hydrogen proton exchange or polymer electrolyte membrane (PEM) Fuel Cell is a clean, efficient way to produce electrical energy from Hydrogen. Its only emission is water, as shown in Figure 1, and it is 2-3 times more efficient than the internal combustion engine.¹ However, the Hydrogen PEM Fuel Cell relies on an expensive platinum catalyst which makes commercial use of the fuel cell impractical.

Previous research has shown that coating the Nafion 117 polymer electrolyte membrane with a film of gold nanoparticles (NP) platelets (dia. ~2.5 nm) increases the performance of the fuel cell.² This may increase the energy output to cost ratio, making the fuel cell a more viable energy alternative. However, the mechanism behind this enhancement is unknown.

In order to help clarify our understanding of the pathway by which the Au NPs improve performance, the effect of high temperature on Au NP performance was studied. As shown in Figure 1, a gas bubbler was designed to heat and humidify the hydrogen gas to operate the fuel cell at a temperature of approximately 70°C while keeping the PEM hydrated.

Previous work has shown that, AuPd NPs act as a catalyst to produce hydrogen peroxide, which causes the dissolution of the Pt catalyst and should theoretically decrease the performance and stability of the fuel cell.^{3,4} Individually, however, Au and Pd both enhance the performance of the Pt-based fuel cell.⁵ PtPd, PtAu and PtAuPd catalysts were tested at different temperatures and the performances were compared. In addition, rotating-ring disk electrode analysis was performed to further clarify the counter-intuitive relationship between these three catalysts.

Preliminary results show that the PtAu is more active than Pt alone at low temperatures. At high temperatures, PtAu does not increase energy output, but it does increase the stability of the fuel cell. [Will insert more results here]

Future work includes finding a cheaper replacement for the Au NPs that also allow the fuel cell to run more efficiently at ambient temperatures, eliminating the need for heating and humidification of the hydrogen.

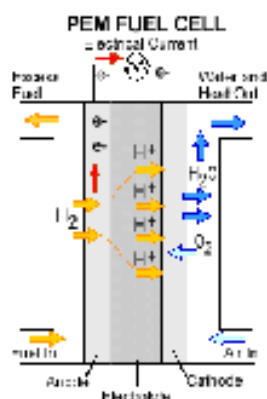


Figure 1: A schematic of the PEM Fuel Cell

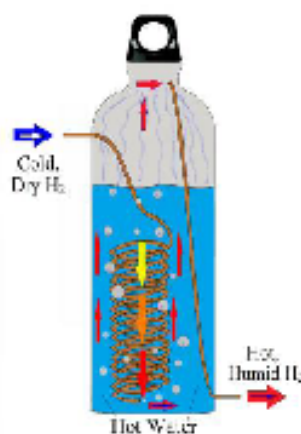


Figure 2: Cross sectional diagram of the H₂ heater and humidifier

¹ Thomas, S., Zalowitz, M., & Gill, D. (2006, December 31). Fuel Cells: Green Power. Retrieved August 8, 2010, from Los Alamos National Laboratory: <http://www.lanl.gov/orgs/mpa/mpa11/Green%20Power.pdf>

² Kao, Chun-Kai. (2007). Nanoparticle Enhancement of Polymer Electrolyte Membrane Fuel Cell Power Output

³ Solsona, B. E., Edwards, J. K., Landon, P., Carley, A. F., & al., a. (2006). Direct Synthesis of Hydrogen Peroxide from H₂ and O₂ Using AuPd. *Chem. Mater.*, 2686-2695.

⁴ Zhang, J., & al., a. (2007, January 12). Stabilization of Platinum Oxygen-Reduction Electrocatalysts Using Gold Clusters. *Science*, pp. 220-222

⁵ Cho, Y.-H., & al., a. (2007). Pd-based PdPt(19:1)/C electrocatalyst as an electrode in PEM fuel cell. *Electrochemistry Communications*, 378-381.

PEM Fuel Cell Efficiency Improvement and Power Output Intensification Through Nanoparticle Catalysis

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Proton Exchange Membrane (PEM) fuel cells, which use hydrogen gas to form H₂O, heat, and energy, are quickly seeming more and more like the most realistic and efficient method of alternative energy due to their high power densities, pollution-free operation, and simple operating conditions (Fig. 1).¹ However, the economic usage of PEM cells is still unrealistic due to the price spent to generate small amounts of energy.

Recent work by Manos Marvrikakis has demonstrated that nanoparticles shaped as flat layered platelets tend to act as great catalysts.² In order to deposit nanoparticles of this shape on the fuel cell membranes we used a Langmuir-Blodgett trough because of its capability to lay a monolayer of nanoparticles on almost all solid substrates. The LB-trough technique is also advantageous due to its precision in terms of a monolayer thickness and its ability to uniformly distribute nanoparticles on the substrate across a monolayer.³

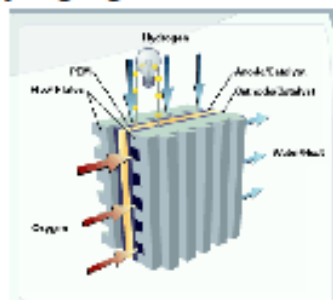


Fig. 1: PEM fuel cell

Source:

http://www1.eere.energy.gov/hydrogenandfuelcells/m/fc_animation_components.html

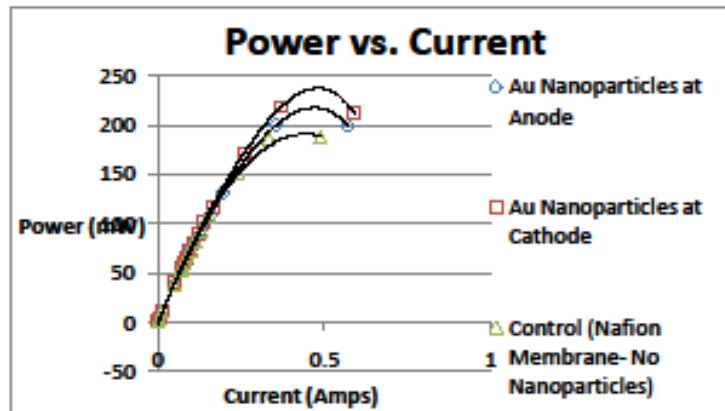


Fig. 2: Efficiency Increase from control to AuNP on Cathode and Anode

membrane on only the cathode side of the fuel cell increased the power output of the cell by approximately 35% (Fig. 2). Our results also showed that coating the membrane with Pd

We synthesized Au, Pd, and Ag nanoparticles and tested their effects when coated on a Nafion-117 membrane using the LB trough. We then tested the increase in efficiency increase of using the nanoparticles-coated membrane by using an ammeter, a flow meter and a voltmeter. Additionally, we tested the difference in efficiency when coating only one side of the membrane. Coating the

¹ Sacca, A, A. Carbone, R. Pedicini, G. Portale, L. D'Ilario, A. Longo, A. Martorana, E. Passalacqua. "Structural and Electrochemical Investigation on Re-cast Nafion Membranes: for Polymer Electrolyte Fuel Cells Application." *Journal of Membrane Science* 278 (2006): 105-113

² Cho, Adrian. "Connecting the Dots to Custom Catalysts." *Science Magazine* 299 (March 2003): 1684-1685

³ "Langmuir and Langmuir-Blodgett Films." *KSV INSTRUMENTS LTD* 107

The effect of Au/Fe₂O₃-TiO₂ on the stability of the Proton Exchange Membrane Fuel Cell

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The study of increasing the efficiency of renewable energy sources (RES) is an integral piece to the puzzle of green engineering. With the onset of the world oil crisis, an increase of greenhouse gases, and the erratic fluctuations of oil prices that harm our consumer society, the turn to RES is not a question of "when" but "how." Hydrogen fuel cells could be the next "combustion engine" of the "green" power revolution. Unlike solar cells and wind turbines, which depend on the availability of sunlight or wind to produce electricity, hydrogen fuel cells are independent of uncontrollable natural patterns and could produce electricity when it is needed of them. Although fuel cells are promising, their current price detracts from their commercial viability to the buyer. Methods of increasing their efficiency and decreasing their cost are essential to their introduction and growth in the consumer market.

Of the leading causes of the inefficiency of the PEM fuel cell is the poisoning of the Platinum catalyst done by the incoming Carbon monoxide. Carbon monoxide has a lower Gibbs free energy of adsorption to the platinum particles on the electrodes than does Hydrogen. Thus, it forms bonds with the Platinum and takes up useful catalytic space for the hydrogen which results in a decrease in efficiency of the fuel cell. Furthermore, Carbon monoxide is impossible to remove without dramatically increasing the cost of the fuel cell.

In this study, Gold nano-particles were loaded onto a Titanium dioxide and Iron(III) Oxide support which was then loaded onto an electrode with the Platinum catalyst. The Preferential Oxidation (PROX) of Carbon monoxide was studied as a function of time (Figure 1). Results indicate that the control performed better than the experimental fuel cells. Further investigation suggests that there was a contamination of the Nafion membrane that was beneficial to the control fuel cell and harmful to the experimental.

Future work includes the introduction of a new Nafion membrane, an increase in the concentration of the particles on the electrode, and an increase in the atmospheric temperature of the fuel cell.

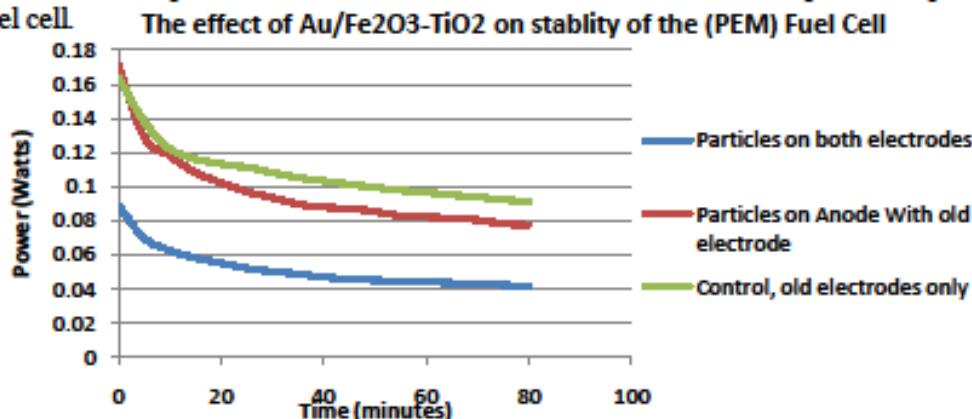
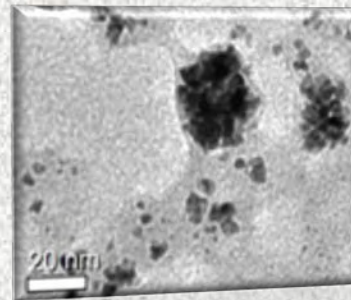
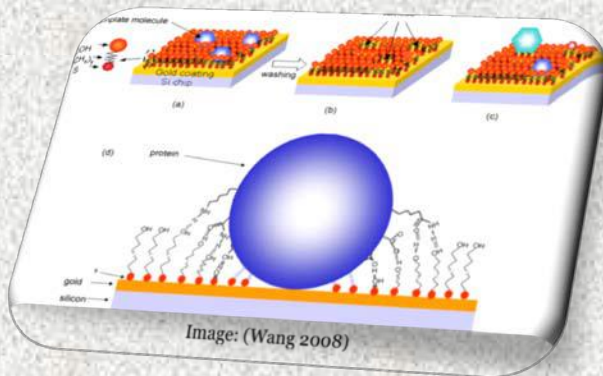


Figure 1: The stability of the fuel cell over an extended period of time

- Yi-Fen Yang, Palanivelu Sangeetha, Yu-Wen Chen. "Au/FeOx-TiO₂ Catalysts for the Preferential Oxidation of CO in a H₂ Stream" *Industrial and Engineering Chemical Research*; American Chemical Society 2009, pp.10402-10407
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Biosensors

SESSION IV



Chair: Sanchita Singal

A Potentiometric Sensor for Virus Detection Based on the Self-Assembled Monolayer Theory

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Vianney Delplace and Miriam Rafailovich, Department of Materials Science and Engineering,
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Pooja Rambhia, Case Western Reserve University

In recent years, interest has grown in potentiometric biosensors due to their quick detection time and reusability when compared to traditional diagnostic tests or polymer imprinting.¹ Little research has been done in imprinting whole pathogens such as viruses, although this method would make disease detection very easy. The ultimate goal of this work is to imprint polio successfully and efficiently, as it belongs to the family Picorniviridae and is therefore closely related to particularly devastating diseases such as Foot and Mouth Disease Virus (FMDV).² Flu A was used in these preliminary experiments because it is relatively easy to obtain.

Detection was done in a 2 mL petri dish instead of the standard 10 mL beaker because of the low concentration of virus. Thiol concentration was manipulated based on previous research showing that too little thiol resulted in an excessively high stabilization value, and that too much thiol resulted in an excessively low stabilization value. The optimum concentration was determined to be 0.0075 mg/mL. The upper limit of detection of virus was determined to be 150 μ L.

Once the proper concentrations of both virus and thiol solution were determined, wafers were incubated for varying lengths of time. The majority of preparation time for each sensor is for incubation, so lowering this greatly increases the applicability of this method of disease detection. Wafers incubated for less than one hour showed no jump. Wafers incubated for one hour showed a more extreme jump than the wafers incubated for two hours, signifying that the optimum time of incubation for this virus is roughly one hour.

Imprints of the Flu A virus are successful about seventy percent of the time thus far. The

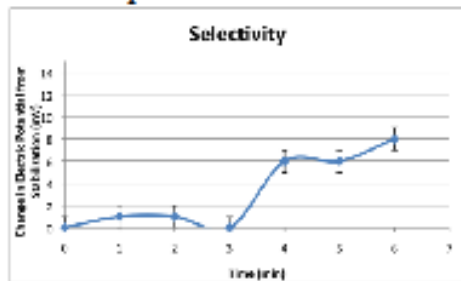


Figure 1: addition of denatured virus at 0 min, addition of normal virus detection solution at 3 min.

graphs of potential vs. time show an initial jump with the addition of detection solution, and then a small decrease, with stabilization at a less extreme jump. This is presumably due to the large upper limit of detection solution of the virus (7.5% volume increase).

In order to verify imprinting, the virus was boiled for one hour at 80 °C in order to denature it and make it unsuitable for detection. No jump was observed when the denatured virus was injected for detection (see figure 1). To make sure the chip used for this experiment was imprinted, normal virus detection solution was injected afterwards, and a jump was observed.

Future work includes imprinting polio as a better model for FMDV, and using the contact angle to verify the upper limit of detection for Flu A. The AFM and SEM will also be used to show the differences between the gold wafer alone, the gold with the monolayer of thiol, and the monolayer imprinted with a virus.

¹Wang, Yantian, et al. "A potentiometric protein sensor built with surface molecular imprinting method." *Biosensors and Bioelectronics* 24 (2008):162-166. Web. 7 Aug. 2010.

²Hyypiä, Timo, et al. "Molecular Pathogenesis of Picornavirus Infections." *Virology* (2007): n. pag. Web. 6 Aug. 2010.

Towards a Theragnostic Approach for Multimodal Cancer Detection and Tumor-Targeted Drug Delivery

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Despite the progress that has been made towards improving survival rates, cancer continues to be the second most devastating disease in the United States, taking nearly half a million lives each year. Consequently, it is essential to develop efficient methods for the early detection, and the treatment of cancer.

As part of the first aim of our study, we sought to engineer a potentiometric biosensor through molecular imprinting for early cancer diagnosis. In specific, current molecular detection of pancreatic cancer relies on tedious and expensive immunoassay methods for a myriad of cancer markers.¹ Instead, we developed a versatile biosensor through a self-assembled monolayer (SAM) of thiol (11-mercapto-1-undecanol) and gold (Au) (See Figure 1). We thus engineered an improved approach to diagnostic methods by imprinting the rapid and inexpensive Au SAM sensor with two specific biomarkers, matrix metalloproteinase-7 (MMP-7) and carcinoembryonic antigen (CEA), both of which have been linked to the onset and progression of pancreatic cancer. The potentiometric biosensor was fabricated using separate solutions of purified CEA and MMP-7 molecules and was subsequently tested using the respective purified solutions, allowing us to establish standard calibration curves. The biosensor then successfully assayed the pancreatic cyst fluid samples of 9 patients (both benign and malignant), determining the specific concentrations of both biomarkers within each sample to parts per billion accuracy.

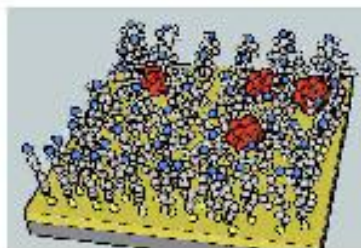


Figure 1: the SAM with organic thiol molecules and proteins for templating (shown in red).

In line with the second aim of the experiment, we developed a novel composite thermoreversible and biocompatible hydrogel platform for drug delivery applications. Current standards for cancer treatment and

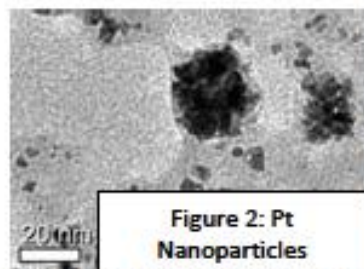


Figure 2: Pt Nanoparticles

eradication, which include chemotherapy, radiation therapy, and surgery, are largely unselective for cancerous tissue, often compromising the integrity of healthy tissue when injected intravenously. Additionally, cancerous cells overexpress Hyaluronan receptors (CD44 and RHAMM) on their cell membranes.² A control gel composed of Hyaluronic Acid crosslinked with Laponite clay was loaded with a substitute drug, Salicylic Acid (SA) dissolved in 95% ethanol. The optimal concentration for gel stability was determined to be 0.01% using UV-Vis spectroscopy. A short-term drug release test was performed in Phosphate Buffer Saline (PBS) at 30 min intervals. The test did not show significant absorbance on the UV spectra, therefore proving HA crosslinked with clay is a slow releasing hydrogel. Next, we coupled our composite hydrogel, consisting of Phrionic F-127 (for thermoreversibility) and Laponite clay (for biocompatibility), with a tumor-targeting moiety, specifically Hyaluronic acid. We then loaded our novel hydrogel with a drug, namely Platinum (Pt) nanoparticles coated with folic acid (Figure 2), and studied release mechanics. We also determined that the IC_{50} concentrations for each of the two pancreatic cancer cell lines, HPAFII (Figure 3) and BXPC3, when exposed to the Pt nanoparticles during a 12 hr period, ranged from 400-500 μ M. In the future, we aim to study the effect of this nanoparticle therapeutic on normal pancreatic cells.

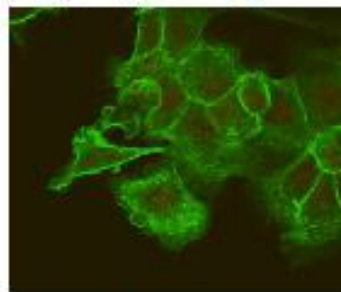


Figure 3: Stressed HPAFII cells

Therefore, we successfully developed an efficient "theragnostic" platform for multimodal cancer detection and therapy.

¹ Wang, Y., Zhou, Y. "A potentiometric protein sensor built with surface molecular imprinting method." *Biosensors and Bioelectronics*. 2008; 24:162-166

² Takada, Moriatsu; Yamamoto, Masahiro; Saitoh, Yoic. "The Significance of CD44 in Human Pancreatic Cancer: I. High Expression of CD44 in Human Pancreatic Adenocarcinoma." *Pancreas*. 1994; 7:6

Detection of Microbial Transglutaminase Using Self-Assembled Monolayer Technique

Alan Czemerinski, The Wheatley School, Old Westbury, NY

Tom Wang, The Wheatley School, Old Westbury, NY

Vianney Delplace, Department of Materials Science & Engineering, Stony Brook University

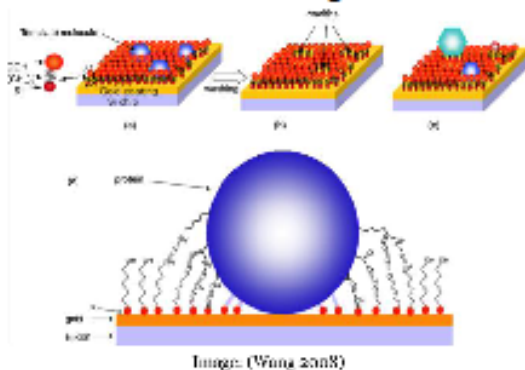
Miriam Rafailovich, Department of Materials Science & Engineering, Stony Brook University

The self-assembled monolayers (SAMs) technique has been applied to the development of biosensors. Using thiol solutions, gold-plated silicon wafers were imprinted with the microbial transglutaminase (mTG) enzyme. It is believed that cells secrete the transglutaminase enzyme to aid in biomineralization. Therefore, differentiating osteoblasts are also hypothesized to do the same. One could easily observe the differentiation of osteoblasts with the detection of the transglutaminase enzyme in cell cultures.

In this study, gold-plated silicon wafers (2.0 x 0.5 cm) were incubated in solutions of the transglutaminase enzyme with different concentrations of thiol. These solutions also consisted of de-ionized water (DIW), which was used to dilute the enzyme that was originally in a powder form. They also contained dimethyl sulfoxide, in which the thiol was dissolved.

The purpose of these incubations was to determine the ideal concentration at which the enzyme could be detected. The detection of the enzyme was established through evaluation of voltage readings from a potentiometer, using the imprinted gold-plated wafer as an electrode. The results from these experiments proved to be inconsistent, which may have been due to the enzyme's propensity to change shape.

Because of this inaccuracy, solutions of the transglutaminase enzyme were boiled at 65° Celsius for 10 minutes for further experimentation. This was done in order to denature the enzyme to prevent it from changing its shape in solution, which would disable it from matching to the imprinted form, leading to the previously inaccurate potential readings. Despite these changes in the procedure, the results remained somewhat unpredictable and it could not be determined if the mTG could be detected using biosensors.



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2. Turner, N., Jeans, C., Brain, K., Allender, C., Hlady, V., Britt, D., From 3D to 2D: A Review of the Molecular Imprinting of Proteins, *Biotechnol. Prog.* (2006) 22: 1474-1489
3. Wang, Y., Zhou, Y., Sokolov, J., Rigas, B., Levon, K., Rafailovich, M., A potentiometric protein sensor built with surface molecular imprinting method, *Biosensors and Bioelectronics* (2008) 24: 162-166

A novel method to detect bacteria using self-assembled monolayers of thiol based on the nano-imprinting theory

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Biosensor technology uses surface molecular imprinting to precisely detect molecules. Molecular imprinting (MI) techniques on two-dimensional surfaces are currently used to detect a wide range of substances using template-shaped cavities in polymer matrices.¹ One type of MI uses self-assembled monolayers (SAMs) produced by a gold-coated silicon chip onto which thiol (11-mercapto-1-undecanol) molecules are chemically bound. During the imprinting process, the target molecules create an imprint in the SAM matrix that is complimentary in size, shape, and hydrophobicity (Figure 1). Biomolecules are detected when they attach themselves to the template, resulting in a change of potential.

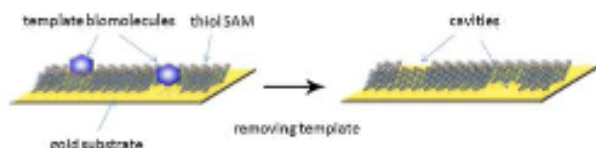


Figure 1. Schematic representation of the template where biomolecules are embedded in a thiol SAM on a gold-coated silicon wafer.¹

This method of detection has been shown to be an efficient and cost-effective technique for proteins.² This study hypothesizes that this technique can be extended for the detection of bacteria as well, which would be greatly beneficial to the field of microbiology and the threat of infectious disease.

It was important to see if the sensor would be able to be imprinted with not only biomolecules alone, but also with media as a consequence of the fact that bacteria often proliferate in media. As a precursor to the detection of bacteria, tests were done to see if the sensor could detect the presence of hemoglobin when in serum. In this study, the chip was incubated with a 0.02 mg/mL hemoglobin-0.005mg/mL thiol solution and was tested with different concentrations of serum and hemoglobin. Results indicated that the sensor was able to detect the presence of hemoglobin even when in serum. Future work will include the imprinting of various bacteria on the sensor and their detection.

¹Wang, Y., Zhou, Y., Sokolov, J., Rigas, B., Levon, K., & Rafailovich, M. (2008). A potentiometric protein sensor built with surface molecular imprinting method. *Biosensors and Bioelectronics*, 24, 162-166.

²Wang, Y., Zhang, Z., Jain, V., Yi, J., Mueller, S., Sokolov, J., & Liu, Z. (2010). Potentiometric sensors based on surface molecular imprinting: Detection of cancer biomarkers and viruses. *Sensors and Actuators B*, 146, 381-387.

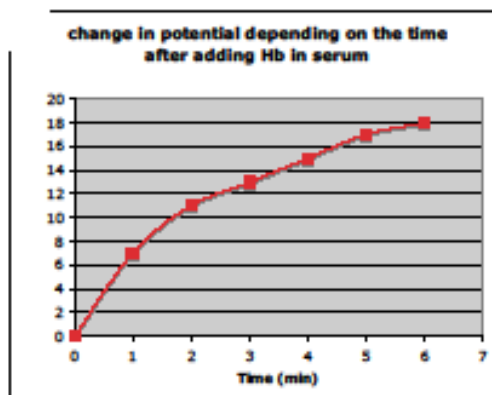
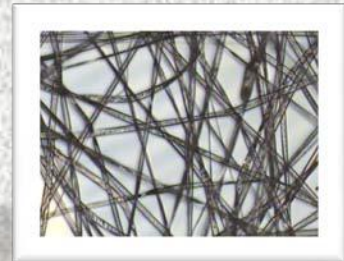
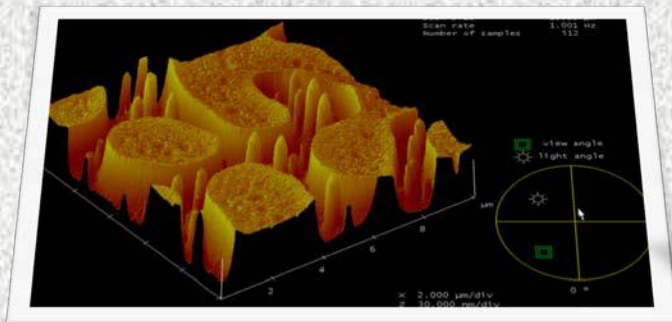


Figure 1. Potentiometric detection of hemoglobin in serum.

Stem Cell Differentiation

SESSION V



Chair: Vladimir Jorukovski

The Mechanical Effect of PMMA Substrates on Dental Pulp Stem Cell Differentiation and Biom mineralization

Leora Apfelbaum, SAR High School; Rachel Shapiro, Yeshiva University High School for Girls
Aaron Akhavan, Yeshiva University

Dr. Miriam Rafailovich, Materials Science Department, Stony Brook University

Dr. Marcia Simon, School of Dental Medicine, Stony Brook University

Aneel Bherwani, School of Dental Medicine, Stony Brook University

The use of scaffolds is a promising technique in the area of tissue engineering. A scaffold, which is comparable to the extra-cellular matrix (ECM) of a tissue, provides a natural environment for cell growth. Dental Pulp Stem Cells (DPSCs) grown on the proper scaffold could potentially be used for tissue repair and regeneration. DPSCs are multi-potent stem cells found in the dental pulp of human teeth. They are readily available and can be induced to differentiate into osteoblasts and bone tissue.¹ DPSCs can be taken from a patient's own body, and therefore the problem of tissue rejection based on finding a donor that is a correct match can be avoided. In this experiment, Poly(methylmethacrylate) (PMMA) was used as the polymer for the scaffolds because it is biocompatible and already FDA-approved for use in medical treatment.²

To create the thin-film scaffolds, silicon wafers were cleaved and solutions of PMMA and toluene were spun cast onto the wafer samples. The solutions used had concentrations of 25 and 30 mg/ml. The fiber samples were prepared using the same procedure, but after spincasting, fibers were electrospun onto the thin film samples. The solution used for electrospinning was a 4g chloroform and PMMA solution that was 20% PMMA by weight. The fibers were electrospun onto the samples in three different alignments: random (Fig. 1), parallel, and crossed (three layers) (Fig. 2). The samples were annealed in a vacuum oven overnight at 150°C.

Afterward, DPSCs were plated on the scaffolds for 21 days to test for biom mineralization. To determine the rates of proliferation of DPSCs on PMMA substrates, growth curves are being created. Cells were plated on PMMA thin-films on silicon wafers, PMMA thin-films on glass cover slips, and PMMA fibers on silicon wafers. The cells were then counted using a hemocytometer. Using optical microscopy, the diameter of the PMMA fibers was determined. Images were taken at 10x and 50x magnifications, and ImageJ was used to measure the fibers in the images. The average fiber diameter in all of the samples was approximately 6 μ m.

Current experiments are running to determine the effect of the substrate on cell biom mineralization and differentiation. Future research involves an experiment to test for biom mineralization of human osteoblasts on PMMA scaffolds, as well as the affect of fiber diameter on the differentiation of DPSCs.

Figure 1: Random PMMA fibers under 10x magnification



Figure 2: Crossed PMMA fibers under 10x magnification



¹ D'Aquino et al. Dental Pulp stem cells: A promising tool for bone regeneration. *Stem Cell Reviews* 4(1): 21-26, March 2008.

² Garrfin. "Biomechanical evaluation of kyphoplasty with calcium sulfate cement in a cadaveric osteoporotic vertebral compression fracture model." *The Spine Journal* 5.5 (2005) 489-93.

The Effect of Hydrogel Environment on Dental Pulp Stem Cell Proliferation and Differentiation

Juliana Coraor, Huntington High School, Lina Lin, Plainview-Old Bethpage John F. Kennedy High School, Divya Bhatnagar and Vladimir Jurukovski, Stony Brook University

Difficulties in tissue transplantation affect millions of people annually. A lack of donors and frequent tissue rejection impede this process's ability to save lives. Tissue engineering is a growing field where a patient's stem cells are used to make replacement tissues¹.

Currently, plastic polymers serve as scaffolds upon which the cells grow and differentiate. However, in case of demand for larger tissue replacements, creating a big scaffold requires a sizable incision for insertion. These polymers are also foreign to the body and therefore are more likely to stimulate an immune response or have other negative effects.

Hydrogels, however, eliminate some of these complications. As hydrophilic polymers that can hold large quantities of water without dissolving², hydrogels are used as 3D scaffolds that can be injected into the damaged site without the invasive surgery and are less likely to induce immune response since the main component of the hydrogels is collagen, protein naturally produced by the body.

More must be learned about how the hydrogel environment affects the behavior of stem cells before tissues can be grown *in vitro*. In order to study this, Dental Pulp Stem Cells (DPSC) were grown on collagen hydrogels with varying stiffness, in presence or absence of dexamethasone (an inducer of osteoblastic differentiation) for 15 days. Afterwards the cells were removed from the gel using collagenase and Trypsin and were plated on glass cover-slips and grown for additional 7 and 15 days in a non-inducing medium, to examine their ability to retain the differentiation potential after the initial "education".

One third of each sample from the initial 15 day growth and incubation were analyzed using confocal microscopy to examine the morphology of the cells and any presence of possible biomineralization (Figure 1a). As shown on Figure 1a, the well stretched green actin fibers indicate the presence of healthy cells, and the presence of bright fluorescent spots might indicate biomineralization. The second third of the samples will be used to confirm biomineralization using mass spectroscopy as evidence of osteoblastic differentiation.

The re-plated cells grown for seven days were examined under a fluorescent microscope (Figure 1b). The preliminary results show that there seems to be evidence of deposits on both the original gels and the glass cover-slips. In the future, we will analyze the re-plated cells after fifteen days and we will use SEM/EDAX to confirm the existence of hydroxyapatite deposits.

¹ Lal, B., Viola, J., Hicks, D., Grad, O. *Emergence of Tissue Engineering*, 11-17.

² Schacht, E. H. (2004). *Polymer Chemistry and Hydrogel Systems*, 22-28.

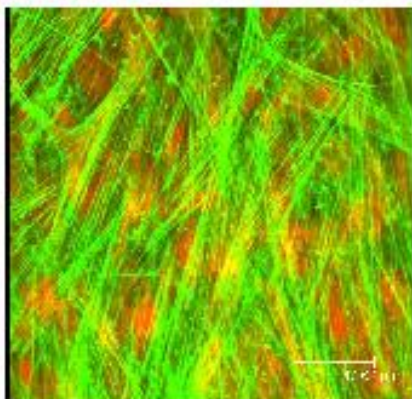


Figure 1a- Gel sample stained and viewed under a confocal microscope. The more intense green specks on the green fibers could be hydroxyapatite deposits.

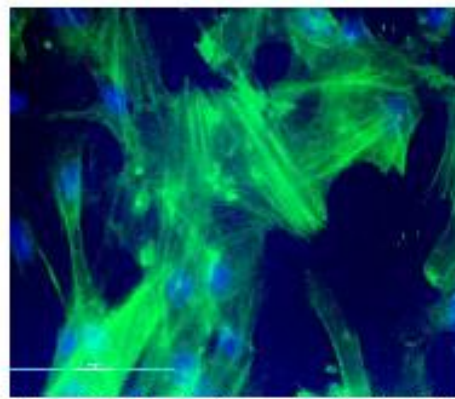


Figure 1b- 7 day plastic sample stained and viewed under a fluorescent microscope. Green dots might indicate biomineralization.

Effects of Titanium Dioxide and Zinc Oxide Nanoparticles on the Proliferation and Differentiation of Human Dental Pulp Stem Cells In Vitro

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Eric Huang, Monta Vista High School, Cupertino, CA

Chien-Hsiu Lin, Vladimir Jurukovski, and Miriam Rafailovich, Department of Materials Science & Engineering, Stony Brook University

Nanotechnology is a promising field of engineering; however, the risks associated with nanoparticles have not been fully explored. In this study we explored the effects of zinc oxide and titanium dioxide nanoparticles on human dental pulp stem cell proliferation and differentiation. From cell counting, we established a comparison between the control and nanoparticle-treated cells. We concluded that zinc oxide and titanium dioxide nanoparticles were extremely toxic to the cells, and were increasingly dangerous as the concentration increased. Additionally, zinc oxide nanoparticles were more harmful to the cells than titanium dioxide.

As for differentiation, we utilized immunofluorescent imaging to search for a potential inhibition of the stem cells' ability to differentiate. The images suggest that zinc oxide seems to completely inhibit the differentiation of dental pulp stem cells, while the control and titanium oxide samples were still able to differentiate when grown with dexamethasone, a corticosteroid that has been shown to induce differentiation (1). However, all types of the nanoparticle-induced cells generally had comparatively disoriented actin fibers, indicating that the cells were affected by their environment (Figure 1).

Given the toxicity of the nanoparticles, we then wanted to see if coatings on the nanoparticles would limit the damage. We used nanoparticles coated with an anionic polymer, antioxidants and a silicon polymer outer wrap. Our cell counting results showed that the coated nanoparticles are less toxic to the cells, but still dramatically inhibit cell proliferation (Figure 1).

Since dexamethasone improved differentiation, we also looked at the effects of dexamethasone on cell proliferation. By plotting a growth curve over the span of one week, we observed that dexamethasone did not any significant effects on cell proliferation.

In the future, we will verify our inferences by staining the cells for osteocalcin, a marker of osteoblasts-like cells (2). Additionally, we will confirm the presence of calcium phosphate using SEM/EDAX analysis.

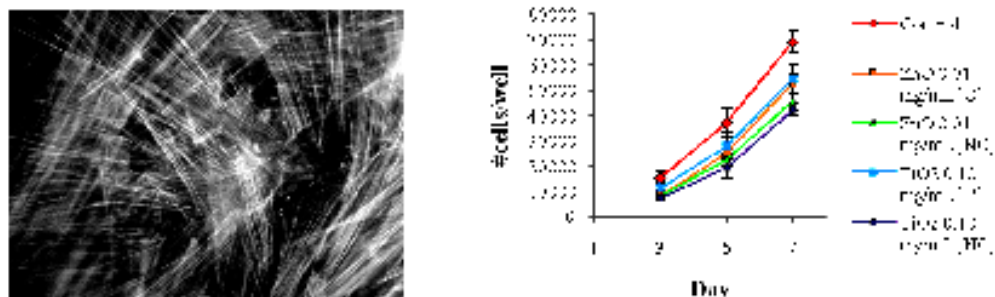


Figure 1: (a) Microscope image of Zinc 0.01 mg/ml cell sample (b) Control, coated, uncoated growth curve

1. Siddappa, R., et al. "cAMP/PKA Pathway Activation in Human Mesenchymal Stem Cells In Vitro Results in Robust Bone Formation In Vivo." *Proceedings of the National Academy of Sciences* 105.20 (2008): 7281-7286.

2. Eghbali-Fatourechi, Guiti Z, et al. "Circulating osteoblast-lineage cells in humans." *The New England Journal Of Medicine* 352.19 (2005): 1959-1966.

Biomaterialization of Dental Pulp Stem Cells in Hydrogels of Differential Stiffness

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The therapeutic potential of stem cells is versatile, with potential applications including treatments for chronic diseases like Alzheimer's and long-term paralysis. In terms of practicality, Dental Pulp Stem Cells (DPSCs) provide multipotent, uncontroversial, and easily obtainable stem cells [1], while hydrogels act as a biocompatible substrate for potential delivery of DPSCs to the treatment site. The ability of DPSCs to differentiate into osteoblast like cells is particularly intriguing and can be potentially used in the regenerative repair of stress fractures and other bone injuries. However, many aspects of the relationship between DPSCs differentiation potential when grown on hydrogels are yet to be explored, including the relationship between substrate stiffness and biomaterialization. Our experimentation seeks to characterize the effects that hydrogel stiffness has on DPSCs' differentiation into osteoblast like cells and biomaterialization.

Based on previous research [2], we hypothesize that hydrogel stiffness is positively correlated with osteoblastic differentiation of DPSCs and biomaterialization levels. The hard gels should induce the most mineralization and therefore have the highest calcium phosphate levels of the samples, while the opposite is true for the soft gels.

Hydrogels of three different hardness (hard, medium, and soft) were made using gelatin and cross-linked with microbial transglutaminase. The stiffness was varied by using 3:1, 25:1, and 125:1 gelatin-to-enzyme ratios. DPSCs were then plated onto each gel and incubated for 15 days in inducing and non-inducing media, and then stained with Alexa-Fluor 488 and propidium iodide and imaged under confocal microscope. In almost all samples, the cells displayed uniformly aligned actin fibers (Fig. 1) probably due to the confluent nature of the cultures. The moduli of the cells were determined using atomic force microscope showing that the cells tend to conform to the stiffness of the gels and it takes about three days for them to adjust (Fig. 2). When incubated in inducing media the cells' moduli were overall higher. Finally, mass spectrometry will be used for quantify the calcium phosphate content to indicate biomaterialization levels.

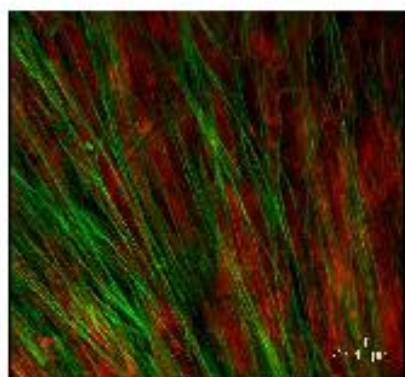


Fig. 1: Cells in 25:1 hydrogel and non-induced media were stained with Alexa-Fluor 488 and propidium iodide and then imaged using the confocal microscope.

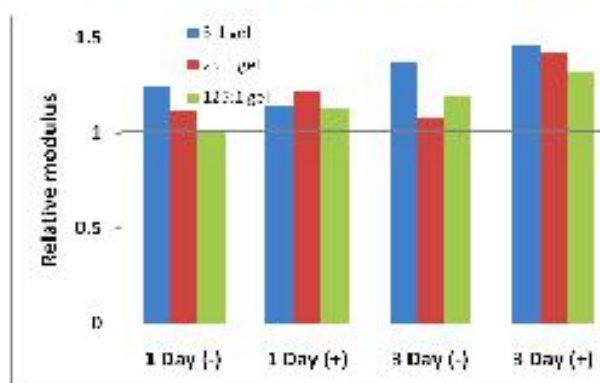


Fig 2. Relative moduli of DPSCs grown on hydrogels with varying stiffness and media conditions for 1 and 3 days, showing slow adaptation to the growing environment.

¹Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000). Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA 97:13625-13630.

²Engler et al., 2006. A.J. Engler, S. Sen, H.L. Sweeney and D.E. Discher, Matrix elasticity directs stem cell lineage specification, Cell 126 (2006), pp. 677-689.

The Effects of Mechanical Nanopatterns on Human Stem Cell Differentiation

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Miriam Rafailovich, Department of Material Science and Engineering,
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Stem cells are of great interest due to their potential in many medical therapies. In this study, human dental pulp stem cells were plated on mechanical nanopatterns in order to study the differentiation and proliferation of the cells on these surfaces. Knowing how the cells differentiate and proliferate can give us control over these processes, and furthermore allow us to direct them. Stem cell transplants can become more efficient and successful as the cells being transplanted are guided towards a certain outcome.

Dental pulp stem cells (DPSCs) are pluripotent stem cells that can be induced to become osteoblasts, neuronal and muscle cells.¹ The material on which the cells are grown can affect cell growth. DPSCs can sense the hardness of the substrate which they are on and grow in response to it. A surface is characterized as "biocompatible" if the cells like the surface. In our research, we study the effects different mechanical nanopatterns have on the differentiation of these cells.

Nanopatterns were created by spin-casting 1:3 polymer blends of polystyrene (PS) and poly(methyl methacrylate) (PMMA) onto silicon wafers (see Figure 1). Nanopatterns were created due to the concept of interfacial tension, the energy at the boundary between two materials. The higher the interfacial tension, the higher the free energy at the interface of two polymers, and vice versa. Because polymers are not compatible with each other, they wish to minimize the interfacial area and ball up therefore.² Then, the ion mill was used to sputter each sample and etch the pattern into the silicon. Finally, the use of a high temperature oven (1000°C) and cleaning the samples in a solution of 1:1:1 H₂SO₄, H₂O₂ and H₂O removed all remains of the polymer, leaving just the etched patterned silicon wafer. The grooves left by the etching were filled with polybutadiene (PB) in one set of samples and ethylene-vinyl acetate (EVA) in another set. The cells were plated on these nanopatterns and their patterns of differentiation and proliferation were studied.

Future work includes analyzing cell growth data and repetition of the experiment on gold-covered silicon wafers.

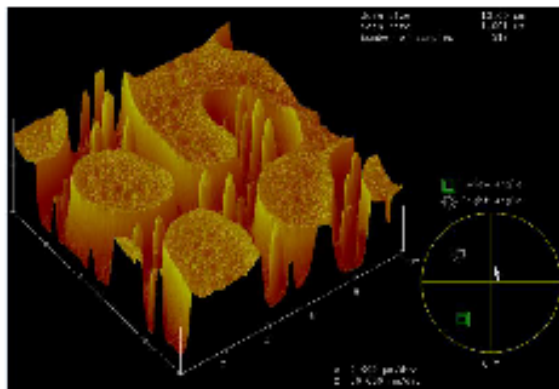


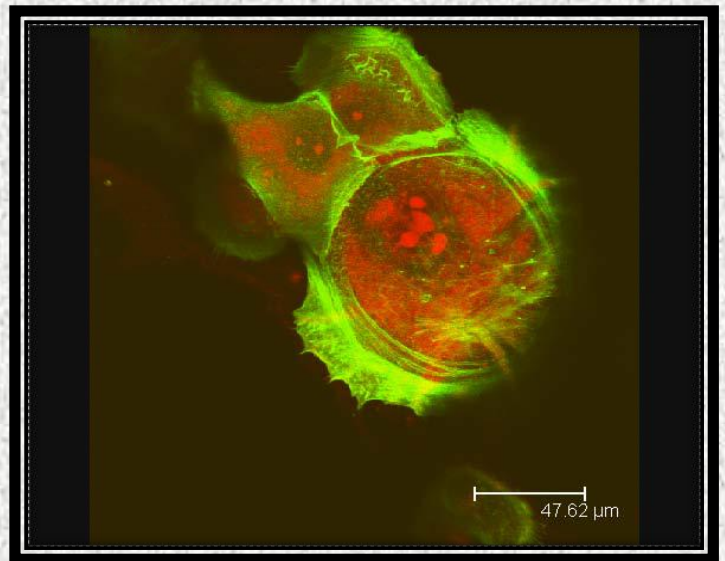
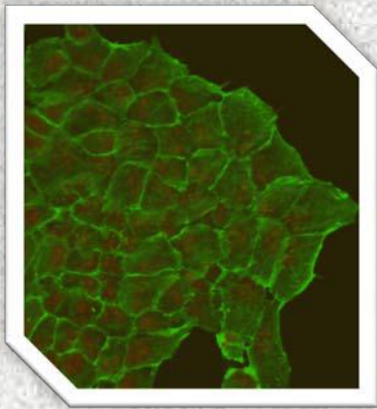
Figure 1: PS : PMMA 1:3 sample on silicon wafer under atomic force microscopy

¹ British Dental Journal; 7/10/2010, Vol. 209 Issue 1, p5-5, 1p

² Jerome, J.; Zhu, S.; Seo, Y. S.; Ho, M.; Parnodet, N.; Gambino, R.; Sokolov, J.; Rafailovich, M. H.; Zaitsev, V.; Schwarz, S.; DiNardo, R. *Macromolecules* 2004, 37, 6504.

Nanoparticles and Cells

SESSION VI



Chair: Anjali Kapur

THE EFFECTS OF GOLD NANOPARTICLES ON HELA CELL MEMBRANES

Penina Safier and Bonnie Rose

Acknowledgments: Dr. Miriam Rafailovich, Dr. Peter Brink, Josh Kaufman, Anjali Kapur, Dr. Wong

siRNA, a form of gene therapy, is an innovative way to cure disease. Nonetheless, it requires a method through which to enter the cell. This treatment can be delivered through injection and viruses, but neither method is foolproof. Our experiment sought to look at the effects of nanoparticle exposure on the membranes of HeLa cells, under the assumption that should the nanoparticles sufficiently weaken the membranes, siRNA could be delivered with greater ease intracellularly.

We tested this theory by using a patch clamp hooked up to a voltmeter. This process facilitates the analysis of ion channels in the plasmalemma. By comparing the graphs created by patches of control cells to the graphs created from patches of cells exposed to 13 nanometer gold nanoparticles and 45 nanometer gold nanoparticles, respectively, we were able to assess the efficacy of said gold nanoparticles on making the cell membrane more permeable. This analysis was based on the supposition that the more permeable the membrane had become, the greater the flow of ions, or current, across it.

Our results showed little difference between the current across our control cells and our experimental cells, suggesting that the nanoparticle exposure did not sufficiently weaken the cell membranes. Nonetheless, further data collection must take place before anything conclusive can be determined.

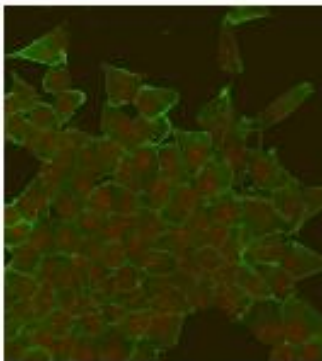


Figure 1.1: Control HeLa Cells, 63x magnification

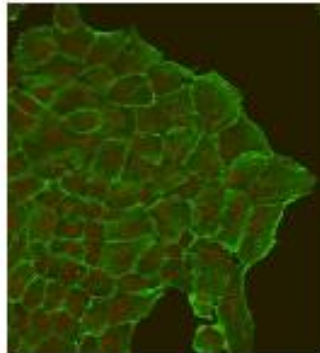


Figure 1.2: HeLa cells exposed to 13 nm nanoparticles

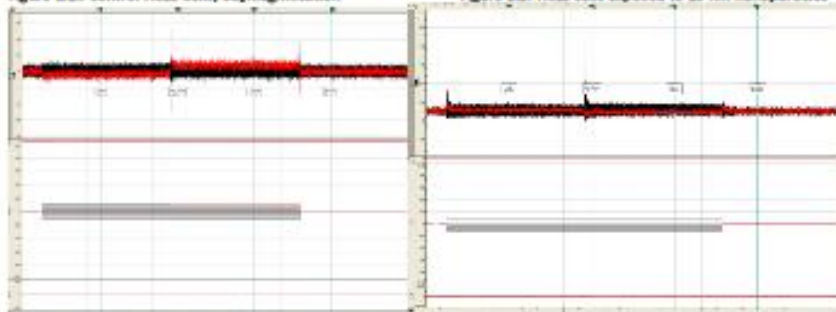


Figure 1.3: Clampex analysis of control patch shows little difference from Clampex analysis of cells exposed to 13 nanometer gold nanoparticles, at right, at left.

Figure 1.4: Clampex analysis of experimental cells exposed to 13 nanometer gold nanoparticles shows little difference from Clampex analysis of control cells, at right, at left.

The Effects of Gold Nanoparticles on Cancerous and Non-Cancerous Bone Cells

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Jeremy Sacks, Hebrew Academy of Five Towns and Rockaway

Helene Sonenberg, Yeshiva University High School for Girls

Kathryn Dorst, Department of Materials Science & Engineering, Stony Brook University

Miriam Rafailovich, Department of Materials Science & Engineering, Stony Brook University

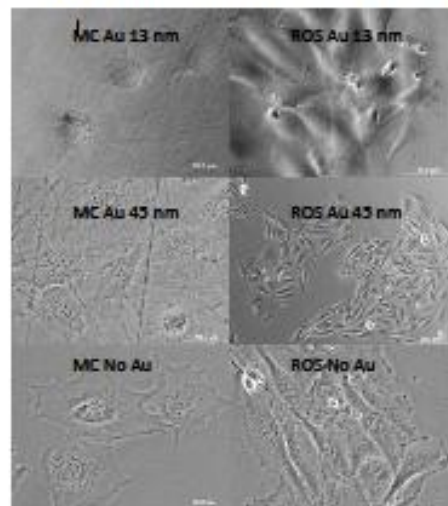
Yizhi Meng, Department of Materials Science & Engineering, Stony Brook University

Nanoparticles have become a field of great interest to scientists. Much is unknown about these substances, such as how they enter cells and how toxic they are. Even though there is evidence that gold nanoparticles are toxic to cells [1], it is not known if the nanoparticles themselves are harmful or if the precursors used during the synthesis of these particles are actually causing the damage to the cells. Since nanoparticles are being used in different common products such as electronics, it is imperative to find out what happens when the nanoparticles penetrate cells. It has been discovered that nanoparticle diameter is integral to the fate of dermal fibroblasts [1]. In the case of both 13 nm and 45 nm gold citrate nanoparticles, the two sized particles entered and formatted in the cells in drastically different ways. The 13 nm particles entered mainly through phagocytosis, while the 45 nm nanoparticles were mainly engulfed by the cell through endocytosis. Additionally, while both size nanoparticles seemed to sequester in vacuoles along the vacuole membranes within the cell, the larger gold nanoparticles were more toxic to the cells compared to the smaller particles possibly due to rupturing of their vacuole containers and allowance of their direct exposure to the cell environment. [1]

Therefore it is our goal to see if cytotoxic effect of gold nanoparticles pertains to osteoblasts as well by culturing MC3T3-E1 and ROS cells in culture medium supplemented with 13nm and 45nm gold nanoparticles or their precursors. We have begun a preliminary growth curve and plan to further this study by analyzing the effects of gold nanoparticles on the biomineralization of MC3T3-E1 and ROS cells over the next 4 weeks and analyze the steps the cells go through after being induced to biomineralize [2].

[1] Mironava, Tatsiana., Hadjiargyrou, Michael., Simon, Marica., Jurukovski, Vladimir., Rafailovich H, Miriam. "Gold nanoparticles cellular toxicity and recovery: Effect of size, concentration and exposure time."

[2] Meng, Yizhi., Qin, Yi-Xian., DiMasi, Elaine., Ba, Xiaolan., Rafailovich, Miriam. "Biomineralization of a Self-Assembled Extracellular Matrix for Bone Tissue Engineering."



Toxicity of Clay Nanoparticles on the Growth and Differentiation of MC3T3 Osteoblasts

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Chi Zhang, Youjun Zhai, and Liudi Zhang, Stony Brook University, NY

Yizhi Meng and Miriam Rafailovich, department of Materials Science & Engineering, Stony Brook University, NY

Widely investigated for their flame retardant properties, clays are often incorporated into polymer nanocomposites as a method for enhancing mineralizing cells.¹ However, companies interested in using these clays must first test their safety levels (especially since they consist of nanoparticles, whose penetrating abilities are often toxic to cells).

In this study, a nanocomposite of silicon and Cloisite 20A, a montmorillonite clay able to improve the thermal and mechanical properties of polymer blends,² was tested for either adverse or beneficial effects on MC3T3 (*Mus musculus*) cells. A monolayer of concentration 1 mg/mL of Cloisite 20A dissolved in Xylene was deposited on a silicon wafer using a Langmuir Blodgett (LB) Trough. The Si/20A wafer and a plain silicon wafer were cut and cells were plated onto each, with composites consisting of no cells acting as controls. Composites were stained with DAPI on days 1, 3, 6, and 8. The cell densities were calculated using a fluorescence microscope, and a growth curve was modeled. On days 2, 8, and 14, the cells were stained with fluorescence (ALP) and viewed under a confocal microscope to check the cells' morphologies. Results indicate that the Cloisite 20A silicon nanocomposite slows the growth of cells and detrimentally affects their differentiation.

Future work includes testing nanocomposites of different clay types and concentrations mixed with polystyrene to determine if a specific concentration would be helpful to the cells.

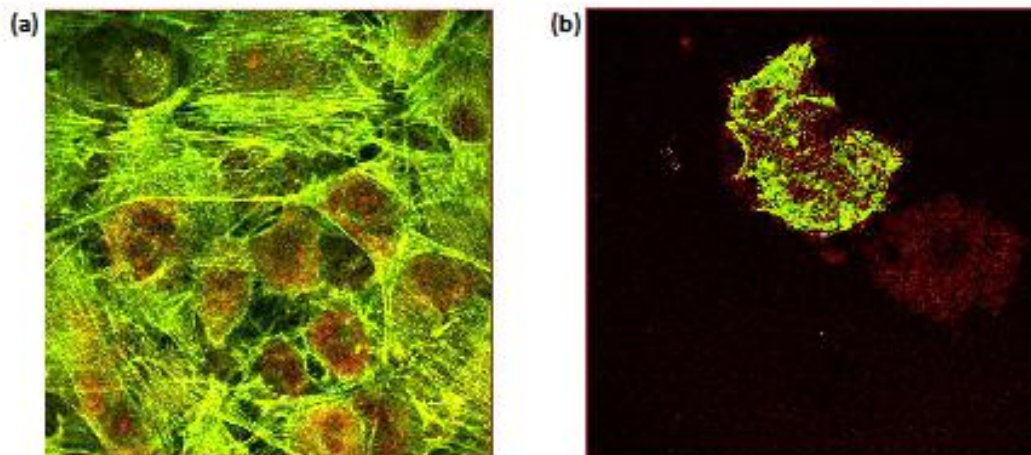


Figure 1: (a) Stained cells on plain silicon wafers, viewed under a confocal microscope day 2 (b) a stained cell on silicon & cloisite 20A, day 2. Cells were rare and deformed

1. Kashiwagi, T.; Harris Jr., R.H.; Zhang, X.; Briber, R. M.; Cipriano, B. H.; Raghavan, S. R.; Awad, W. H.; Shields, J. R. *Flame retardant mechanism of polyamide 6-clay nanocomposites*. (2004). 45, 881-891.
2. Si, M.; Araki, T.; Ade, H.; Kilcoyne, A. L. D.; Fisher, R.; Sokolov, J. C.; Rafailovich, M. H. *Macromolecules* (2006). 39, 4793-4801.

Gold and Platinum Nanoparticles Effects on Dental Pulp Stem Cell Growth

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Evan Schneider, Roslyn High School, Roslyn Heights, NY

Tatsiana Mironava, Department of Materials Science and Engineering

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Many studies in the field of nanoparticles have centered around their effects on cells and their use in cell labeling. Scientists have looked at the different ways in which nanoparticles enter cells, inhabit cells, and exit cells. They have also tried to explain how nanoparticle toxicity can have either advantageous (used in cancer treatments) or detrimental effects on cells due to its general toxicity.

Currently, research on nanoparticles is being devoted to the examination of the different ways in which nanoparticles interact with cells. Research on nanoparticles is also being directed towards using nanoparticles (in cells) as a mode of cell tracking, or cell labeling. In recent studies, the interactions that occur between gold nanoparticles and human dermal fibroblasts were observed, and the uptake of these nanoparticles was detected as a function of time, size, and concentration of the nanoparticles¹. In another study, iron oxide nanoparticles were used to track bone marrow and embryonic stem cells in the brain and spinal cord of a rat in order to prove that nanoparticles can be used as a method of magnetic resonance tracking of implanted stem cells². Both studies used electron microscopy among other methods to observe the cell structure and interaction of the cells with the nanoparticles.

One thing that scientists have not extensively researched is the possible effect that the toxicity of nanoparticles has on cell growth and particularly stem cell differentiation. In this study, gold and platinum nanoparticles were used to observe the way that nanoparticles might hinder or encourage the growth and differentiation of dental pulp stem cells. This study focused on the differentiation of the dental pulp stem cells with and without nanoparticles, and it also tested the effect that nanoparticles had on the cells. In order to detect the interaction between the nanoparticles and the cytoskeleton and nucleus of the cell, confocal and fluorescent microscopy were performed. Fluorescent microscopy was used to better analyze the differentiation of the stem cells and to detect osteocalcin (an early signal of proper differentiation). In order for osteocalcin to be present, dexamethasone (a corticosteroid) is required. This study observed that cells that were treated with nanoparticles produced osteocalcin in inducing media (media containing dexamethasone) whereas the cells that were placed in non-inducing media (without dexamethasone) did not produce osteocalcin, except in the case of the platinum nanoparticles, which is very interesting and will be a subject of future work. These results indicate that nanoparticles do not hinder the early differentiation of dental pulp stem cells.

Further work will include testing different concentrations of nanoparticles on these cells and tracking the presence of these nanoparticles inside of the cells.

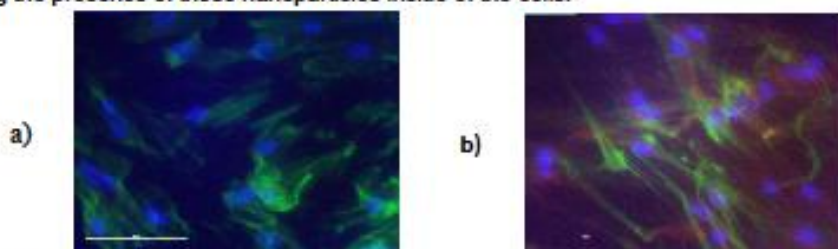


Figure 1: (a) Cells in non-inducing media without nanoparticles that did not differentiate (b) Cells in induced media without nanoparticles that did differentiate.

¹ Mironava, Tatsiana, Hadjiargyrou, Michael, Simon, Marcia, Jurukovski, Vladimir, Rafailovich, H. Miriam. Gold nanoparticles cellular toxicity and recovery. Effect of size, concentration and exposure time. *Nanotoxicology*; March 2010, Vol. 4, No. 1 : Pages 120-137

² Jendelova, Pavla., Herynek, Vit., Urdzikova, Lucia., Glogarova, Katerina., Kroupova, Jana., Andersson, Benita., Bryja, Vitezslav., Burian, Martin., Hajek, Milan., Sykova, Eva. *Journal of Neuroscience Research*, Volume 76 Issue 2, Pages 232-243.

The Modulus of Cancerous and Normal Keratinocytes and the Effect of Titanium Dioxide Nanoparticles and UVA Radiation on their Mortality

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Rebecca Klahr, HAFTR High School, Cedarhurst, NY

Marcia Simon, School of Dental Medicine, Stony Brook University

Miriam Rafailovich, Dept. of Material Science & Engineering, Stony Brook University

Skin cancers are the most frequently diagnosed cancers in the United States. Among these cancers, squamous cell carcinoma is the second most commonly diagnosed. Generally these carcinomas occur in areas of the body directly exposed to UV radiation from sunlight or other sources. Although sunscreen use has been suggested as a means to limit the risk of skin cancers, sunscreens that include nanoparticles of titanium dioxide (TiO_2) may not adequately provide this benefit. UV exposure of these nanoparticles generates reactive oxygen species and peroxide radicals which can modify lipids, proteins and DNA to cause cellular damage. (1, 2)

Recent studies have suggested that changes in cell mechanics can be used as an indicator of cancer and reflect differences in cell function (3). In our study, we are trying to determine [1] whether the viability of normal (DO33) and transformed (SCC12b, SCC13) keratinocytes are affected similarly by TiO_2 (with or without UV exposure), [2] how cellular and matrix mechanic of normal and transformed cells are altered by TiO_2 and [3] whether TiO_2 alters the effects of UVA exposure of normal and transformed cells. In an effort to understand the nature of cancer progression we have also carried out these experiments with immortalized, non-transformed keratinocytes (SF-44). Future experiments will explore the effect of the extracellular matrix on cell mechanics. For this purpose we will measure the modulus of normal cells grown on the cancer cell matrix and cancer cells grown on the normal cell matrix.

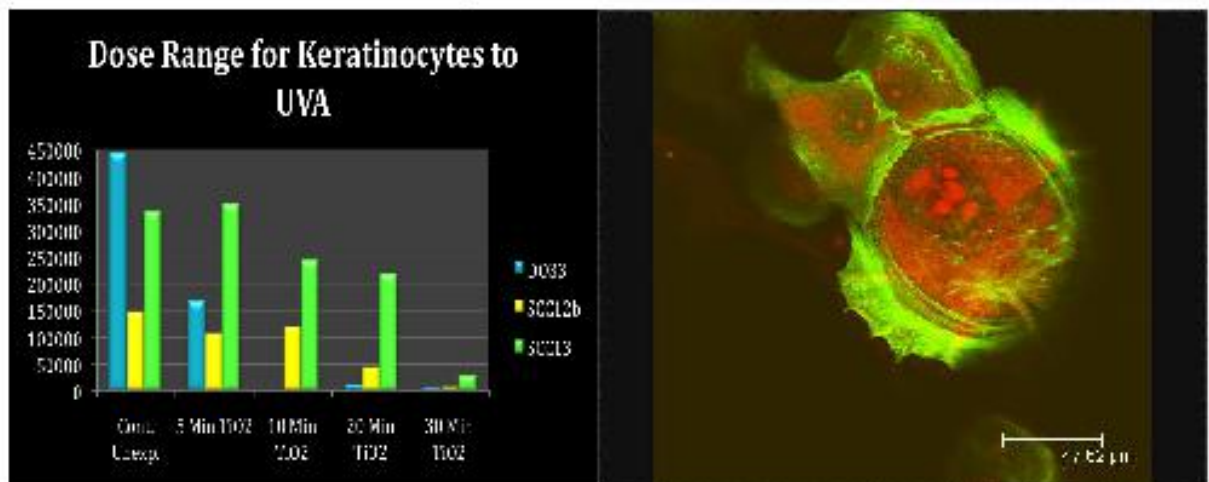


Figure 1: (a) Dose range for Keratinocytes exposed to UVA radiation (b) Confocal Image of DO33 cell at 63x magnification.

1. Gross, Neil D. "Skin Cancer: Squamous Cell Carcinoma." *eMedicine*. N.p., n.d. Web. 17 July 2010. <<http://emedicine.medscape.com>>.
2. Kiss, Borbala. "Investigation of micronized titanium dioxide penetration human skin xenografts and its effect on cellular functions of human skin-derived cells." *Experimental Dermatology* (Dec. 2007): 659-667. Web. 3 Aug. 2010.
3. Parnodet, Nadine. "Detecting Cancer Cells in Normal Tissue by Scanning Force Modulation Microscopy." *Microscopy and Analysis* (Mar. 2008): Print.

Synthesis of Elastomeric Polymer Blends

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Maxwell Plaut, Massachusetts Institute of Technology

Miriam Rafailovich, Department of Materials Science & Engineering, Stony Brook University

Ethylene vinyl acetate (EVA) is a copolymer currently used for a variety of applications. EVA is used because of its many useful properties such as toughness, good adhesion, and stress crack resistance.¹ It also poses no threat to one's health. It has the potential to replace the more dangerous polymer, PVC in transmission cables. But it would be better suited for this purpose if it were made flame retardant and more flexible. This may be possible by creating a polymer blend that incorporates EVA. Blending polymers is a means of creating new materials which combine the valuable qualities of each of the components.

In this experiment, EVA was blended with the polymers, Polystyrene (PS) and Polycaprolactone (PCL). Spin casting was used to create thin films to determine the miscibility of these polymer blends. Atomic force microscopy (AFM) allowed the contact angle to be measured. A low contact angle, averaging 7.947° was measured for the EVA/PS blend, indicating that EVA and PS are miscible. The AFM image of the blend shows droplets of one polymer in the other polymer so they are not perfectly miscible (Figure 1 a). In addition to trying to mix polymers by themselves, clays were also added because clays reduce interfacial tension, making polymers more miscible.² The average contact angle for the EVA/PS blend mixed with cloisite clay 20A was 28.073° , which is significantly higher than the contact angle of the blend without the clay. This is visible in the AFM image because there are more droplets in the image of the blend with the clay than in the image of the blend without clay, indicating that the blend is less miscible with the clay (Figure 1b).

In the future, the EVA/PS blend will be tested in bulk and the miscibility of the EVA/PCL blend will be determined, possibly allowing it to be tested also. Another goal will be giving EVA self healing properties.

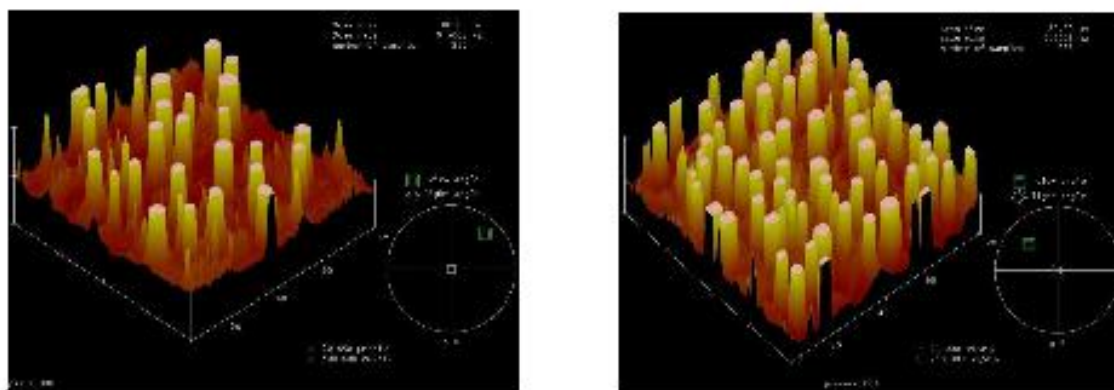


Figure 1: (a) EVA/PS blend viewed with AFM (b) EVA/PS blend with 2% cloisite clay 20A

¹ Mark, James E., ed. *Polymer Data Handbook*. N.p.: Oxford University Press, 1999.

http://www.qmc.ufsc.br/~minatti/docs/20061/polymer_data_handbook.pdf

Web. Aug. 2010.

² Ade, Harald, Winesett D.A., Smith A.P., Qu S., Ge S., Sokolov J., Rafailovich M. "Phase Segregation in Polymer Thin Films: Elucidations by X-Ray and Scanning Force Microscopy." *Europhysics Letters* 45(1999): 526-532.

The Crystallization Rate of Polylactide (PLA): Nanocomposites and Copolymerization

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Maxwell Plaut, MIT

Miriam Rafailovich, Department of Materials Science & Engineering, Stony Brook University

Polylactide (PLA), derived from the lactic acid of renewable sources such as corn and sugarcane, is an eco-friendly substitute to plastics such as Polystyrene (PS), Polyethylene (PE), Polypropylene (PP), Polyethylene Terephthalate (PET) Polyvinyl Chloride (PVC), and other types of polymers. Serving as a plastic that is 100% biodegradable, PLA is able to save up to 65% of energy in production methods and 68% less toxic greenhouse gases, allowing PLA to join the global trend towards the “green” movement as an alternative energy plastic.¹

Although PLA presents itself as a solution to the petroleum-based, indecomposable plastics currently in use, it is flawed in its ability to withstand high temperatures. With a glass transition temperature at 58°C and a melting point at 152°C, PLA is unable to be used under conditions that require excessive amount of heat.² This property makes PLA an impractical alternative to other types of commonly used polymers, so the emphasis in this project is set on increasing the crystallization of PLA to improve PLA’s melting temperature.

For this study, cleaved Silicon wafers were used to make thin films using PLA/2% Cloisite Clay 20A, PS/PLA, PS/PLA/2% Cloisite Clay 20A, PLA/Ecoflex, PLA/RDP, PLA/PS/RDP, PLA/Ecoflex/RDP, PLA/Ecoflex/2% Cloisite Clay 20A blends. The thin films were then scanned using the Atomic Force Microscope (AFM), and the contact angle was calculated from the scans. The contact angle allowed the miscibility of each polymer blend to be determined. From looking at the AFM scans, the polymer blend with more droplets visible were more immiscible than the polymer blend with fewer droplets evident (Figure 1). If two substances mix well together, then they are considered miscible; if they do not, then they are immiscible.

Future work includes conducting tests in bulk to increase PLA’s flame retardancy and using copolymerization in which PLA will be blended with other polymers to increase the rate of crystallization.

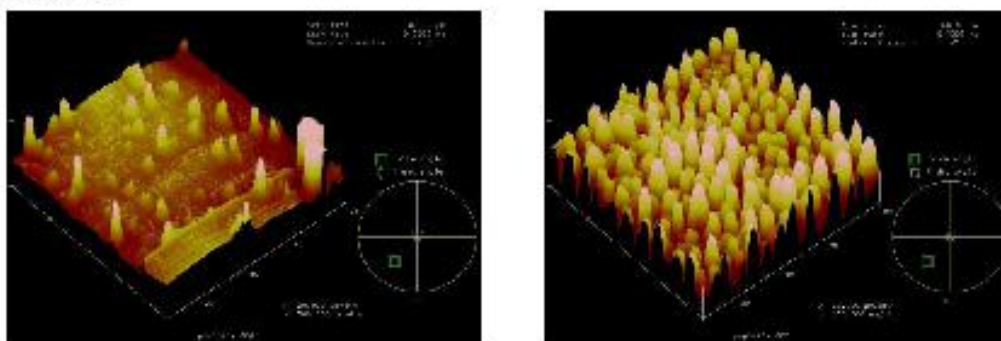


Figure 1: (a) 3-D AFM scan of a PLA/2% Cloisite Clay 20A blend on a thin film dipped in a Langmuir-Blodgett trough (b) 3-D AFM scan of a PS/PLA/2% Cloisite Clay 20A blend on a thin film

¹ Royle, Elizabeth. “Corn Plastic to the Rescue.” August 2006. Web. 8 August 2010.

² Averous, Luc. “Bioplastics - Biodegradable polyesters (PLA, PHA, PCL ...).” University of Strasbourg, May 2009. Web. 8 August 2010.

Mechanical, thermal, and morphological characterization of biodegradable Ecoflex and Poly(lactic acid) (PLA) blends with RDP soaked cloisite clay

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Kai Yang, Dr. Miriam Rafailovich, Dr. John Jerome

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Mariah Nicole Geritano SUNY Stony Brook

In recent years, the demand and interest in biodegradable materials for use in packaging, agriculture, medicine, and other areas has seen a large increase¹. Biocomposites such as Ecoflex and poly(lactic acid) (PLA) are of special interest due to their relatively inexpensive cost to produce, mechanical strength, and their ability to biodegrade completely². However, the applications of these polymers are limited by their low flame retardancy. To solve this, Ecoflex and PLA nanocomposites were made with a novel additive, resorcinol bis(diphenyl phosphate) cloisite soaked clay. This study aims to create these blends on the bulk and thin-film level in order to improve the flame retardancy of the polymers for further industrial applications.

Homogenous blending of Ecoflex and PLA with RDP soaked cloisite clay was done using a C.W. Brabender. In this study, four concentrations of RDP soaked clay and Ecoflex/PLA were used, 100% Ecoflex/PLA and 0% RDP clay, 99% Ecoflex/PLA and 1% RDP clay, 95% Ecoflex/PLA and 5% RDP clay, and 90% Ecoflex/PLA and 10% RDP clay. Tensile testing and DMA revealed that as the concentration of RDP clay increased, the tensile strength of the polymer blends decreased (Figure 1) and the stiffness increased (Figure 2). However, the resistance of the blends to flames showed great improvement as there was less blackening of the polymer and reduced dripping with increases in the concentration of RDP clay.

Future work will focus on the interaction between Ecoflex, PLA, and RDP clay on the nanoscale level. In order to determine if the RDP soaked clay interacts favorably with Ecoflex and PLA, thin films of Ecoflex and PLA will be synthesized through spin-casting on a monolayer of RDP clay deposited on a silicon wafer using a Langmuir-Blodgett trough. Atomic force microscopy will be done before and after annealing of the polymers to look for phase separation.

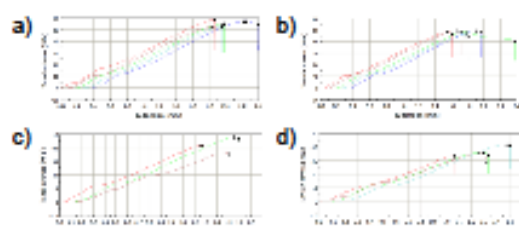


Figure 1. Tensile test results for a) 100%PLA b) 99%PLA 1%RDP c) 95%PLA 5%RDP d) 90%PLA 10%RDP

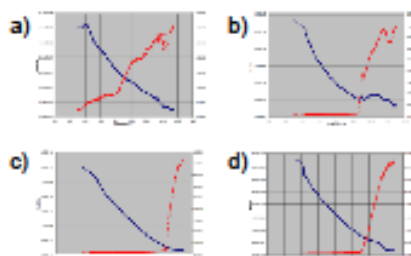


Figure 2. DMA test results for a) 100%Ecoflex b) 99%Ecoflex 1%RDP c) 95%Ecoflex 5%RDP d) 90%Ecoflex 10%RDP

¹ Kolybaba, M., Tabil, L.G., Panigrahi, S., Crerar, W.J., Powell, T., Wang, B., 2003. Biodegradable Polymers: Past, Present and Future. CSAE/ASAE Annual Meeting Paper No. RRV03-0007, Fargo, ND, October 3–4.

² BASF. "Ecoflex- the entirely biodegradable plastic." *plasticportal.net*. BASF, 2002. Web. 10 Aug. 2010.

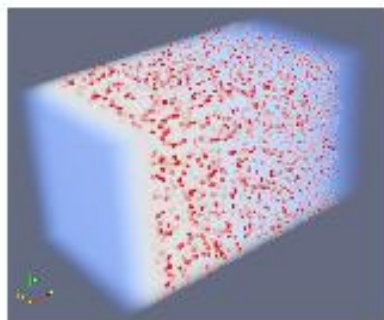
<<http://www2.basf.us/PLASTICSWEB/displayanyfile?id=0901a5e1801359>>.

SIMULATING HEAT TRANSFER IN POLYMER COMPOSITES USING THE LATTICE BOLTZMANN METHOD

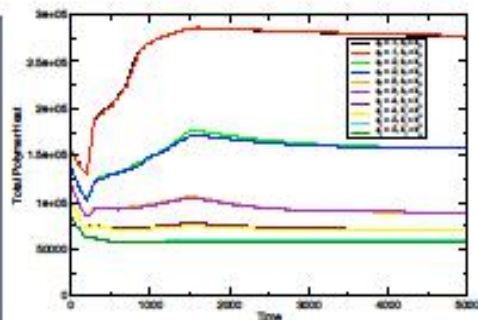
Arpon Raksit, *Commack High School, Commack, NY*

Dilip Gersappe, Joseph Ortiz and Miriam Rafailovich, *Department of Materials Science & Engineering, Stony Brook University, Stony Brook, NY*

While polymers and plastics have many advantages as materials—e.g. their strength, flexibility and low cost of production—there are also disadvantages, including polymers relatively low ignition temperatures and high combustibility¹. In an effort to reduce the flammability of commonly used polymers with and create more fire-safe materials, research is being done on the use of flame retardants within polymers. To date, not much is known about the physics and dynamics of this process; without a theoretical understanding of how polymers act in high heat environments, polymer-flame retardant composites cannot have optimal characteristics. Simulating the effect of flame retardants on the spread of heat throughout a polymer may provide a better understanding on how to most effectively use these flame retardant materials. Using the lattice Boltzmann method (LBM), a highly parallelizable computational technique for simulating fluid flow², a model is developed in both two and three dimensions to simulate heat diffusion from a heat source to sink in a system comprised of an ignitable polymer matrix and flame retardant filler particles. An initial model was developed, using insulating particles as flame retardants to prove the applicability of the LBM in studying heat flow in polymers: it was clearly seen that as the concentration of flame retardants increased, polymer combustion and heat propagation decreased. A more complex flame retardant model was then developed, where filler particles absorb heat from the surrounding polymer and where heat is conducted through connected flame retardant structures (Fig. 1a). By varying the volume fraction of these particles, along with their thermal conductivity, specific heat, shape and location, the effect on polymer combustion is studied (Fig. 1b).



(a) Three dimensional model with heat absorbing and conducting filler particles



(b) The effects of flame retardant concentration and conductivity on heat dissipation in the polymer matrix

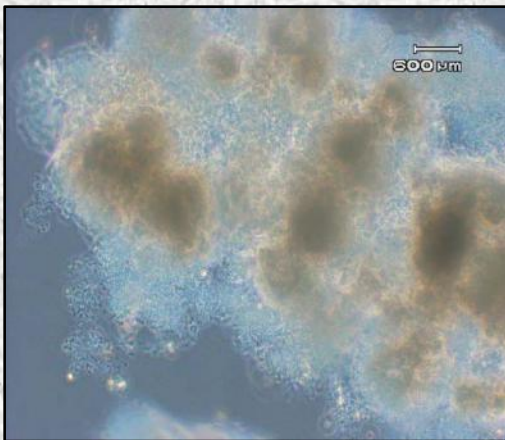
Figure 1: Data collected from the developed model

¹Fu-Yu Hsieh, Joel M. Stoltzfus, and Harold D. Boeson. Note: Autoignition Temperature of Selected Polymers at Elevated Oxygen Pressure and Their Heat of Combustion. *Fire and Materials*, 20:301303, 1996.

²Shiyi Chen and Gary D. Doolen. Lattice Boltzmann method for fluid flows. *Annual Review of Fluid Mechanics*, 30:329364, 1998.

Environmental Remediation

SESSION VIII



Chair: Lourdes Collazo
Tiara Marshall

The Effect of Ocean Acidification and Oil Spills on *Emiliana Huxleyi* Transparent Exopolymer Particles

Kunal Sangani and Mishka Gidwani

Fayetteville-Manlius High School and Neuqua Valley High School

Lourdes Collazo, Xiaolan Ba, and Dr. Miriam Rafailovich

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Dr. Cindy Lee and Carolina Cistern, Department of Marine Sciences, Stony Brook University

Increased atmospheric carbon dioxide has been the focus of marine scientists for the resulting phenomenon of ocean acidification, which occurs when CO_2 reacts with seawater to form carbonic acid. This has important ramifications for marine ecosystems - reduced calcification in phytoplankton and corals, loss of biodiversity in shallow reefs, and a brighter, noisier ocean.^[1] The calcium carbonate shells of corals also dissolve in acidic conditions, harming these organisms and releasing large amounts of trapped CO_2 back into the ocean.^[2]

One important mechanism for the removal of carbon dioxide from the atmosphere involves marine phytoplankton. As these organisms photosynthesize, they store atmospheric CO_2 as organic matter. When stressed, phytoplankton secrete a polysaccharide known as TEP (Transparent Exopolymer Particles). TEP acts as a glue, creating aggregates of coccolithophores and debris (Figure 1-a). As these aggregates become larger, they sink and sediment on the ocean floor, and their organic carbon is not returned to the atmosphere.^[3]

We cultured *Emiliana Huxleyi*, a coccolithophore species 3-7 μm in diameter. We engineered an apparatus to achieve optimal conditions for cell growth. Using HCl and NaOH, we titrated the pHs of each culture - one was adjusted to a pre-industrial pH of 8.3, another was left at 8.0, and the last was changed to 7.7, the estimated ocean pH in year 2200.

We stained our cultures with Alcian Blue and measured light absorbance to determine the amount of secreted TEP. We also measured rheologies to see the impact of pH on the mechanical properties of TEP. We used the Atomic Force Microscope to measure changes in cell and TEP mechanics between pHs, specifically the “stickiness” of the cell. This testing proved challenging as *E. Hux.* has no binding sites and would not adhere strongly enough to be viewed (Figure 1-b).

Oil spills cause incalculable damage to nearby ecosystems. We observed the effect of soluble oil dispersal through the photic zone on aggregation. We prepared two roller tanks - one with seawater and one with seawater and soluble oil. The tank motion simulated natural ocean currents and promoted aggregation, allowing us to measure the time till aggregation and the nature of the aggregates. We compared these tanks to find the effect of oil on aggregation.

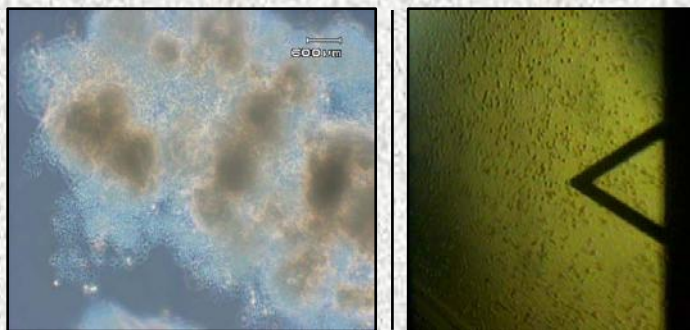


Figure 1: (a) A large aggregation of yellow-orange *Emiliana Huxleyi* cells within white TEP (b) Several *Emiliana Huxleyi* cells adhering to a PVP thin film on a silicon wafer

^[1] Doney, S. C., W. M. Balch, V. J. Fabry, R. A. Feely. 2009. Ocean Acidification: A critical emerging problem for the ocean sciences. *Oceanography*, Vol. 22, No. 4: 16-25.

^[2] Riebesell, U., I. Zondervan, B. Rost, P. D. Tortell, R. E. Zeebe, F. M. Morel. 2000. Reduced calcification of marine plankton in response to increased atmospheric CO_2 . *Nature*, Vol. 407: 311-313.

^[3] Engel, A., B. Delille, S. Jacquet, U. Riebesell, E. Rochelle-Newall, A. Terbrüggen, I. Zondervan. 2004. Transparent exopolymer particles and dissolved organic carbon production by *Emiliana Huxleyi* exposed to different CO_2 concentrations. *Aquatic Microbial Ecology*, Vol. 34: 93-104.

Population and Degradation Studies of Marine Bacteria *Marinobacter aquaeolei* strain VT8

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Brentwood High School, Brentwood, New York¹. Cornell University². Stony Brook University³.

Marinobacter aquaeolei strain VT8 is a species of bacteria that is found in throughout the water column and in the deep ocean. *M. aquaeolei* able to utilize organic acids and amino acids and the VT8 strain has the ability to use n-alkanes as its sole carbon source^[1]. This bacteria plays a vital role in its marine environment by its ability to degrade n-hexadecane, pristane, and some crude oil components.

Marinobacter aquaeolei was initially cultivated in halomonas medium (HM) at 30°C for all studies. A growth curve was then conducted with the *M. aquaeolei* in suspension in HM, HM supplemented with small percentages of crude oil obtained from Sigma Aldrich, and water with limited nutrients also supplemented with crude oil. This was to assess the bacteria's growth while in different mediums. The measurements of amount of bacteria were obtained using a cell density meter measuring the absorbance at 600 nm.

Degradation studies were then conducted in 6 well plates. 100 µl of a constant amount of *Marinobacter aquaeolei* was placed in each well containing 5 mL of the halomonas medium and crude oil to assess *M. aquaeolei's* degradation ability of various amounts of crude oil over a span of 27 hours.

[1] Huu, N. et. Al. "*Marinobacter aquaeolei* sp. nov., a halophilic bacterium isolated from a Vietnamese oil-producing well" International Journal of Systematic Bacteriology. (1999)

Immobilization of *E. coli* K-12 for Future Oil Remediation Applications

William Genova¹, Lauren Herrera¹, Tiara Marshall², Lourdes Collazo³, Miriam Rafailovich³

¹Brentwood High School, Brentwood NY 11717

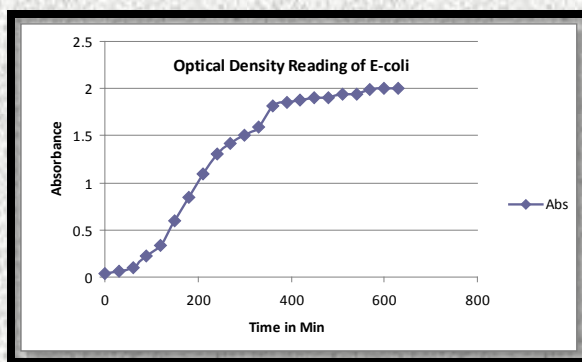
²Cornell University, Ithaca NY 14850

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Although the *Deepwater Horizon* has stopped, millions of tons of crude oil are left spilt across the Gulf of Mexico. The hard and tedious task that lay ahead is to remediate the oil-stricken waters. To stray away from hazardous oil-remediating techniques, a novel, environmentally safe method to clean-up oil needs to be instituted.

A strategy that has been costly and challenging, but has shown promise, is the encapsulation of oil-degrading microbes. This method will allow for a system to be created that will not harm the environment and expose it to an abundance of outside microorganisms. The purpose of our research is to examine if the embedment of microbes within a hydrogel is possible for future oil-remediation strategies.

Figure 1: Growth Curve of *E. coli* K12 using absorbance



The bacterium utilized for immobilization was *Escherichia coli* K-12. The gram negative rod-shaped bacterium is a widely used model organism that will represent the capabilities of other microorganisms while encapsulated. A growth curve was created for the *E. coli* within Lysogeny broth media and the absorbance was found every half hour for ten and a half hours. *E. coli* reached optimal absorbency at hour ten, which meant the bacteria was at its highest population (Figure 1).

Currently *E. coli* is being immobilized with the Calcium alginate gel beads. Utilized due to it not having any toxic effects on organisms, the Calcium alginate beads will provide a cross-linked polymer for the *E. coli* to be embedded on. *E. coli* is embedded in the beads by being placed in LB media that is combined with Sodium Alginate. The mixture is then added drop-wise to Calcium Chloride to create the Calcium Alginate beads. Experiments are being currently done to examine the survival of *E. coli*, and the percentage of *E. coli* being immobilized within the Calcium Alginate beads.

E. coli is also being encapsulated in Pluronic F-127. This copolymer is a thermo reversible gel that gels at body temperature. The polymer is widely used for drug delivery and tissue engineering due to it not being biodegradable, but we are examining if it can be applicable in encapsulating bacteria. To do so, we immobilized the *E. coli* into the Pluronic while it is in its liquid stage. It is then placed in the incubator to allow the uncrossed linked co-polymer to solidify. Proliferation tests will ensue to examine the capability of the Pluronic as an effective polymer.

Once tests are completed, results will show whether or not future applications are possible. If they are, embedment of the oil-degrading bacteria, *Marinobacter aquoli* will follow and the same tests will be repeated to ensure that the bacteria can survive. Oil degradation tests within the hydrogels will also be explored to ensure the bacteria can remediate within the gels.








[1] Sun, Kunshan. "Thermogelling Aqueous Fluids Containing Low Concentrations of Pluronic F127 and Laponite Nanoparticles." *2010 American Chemical Society*(2010): n. pag. Web. 8 Aug 2010. <http://complexfluids.umd.edu/papers/64_2010g.pdf>.

[2] Tao, Xue-Qin et al. "Rapid Degradation of Phenanthrene by Using Sphingomonas sp. GY2B Immobilization in Calcium Alginate Gel Beads" *Environmental Research and Public Health* (2009)

EVERYDAY STARTS WITH A GROUP MEETING

CHECK SCHEDULE DAILY!

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
Week of 6/28	28 10:00 AM General meeting 11:00 AM Srinivas Pentyala 12:00 PM Michael Hadgyargyrou 1:00 PM Lunch 3:00 PM ID cards and campus tour	29 10:00AM General meeting 11:00 AM Kim Auletta Chemical waste 12:00 PM Hazardous Waste, Jeff Carter 1:00 PM Lunch 1:45 PM Biological Hazard Bob Holthausen 2:30 PM "Occupational Exposure, Louis Mancuso 3:30pm ID cards and campus tour	30 10:00 AM General meeting 10:30am DNA: Jonathan Sokolov 11:30 AM Vladimir Jurokowski 12:30 AM On-site Training and Lunch: Group I 1:30 PM On-site Training and Lunch: Group II 3:15 PM Safety Quiz	1 10:00 AM General meeting 10:30 AM Donna Tuminello: Patents 11:30AM "Biom mineralization-nature's nanomaterials, studied by synchrotron x-rays <i>Elaine Dimasi</i> 12:030 PM Lunch 1:45 PM <i>Facilities Tour</i>	2 10:00 AM General meeting 11:30 AM Learning Science Databases / Excel Tutorial 1:30PM Pizza Lunch/ Journal Club 
Week of 7/5	<p>HAPPY 4TH OF JULY!</p> 	6 10:00AM General meeting 10:30 AM Peter Brink: Patch Clamping 11:15 AM Distribution of lab boxes, lab notebooks, storage, etc. 1:00 PM Lunch 2:00 PM Ellipsometer: Jonathan Sokolov	7 10:00 AM General meeting 10:30 AM Cindy Lee 11:15 AM Dr. Doug Foerth 12:00 PM Lunch 1:00 PM Spinning/Journal Club 2:30 PM Journal Club/ Spinning	8 10:00 AM General meeting 10:30 AM Steve Schwarz 11:15 AM Thomas Cubeaud 12:00 PM Lunch 12:30 PM Spinning and Journal Club	9 10:00AM General meeting 10:30 AM Richard Gross 11:30 AM T.A. Vanakatesh 12:30 PM S 1:00 PM Pi 
Week of 7/10	12 10:00 AM General meeting 10:30 AM Kalle Levon 11:15AM Brook Ellison 12:00 PM Charles Fortman 12:30 PM Lunch 1:30 PM Project distribution./ Spinning lecture /Data Processing	13 10:00AM General meeting 10:30 AM Marcia Simon 11:30 AM Dillip Gersappe 12:30 PM Lunch 1:30 PM Project Distribution	14 10:00 AM General meeting 10:30 AM Yizhi Meng 11:30 AM Richard Clark 12:30 PM Lunch 1:30 PM Project Distribution	15 <p>ATLANTIS MARINE WORLD AQUARIUM TRIP</p> 	16 10:00AM General meeting 10:30 AM Mr. Allen Sachs Science Competition and Required paperwork 1130AM Statistics: Miriam Rafailovich 12:00 PM Pizza Lunch 

<p>Week of 7/19</p>	<p>19 10:00 AM General meeting 10:30 AM WORK!</p>	<p>20 10:00AM General meeting 11:00 AM Leishmaniasis: Dr. Adil Allahverdiyev 11:30 AM LUNCH! 12:30 PM WORK!</p>	<p>21 10:00 AM General meeting 10:30 AM WORK!</p>	<p>22 10:00 AM General meeting 10:30 AM WORK!</p>	<p>23 10:00AM General meeting 10:30 Speakers: Spinning Group 12: 00 Pizza Lunch</p> 
<p>Week of 7/26</p>	<p>26 10:00 AM General meeting 10:30 AM WORK!</p>	<p>27 10:00 AM General meeting 10:30 AM WORK!!!! 11:30 AM LUNCH! 12:30 PM WORK!!!!</p>	<p>28 10:00 AM General meeting 10:30 AM WORK 4: 00 Baseball Game</p>	<p>29 10:00 AM General meeting 10:30 AM WORK</p>	<p>30 10:00 AM General meeting 10:30 AM Student Presentations 11:30 AM BBQ</p> 
<p>Week of 8/2</p>	<p>2 10:00 AM General meeting 10:30 AM WORK!!!!</p>	<p>3 10:00 AM General meeting 10:30 AM WORK!!!!</p>	<p>4 10:00 AM General meeting 10:30 Mary Cowman 11:00 AM Student Presentations WORK 4:00 PM Baseball Game</p> 	<p>5 10:00 AM General meeting 10:30 AM WORK 6PM Fishing Trip! & BBQ</p> 	<p>6 10:00AM Group Meeting 11:00 AM Student Presentations Pizza Lunch</p> 
<p>Week of 8/9</p>	<p>9 10:00 AM General meeting CANOE TRIP!</p> 	<p>10 10:00AM General meeting 11:00 AM Student Presentations WORK</p>	<p>11 10:00 AM General meeting 11:00 AM Student Presentations WORK</p>	<p>12 10:00 AM General meeting 11:00 AM Student Presentations WORK</p>	<p>13 10:00 AM – 1:00 PM GARCIA SYMPOSIUM SAC Ballroom A</p> 

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Dr. Yizhi Meng

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Dr. Richard Fine

Miss Veronica Collazo

Miss Lauren Collazo

Mrs. Manuela Sherrard

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