



Garcia MRSEC

Polymers at Engineered Interfaces

SUNY Stony Brook • Polytechnic University • CUNY • North Carolina State University



RESEARCH SCHOLARS PROGRAM 2008

This Program is sponsored in part
by the National Science Foundation.





Garcia MRSEC

NSF

Polymers at Engineered Interfaces

SUNY Stony Brook • Polytechnic University • CUNY • North Carolina State University

"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".



Jonathan
Sokolov



Miriam
Rafailovich

Miriam Rafailovich
Professor, Garcia MRSEC

Jonathan Sokolov
Professor, Garcia MRSEC

Research Experience for Teachers



*Research Experience
for
Undergrads*



Alan Deroine



*K
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n*



Nicole Elstein



Daniel Gross



Daniel Katz



*Christina
Kalarickal*



J
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Alicia Franco



Wanyi (Ruby) Yen



Adam Fields



Aryeh Sokolov



Grads & Post Docs



Yantian Wang



Chunhua Li



Xiaolan (Nancy) Ba



Divya Bhatnagar



Chung-Chueh Chang



Stancy Joseph



Jaseung Koo



CHIEN-HSIU LIN



Ying Liu



Tatiana Mironava



Rachel Mu



Seongchan Park



ANGELINA



Jason Yang



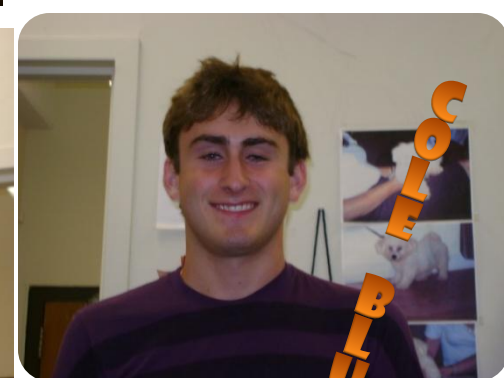
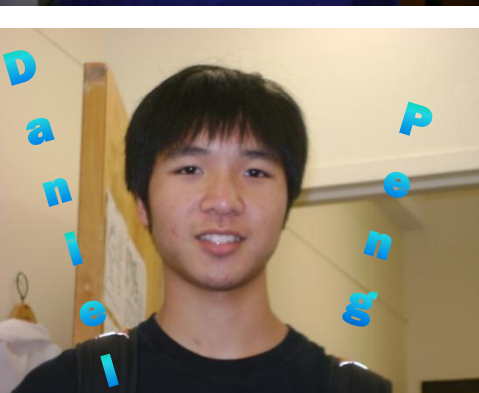
Lourdes Collazo

STAFF

Vladimir Zaitsev

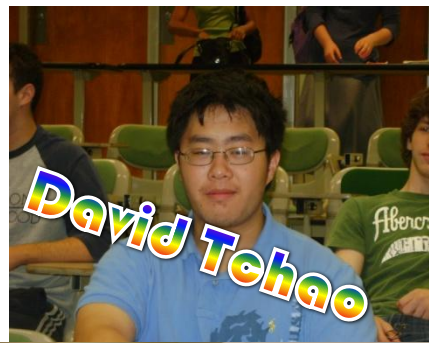


HIGH SCHOOL SUMMER SCHOLARS 2008





Darren Huang



David Tehao



Divya Chhabra



Elias Goodman



Elizabeth Jacob



Elias
Iebovits



IRSAL ALSANEA



Jacqueline Belizar



Georgios Mourdoukoutas



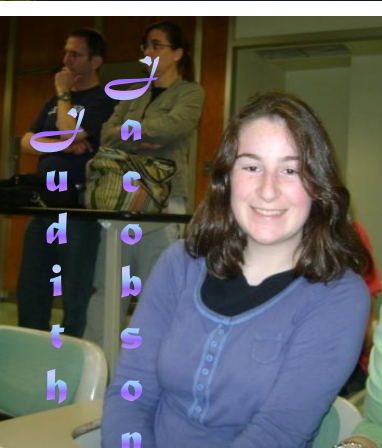
Jake Bryant



Jeremy Joven



Jaclyn Schein



Jacobson



Jisha Thomas



Jibrán Durán



Julia Budassi



Lara Fourman



Mariah Geritano



Matthew Hung



Maxwell Plant



MEGAN GARBER



Melody Jan



Michael Tischler



Michelle Bayofsky



Naveen Murali



Nicholas Mubchan



Neil Muir



Neil Patel



Paul Masih Das



Pooja Ramblhia

Rachelle Ludwick



Sojeung (Sally) Park

Rida Malick



Rishi Ratan



Sanchita Singal



Samantha Laidson



Samuel Kim



Sarah Snowy



Sarajane Gross



Sarah Schnoll



Sowmya Sundaresh



A-ien Jung



Tiya Nandi



Daniel

Younger



Susie Ahn



Walter Gurzynski



Wenting Cao



Life in the Lab



Brookhaven National Lab



Our Fishing Trip





RESEARCH SCHOLARS PROGRAM 2008

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Photo credits: Mariah Geritano



Summer Research Symposium Program

Friday, August 15,

SAC Ball Room A

10:00-10:15 Welcome Miriam Rafailovich



Greetings: Representative from Senator Hillary Clinton's Office

“Concert for Violin, String & Continuo by A. Vivaldi”

Musical Arrangement: John Jerome, Conductor

The Students of THE GARCIA PROGRAM (Violin: Melody Tan, Darren Huang, Paul Masih Das; Viola: Nicholas Mulchan, Susie Ahn; Cello: Samuel Kim)

10: 15-10:25 **Opening Address:** Philip Cummins, PhD
Executive Director of Physical Chemistry and Polymer Technology, Estee Lauder Companies,

10:25-10:30 Research Experience for Teachers Address: Dr. Joanne Figueredo
“Teaching Research Skills”

Session I: Nanocomposites



10:30- 10:50 **Thin Films**
Chair: Robert de la Cruz, Valley Stream H.S.

Planarization of TFT arrays Using Spin-Cast PMMA- Carbon-Black Nanocomposites

Judith Jacobson, Torah Academy for Girls

Physical Properties of PMMA, PS, and PVC Blends
Maxwell Plaut, Rambam Mesivta H.S.; Jaclyn Schein, Lawrence HS

Processing Bulk Polymers

Chair: *John Iraci, Cornell University*
Sergei Kolchinsky, Cooper Union

Characterizing the effect of scCO₂ on tri-blend polymer composites
Rachelle Ludwick, Half Hollow Hills HS West & Byron Smith, Chaminade

Nanoclay Polymer Composites: Biodegradability and Flame Retardancy
David Tchao, High School West Half Hollow Hills, Elizabeth Jacob, The Wheatley School; Neil Muir, Uniondale High School; Omar Waqar, Half Hollow Hills High School East

Toxicity Studies

Observing Dermal Fibroblast Growth on Multiple Clay-Polymer Blends
Mariah Geritano, Plainview Old-Bethpage John F. Kennedy High School
Walter Gurzynski, Locust Valley High School

The Synthesis of Advanced Silica-Silver Composite Particles For Antimicrobial Purposes
Daniel Chun, Georgios Mourdoukoutas, JFK High School,

Session II: Alternate Energy Sources

10:50-11:00

Alternate Energy Sources

Chairs: *Daniel Katz, Cornell University*
Kenny Kao, Stanford University
Christina Kalarikal, Stony Brook University

Development of a Chemical Mechanism to Stabilize Methane Hydrate For Harnessing Sub-Oceanic Natural Gas Reserves
Daniel Fourman, Ward Melville High School

Creating a Viable Source for Alternative Energy: Optimizing Methane Hydrate Formation, Stability, and Uptake via Natural Organic Matter
Darren Huang, Herricks H.S. Wenting Cao, Mission San Jose HS,
Benjamin Parnes, HAFTR High School

Nanoparticles as Catalysts in Polymer Electrolyte Membrane (PEM) Fuels Cells and Direct Methanol Fuel Cells
Divya Chhabra, Ardsley High School, Sarah Schnoll, Stella K.



Session III: Biomolecule Detection

11:00-11:10:

Biomolecule Detection

Chair: *Eliana Pfeffer, University of Chicago*

Characterizing single and double stranded DNA

Irsal Alsanea, & Julia Budassi, Long Beach High School

Molecular Imprinting of a Biosensor to Recognize Biomarkers for Early Cancer Detection

Megan Garber, Ashley Khalili & Danny Younger, North Shore Hebrew Academy



Session IV: Drug delivery systems

11:10-11:20:

Drug delivery systems

Chairs: *Robert Winston, Thomas Edison*

Fabricating Temperature Sensitive N-isopropylacrylamide Gels for use as topical delivery systems

Cruz Granados, Long Beach High School,

Engineering and Rheological Modeling of Hydrogels for Controlled Drug Delivery via Folate-Coated Platinum Nanoparticles

Naveen Murali Staples High School; Elias Lebovits, Ramaz Upper School; Daniel Peng, Monta Vista High School,

Session V Cell surface interactions

11:20-11:30

Cell surface interactions

Chair: *Alex Ramek, Harvard University*

An Analysis of Polymer Substrates as Inducing Surfaces: Novel Implications for the Optimal Control of Stem Cell Differentiation

Michael Tischler, Jericho High School

The Influence of Polybutadiene on the Proliferation and Mechanics of Keratinocytes in the absence of 3T3S

Jackie Belizar, Miller Place H.S. Reena Glaser, Smithtown H.S. West; Matthew Hung, Smithtown H.S. West

Layer-by-Layer Assembly of a Bilayer Tissue Through the Harvesting of Cell Sheets On Photosensitive Polymer Substrates

Susie Ahn, Herricks High School; Tiya Nandi, Jericho High School

Session VI: Properties of Electrospun Fibers

11:30-11:40

Properties of Electrospun Fibers

Chair: Yusung John Lim, MIT

Enhancement of Electrospun Fibers and Polymer Thin Films with Polyhedral Oligomeric Silsesquioxanes

Neil Patel, Herricks High School; Jeremy Joven, Valley Stream Central High School

Effect of Polymer and Solvent Blends on Electro-spinnability and the Physical Properties of Electrospun Fibers

Melody Tan, Valley Stream Central HS, Jake Bryant, Ward Melville HS

Low-Cost and efficient method to create highly conductive nanofibers

Rishi Ratan, Jericho Senior HS, Suk Yong(Allen) Jung, Plainview-Old Bethpage, JFK HS

Session VII: Fibrillar Scaffolds

11:40-11:55:

Fibrillar Scaffolds

Chair: Alex Ramek, Harvard University

Effects of Electrospun PMMA/TiO₂ Fibers on Cells and DNA

Michelle Bayefsky, Ramaz High School

Dermal Fibroblast Cell Migration on 3-Dimensional Electrospun PMMA Fibrous Scaffolds

Sarajane Gross, Elias Goodman, North Shore Hebrew Academy High School,

Mechanism of fibrinogen fiber formation on Poly(methyl methacrylate) (PMMA) fibers,

Jisha Thomas, New Hyde Park Memorial High School

Characterizing Interactions between Fibrinogen Dimers during Fiber self-assembly

Brandon Hsu, Monta Vista H.S, Cupertino, CA

Self-Assembly of Fibrinogen Clots: A Study of Biomaterials in Contact with Blood

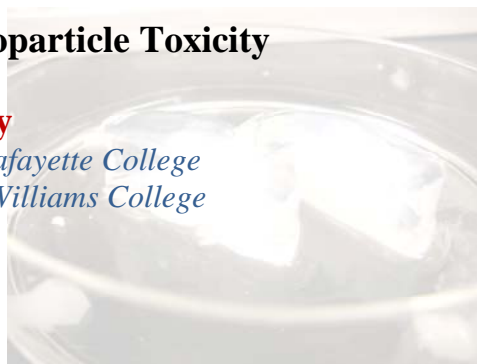
Pooja Rambhia, Jericho High School; Sara Snow, Stella K Abrahams HS.

Session VIII: Nanoparticle Toxicity

11:55-12:10

Nanoparticle Toxicity

Chair: Nicole Elstein, Lafayette College
Daniel Gross, Williams College



Comparative Study of the Effects of Nanoparticle Toxicities on Normal and Cancerous Cells

Sowmya Sundaresh, Hicksville High School

Colloidal Gold Nanoparticle Penetration in MC3T3-E1 Osteoblasts: Effect of Particle Size on Cell Proliferation and Structural Morphology *in vitro*

Samuel Kim, Syosset High School

Methods of photocatalytic activity reduction of titanium and zinc oxides nanoparticles: control of reactive oxygen species

Cole Blum and Samantha Larsen, Long Beach H.S.

Gold Nanoparticles: Protein Adsorption and Nanoparticle Uptake

Sally Park, JFK High School; Jibrán Durán, Bayside H.S.

The Effects of TiO₂ and ZnO Nanoparticles on Chemically-Induced Dental Pulp Stem Cells and MC-3T3 Osteoblasts

Rida Malick and Paul Masih Das, Lawrence H.S.; Nicholas Mulchan, John F. Kennedy High School

Session IX: Biomineralization

12:10-12:30

Biomineralization

Chair : *Kate Dorst, Stony Brook University*

Aryeh Sokolov, Queens College

Biomineralization on Sulfonated Polystyrene Surfaces for Bone Tissue Engineering

Lara Fourman John F. Kennedy High School

Organization and Biomineralization of Osteoblasts on Superparamagnetic Nanocomposites for Bone Tissue Engineering

Sanchita Singal, Herricks High School

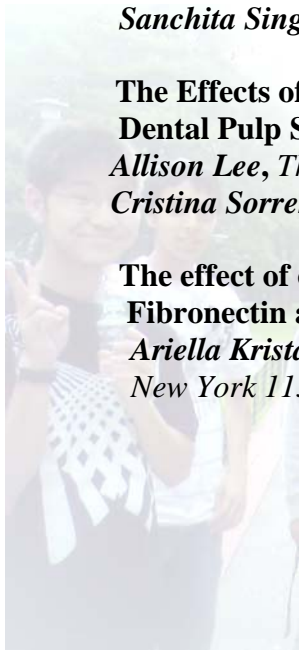
The Effects of a Sulfonated Polystyrene Thin Film Scaffold on Human Dental Pulp Stem Cells: Reproduction, Proliferation, and Differentiation

Allison Lee, The Wheatley School

Cristina Sorrento, Locust Valley High School

The effect of collagen on biomineralization through its interaction with Fibronectin and elastin

Ariella Kristal, Solomon Schechter High School of Long Island, Glen Cove, New York 11542



*Please enjoy lunch provided by
Wing Wan of West Hemsstead*

Nanocomposites

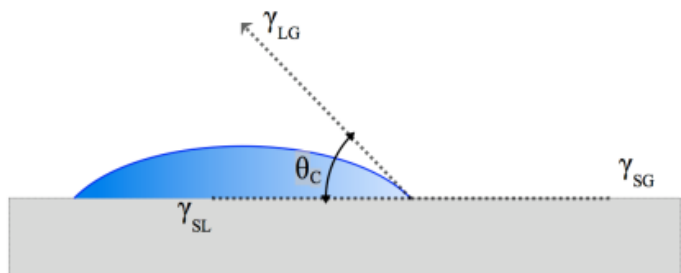
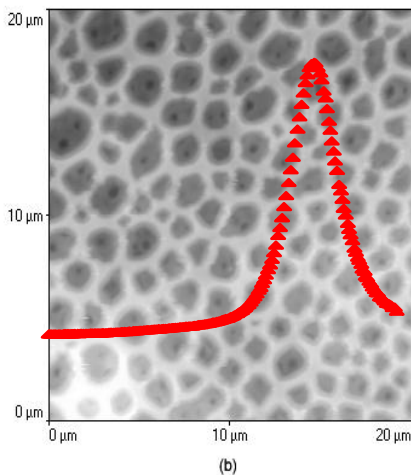
Session I

Chairs: Robert de la Cruz

John Iraci, Sergei Kolchinsky

Garcia Staff Mentors:

Seonchang Pack, Jasoung Koo



Planarization of TFT Arrays Using Spin-Cast PMMA- Carbon-Black Nanocomposites

Judith Jacobson, *Torah Academy for Girls*

Jennifer Segui, Dr. Miriam Rafailovich, Dr. Joanne Figueiredo

Garcia Center for Materials Science and Engineering Research, SUNY Stonybrook

Solid state electronic devices including displays and sensors frequently employ thin-film transistor (TFT) arrays to create a digital image. TFT arrays are rows of solid state switches formed from the sequential deposition and patterning of many layers ultimately resulting in a variable surface topography that can adversely affect device performance. We are ultimately interested in the use of polymer nanocomposites to achieve the desired degree of planarization (DOP) and electrical properties required by a particular device.

Many materials that possess self-leveling properties are very good electrical insulators. PMMA (Polymethyl-Methacrylate) is one such material. Our goal is to reduce the volume resistivity of the polymer (PMMA) by adding the conductive filler carbon black. In previous work, PMMA was spun on bare silicon gratings with square-wave patterns resulting in substantial attenuation of the surface variations (1). We are interested to investigate the effect that filler particles have on the ability of polymers to planarize a variable surface. The nanocomposite in this study is being used to understand its fundamental physical properties so that we may apply them to more sophisticated electronic materials.

In the present investigation, we prepared solutions of PMMA and carbon black in different solvents via mixing and sonication to break up carbon black aggregates. Films were prepared via spin casting at varied spin speeds on methanol cleaned silicon wafers followed by annealing in vacuum for different lengths of time. The thickness and surface morphology of our films was characterized using AFM. Four point probe measurements were performed to study the effect of film thickness and filler fraction on the volume resistivity of our samples. Thus far, we have studied the effect of annealing time, spin speed, PMMA concentration, carbon black filler fraction, and the impact of different solvents on film morphology and volume resistivity.

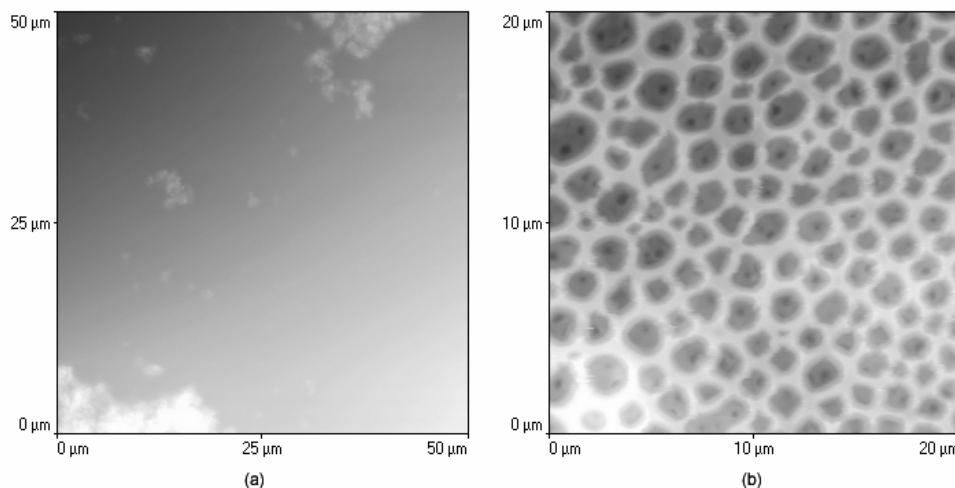


Figure 1. AFM images of films cast from 7 wt% PMMA in (a) toluene and (b) THF with a carbon black filler fraction of 1 wt% prepared using a spin speed of 1000 RPM and prior to annealing. Images demonstrate the improvement in carbon black dispersion when THF is used as a solvent.

Further work will include the effect of different molecular weights of PMMA and different aggregate sizes of carbon black to determine the effect on physical and electrical properties of the films. PMMA-CB composites will finally be tested on surfaces with patterned square wave gratings to confirm the effect of filler particle addition on the ability of PMMA to level variable surface topography.

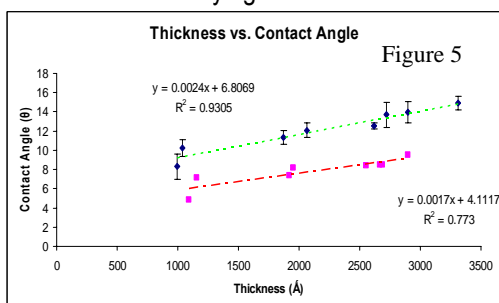
(1) Polymer Thin Films on Patterned Si Surfaces- Zi Li, Dr. M.H. Rafailovich, Dr. J. Sokolov, 1998

Physical Properties of PMMA, PS, and PVC Blends

Maxwell Plaut¹, Jaclyn Schein², Adam Fields³, John Lim⁴, Alex Ramek⁵, Dr. John Jerome⁶, Dr. Miriam Rafailovich⁷
 Rambam Mesivta, Lawrence, NY¹, Hebrew Academy of the Five Towns and Rockaway, Cedarhurst, NY²
 Yale University, New Haven, CT³, MIT, Cambridge, MA⁴, Harvard University, Cambridge, MA⁵
 Suffolk County Community College, Brentwood, NY⁶, SUNY Stony Brook, Stony Brook, NY⁷

The blending of polymers provides a means of producing new materials which combine the valuable characteristics of all the constituents. The technology of blending is now advancing at a rapid pace and is proving to be useful in the recycling of plastics, as well as coatings in various fields. These coatings serve to protect mild steel structures from corrosive environments, control drug delivery systems, as well as coat pellets. There have been many previous research studies conducted on two components, but there have not been known projects that studied three components.

PMMA/PS/PVC has many different possible combinations, so we are using a systematic approach to analyze the physical properties. We are making PS/PVC, PMMA/PVC, and PS/PMMA/PVC blends in different ratios at varying concentrations.



In our binary systems, we measured contact angle (Figure 4) (using an Atomic Force Microscope) as a function of thickness (measured using an angle as a function of CO₂ and thickness. We observe a

significant difference in the contact angle when the blend is exposed to supercritical CO₂ (Figure 5).

Interfacial Energy was measured using Young-dupre's equation. In addition, we calculated the Neuman Angle for each sample.

$$\text{Neuman Angle: } \gamma_S = \gamma_{LS} \cos \beta + \gamma_L \cos \theta$$

In order to determine which polymer was the under layer and which polymer was creating the bubbles in the PS/PVC blends, we had to use a differential solvent. We dipped a silicon wafer with a PS/PVC blend in toluene for ten seconds, thereby dissolving the PS. From this, we were able to determine that the bubbles were the PS (Figures 1,2,3).

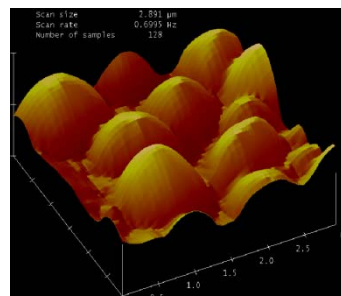


Figure 1 – PS/PVC Blend

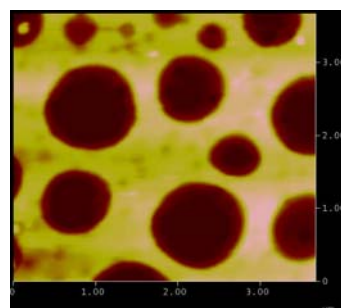


Figure 2 – PS/PVC Blend with PS dissolved using toluene

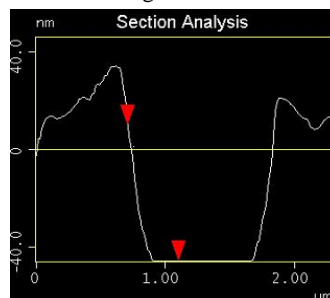


Figure 3 – Section Analysis of figure 2

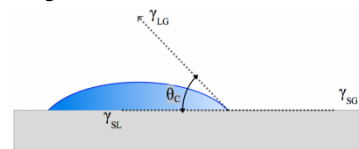


Figure 4 – Contact Angle Diagram¹

References:

- A.M. Higgins et. al. Surface segregation and self-stratification in blends of spin-cast polyfluorene derivatives, Journal of Physics: Condensed Matter, 2005.
- C. Ton-That, Surface feature size of spin-cast PS/PMMA blends, Polymer, 2002.
- D. Briggs et.al. Surface morphology of a PVC/PMMA blend studied by ToF SIMS, Surface and Interface Analysis, 1996.
- J.J. Schmidt, Surface studies of polymer blends. 2. an esca and ir study of PMMA/PVC homopolymer blends, macromolecules, 1989
- S.T. Jackson, Surface Morphology of PVC/PMMA blends, J. Mater. Chem, 1992.

¹ http://en.wikipedia.org/wiki/Contact_angle

Characterizing the effect of scCO₂ on tri-blend polymer composites

Rachelle Ludwick and Byron Smith; Half Hollow Hills HS West and Chaminade High School

Seongchan Park and Dr. Miriam Rafailovich, Department of Material Science and Engineering, Stony Brook University; Sergey Kolchinskiy, The Cooper Union; John Michael Iraci, Cornell University

A growing need for plastics in society has led us to a point where used plastics are voluminous in landfills. However, burning these plastics is detrimental to air quality. Thus it is important to find ways to recycle these plastics. Blending plastics is a way to continue the lifespan of a plastic and enhance polymer characteristics¹, such as tensile strength, or shear modulus. However, polymers do not blend well because they are essentially immiscible. Supercritical carbon dioxide (scCO₂) can be used as an environmentally friendly solvent by creating regions of higher density to blend the interphases of two polymers². scCO₂ was tested for its effectiveness in compatibilizing a three polymer system of high impact polystyrene (HIPS), poly(methyl methacrylate) (PMMA), and poly vinyl chloride (PVC), which are widely used for industrial and commercial applications. Our goal is to effectively blend these polymers with scCO₂ and clay and prove they are better compatibilized after being exposed, thus creating a novel method for the recycling of plastics.

HIPS, PMMA, and PVC are blended in varying concentrations using a C.W. Brabender Plasti-Corder. Nine binary blends are made, following ratios of 1:1, 1:3, and 3:1 for all combinations. Then, ternary blends are made in ratios of 9:9:2, 9:2:9, and 2:9:9. Furthermore, blends are made with Cloisite 20A in a ratio of 9:1 polymer to clay by mass to increase compatibilization, and to mimic the prevailing influence of clays in modern plastics. Samples are tested via Dynamic Mechanical Analysis (DMA), the Instron machine, and Differential Scanning Calorimetry (DSC) in order to measure tensile strength, shear modulus as a function of temperature, Young's modulus, and compatibilization through glass transition temperature. The Atomic Force Microscope and the Scanning Electron Microscope will be used to observe the interphase blending.

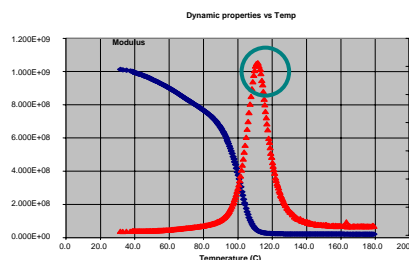
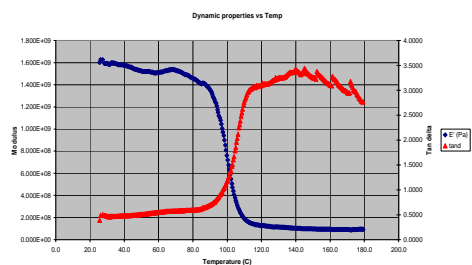


Figure 1 (left): 2:9:9 blend of PMMA:HIPS:PVC [Pre-super critical CO₂ Exposure]

Figure 2: Post scCO₂ exposure (T_g circled)

These two DMA graphs exemplify the change by scCO₂ exposure. After exposure, a single glass transition temperature is readily identified in Fig. 2 whereas in the Fig. there appear to be multiple glass temperatures. This indicates compatibilization of polymers by scCO₂.

¹Tomasko, D.; Li, H.; Liu, D.; Han, X.; Wingert, M.; Lee, J.; Koelling, K.; "A Review of CO₂ Applications in the Processing of Polymers"; *Ind. Eng. Chem. Res.* 2003 Volume 42, 6431-6456
²Cansell, F.; Aymonier, C.; Loppinet-Serani, A.; "Review on material science and supercritical fluids"; *Current Opinion in Solid State & Materials Science.* 2004 Volume 7

Establishing Differences Between Various Nylon Nanocomposites in order to Show Nanocomposite's advantage to Regular Polymers

David Tchao, High School West Half Hollow Hills Long Island New York

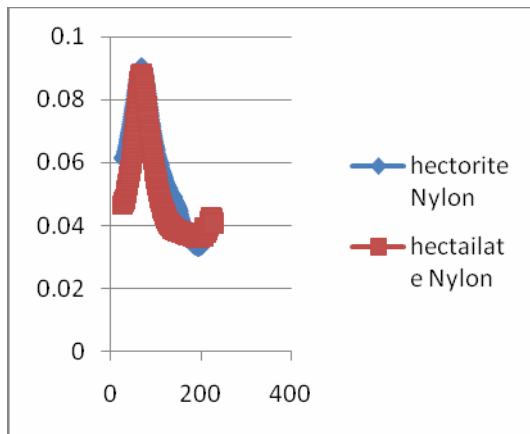
David Abecassis, SUNY at Stony Brook

Nanocomposite polymers are new developments that are rapidly changing society. The novel nanosized clay to polymer mechanism not only gives these polymers improves properties, but also expands their applicability. Some examples of these properties include greater shear or tensile strength, greater temperature resistance, and increased biodegradability.

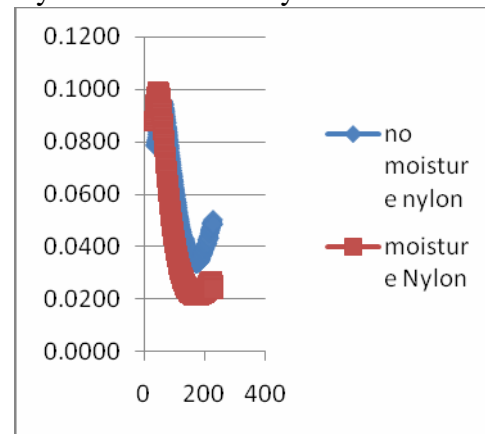
Our research aims to find and note the differences between pure polymer Nylon and the diverse nanocomposite nylon blends as a way to study the effects of clays (Hectorite and Hectalite), such as their effect on mechanical properties, and their potential usefulness in nylon, a polymer hardly studied previously. To test out the differences between these polymers we dried out the samples using a vacuum oven, melt-mixed all the samples at 230° C using a Brabender, molded them with a Heat Press, and then tested the mechanical and thermal properties as a function of temperature with the Dynamic Mechanical Analysis (DMA). In addition, pure nylon samples were prepared both with and without moisture to examine the effects of water on the polymer matrix.

The DMAs of the Hectorite and Hectalite samples (95wt% nylon 5wt% clay) show slight variations despite the fact that the two clays are actually the same. In the moisture comparison, nylon with no moisture has a slightly higher glass transition temperature (the peak of the function). Nylon EDB-T and Hectalite Nylon have diverse tan theta before 50° but it slowly become more same at 120°.

Figures 1 (left) and 2 (right): Tan theta of nylon blends as a function of temperature (°C) Hectorite vs. Hectalite



Nylon moisture vs Nylon no moisture



<http://www.irishscientist.ie/2002/contents.asp?contentxml=02p197b.xml&contentxml=is02pages.xml>

Contact Dr Jenny Melia,

Materials Ireland Polymer Research Centre, Physics Department,
Trinity College Dublin;

Nanoclay Polymer Composites: Biodegradability and Flame Retardancy

Elizabeth Jacob, The Wheatley School; Neil Muir, Uniondale High School; Omar Waqar, Half Hollow Hills High School East

Seongchan Park and Dr. Miriam Rafailovich, Department of Material Science and Engineering, Stony Brook University; Sergey Kolchinskiy, The Cooper Union; John Michael Iraci, Cornell University

In recent years, many materials designed for short-term use (e.g. packaging, hygiene, etc.)¹ have been created using long-lasting, non-biodegradable plastics, and the resulting plastic waste management problems have only exacerbated current environmental troubles². Efforts have been made to produce such biodegradable plastics, for use as industrial structural components². One of the main shortcomings of a biodegradable plastic is its flammability, which makes it difficult for it to meet industry standards². To overcome this issue, flame retardant polymers have also been developed. Regrettably, most flame retardant polymers are not only non-biodegradable but also contain toxic additives. Moreover, the flame retardant polymer additives can also accumulate in the human body, causing multiple health risks³. As a result, biodegradable, flame retardant polymers are currently in high demand.

Phosphorus-based ones like resorcinol diphenyl phosphate (RDP)⁴, which replace toxic additives, are used in combination with nanoclays to test thermal, mechanical, and flame retardant properties of biopolymers. Additives were melt-intercalated and pressed with the base polymers polylactic acid (PLA), polyester (Ecoflex), and an RDP/Starch/Glycerin blend in varying concentrations in a C.W. Brabender Plasti-Corder and Carver Heat Press. The resulting samples were tested for mechanical properties, thermal properties, flame retardancy, and biodegradability.

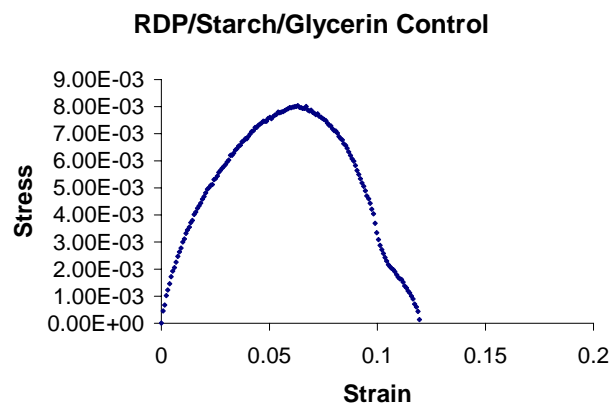


Figure 1

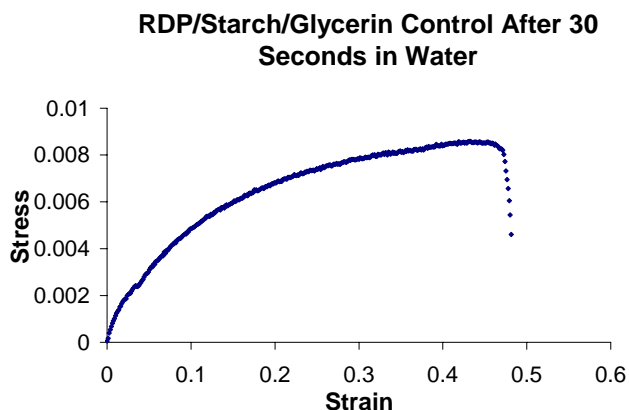


Figure 2

To test biodegradability, the RDP/Starch/Glycerin control samples were submerged in water at intervals of 15, 30, and 45 seconds. As shown in the figures above, the sample submerged in water for 30 seconds shows increased tensile strength, modulus, and toughness. The improved tensile test results may be attributed to the plasticizing nature of water, which would improve the mechanical properties of the sample.

In further research, we plan to perform flame tests, biodegradability, and further tensile and mechanical tests.

¹ Averous, Luc. "Biodegradable polymers." *Information on Biopolymers of Bioplastics*. Apr. 2007. 21 July 2008 <<http://biodeg.net>>.

² Matkó, Sz., A. Toldy, S. Keszei, P. Anna, Gy Bertalan, and Gy Marosi. "Flame retardancy of biodegradable polymers and biocomposites." *Polymer Degradation and Stability* 88 (2005): 138-45.

³ Siddiqi, M. A.; Laessig, R.H.; Reed, K.D.; *Clinical Medicine and Research*. 2003, Volume 1, 281-290.

⁴ Price, Dennis, et al. "Flame retardance of poly(methyl methacrylate) modified with phosphorus-containing compounds." *Polymer Degradation and Stability* 77 (2002): 227-233.

Observing Dermal Fibroblast Growth on Multiple Clay-Polymer Blends

Mariah Geritano, Plainview Old-Bethpage John F. Kennedy High School

Walter Gurzynski, Locust Valley High School

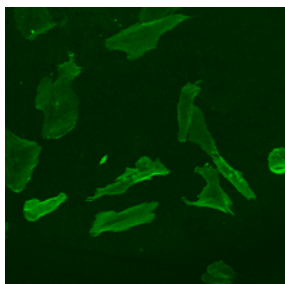
Miriam Rafailovich, Tatsiana Mironava, Lourdes Collazo, Seongchan Park, and Sergey Kolchinskiy, Stony Brook University

Our experimentation aimed to observe Dermal Fibroblast growth on various Poly(methyl) methacrylate (PMMA)- clay nanocomposite surfaces. Clay nanocomposites have grown in popularity due to their enhanced mechanical and thermal properties, but little is known about their cytological properties. One study in particular, performed by Hao Liaw et.al. showed the basic fundamentals of the formation of Poly (methyl) methacrylate molds and testing its dynamic characteristics, but none have gone as far as examining its effect on Dermal Fibroblasts.

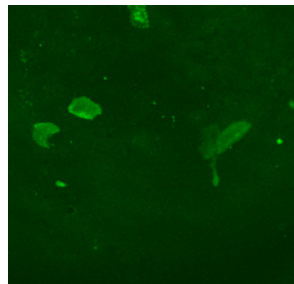
We used three different clay samples in this study, Cloisite Na⁺ Clay, Cloisite 20A Clay and RDP-soaked Halloysite nanotubes. We first made the clay-polymer blends using the C.W Brabender, and then used a Heat Press to form the circular molds used to plate cells as well as larger circular molds that was used for Confocal imaging. We then sterilized the molds and placed them into Multiwell™ 24-well plates and cultured CF-29 Dermal Fibroblasts onto them. The molds were incubated for 24-hours and then counted on days 1, 4 and 5. On day 5 we used the Confocal microscope to view the larger molds. Due to the excessive death of the cells, we aborted the trial and re-sterilized the molds. We then added Fibronectin, a protein that aids in the binding of the cell to the surface, and replated the cells.

We found that all clays, including the PMMA control (Fig.1), showed a decrease in cell counts. The clays which had the largest cell count drop within 4 days were the PMMA and RDP-soaked Halloysite nanotubes (Fig.3) and PMMA and Cloisite Na⁺, with the PMMA and 20A (Fig.2) showing the least detrimental effects on the cells.

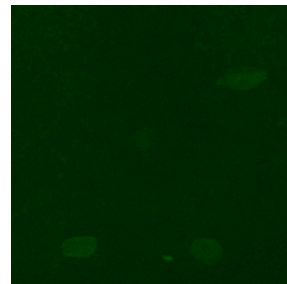
We are currently counting and observing the second trial, which contains Fibronectin. We hope to re-run these experiments at least three more times, to obtain a more valid result and conclusion. We also plan on repeating this experiment with different types of polymer to see if the polymer has an effect as well on the cell growth.



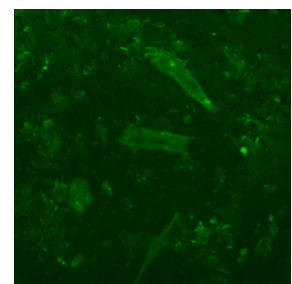
PMMA control
Confocal Image
(Fig.1)



PMMA+20A
Confocal Image
(Fig.2)



PMMA+RDP
Confocal Image
(Fig.3)



PMMA+Sodium
Confocal Image
(Fig.4)

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The Synthesis of Advanced Silica-Silver Composite Particles For Antimicrobial Purposes

Daniel Chun¹, Georgios Mourdoukoutas¹, Dr. Lourdes Collazo², Dr. Miriam Rafailovich², Sean Moore², Dr. Vladimir Zaitsev²

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There is a constant demand for antimicrobial materials to protect human beings from being infected by the microorganisms found in our environment. Silver ions and metallic silver have been identified as having antimicrobial properties. First, silver inactivates enzymes and proteins by oxidizing thiol groups (-SH) to disulfide bonds. Second, silver inhibits cell division and structurally damages the outer cell layers and cytoplasmic contents. Third, silver has a denaturation effect on DNA, resulting in a loss of replication abilities. Ag nanoparticles, however, easily aggregate and lose its antimicrobial properties. In order to maintain silver's antimicrobial properties and prevent aggregation, 1 μm spherical silver-silica particles functionalized with thiol groups were synthesized.

Two methods of synthesis were used: the well-known Stober method and a modified aqueous process. The Stober method called for a reaction in ethanol, where tetraethyl orthosilicate (TEOS) acted as a precursor, 3-mercaptopropylmethoxysilane (MPTMS) contributed the thiol groups, and ammonium hydroxide acted as a catalyst. To attach the silver, the silica particles underwent the polyol process, a chemical reduction method where ethylene glycol, the polyol used, redispersed the silica particles and reduced the silver onto the surface of the silica particles. Poly(vinylpyrrolidone) (PVP) acted as a nucleation-promoting agent.

In the modified aqueous process, the reaction took place in water, where MPTMS silica spheres functionalized with thiol groups formed. Water was used to redisperse the silica particles and sodium borahydride acted as the reducing agent. Scanning electron microscopy (SEM) images showed the Stober method was unable to accurately control the size of the particles (Fig. 1), while the modified aqueous process accurately synthesized monodispersed 1 μm sized particles (Fig. 2). Energy dispersive X-ray spectroscopy (EDX) determined that silver was present on the aqueous particles, possibly as a coating on the outer surfaces.

Future work will consist of inserting the silica-silver composite particles into a polycarbonate (PC) matrix. Then, cells and bacteria will be plated onto the PC-silica-silver to determine its effect on living organisms and antimicrobial properties.

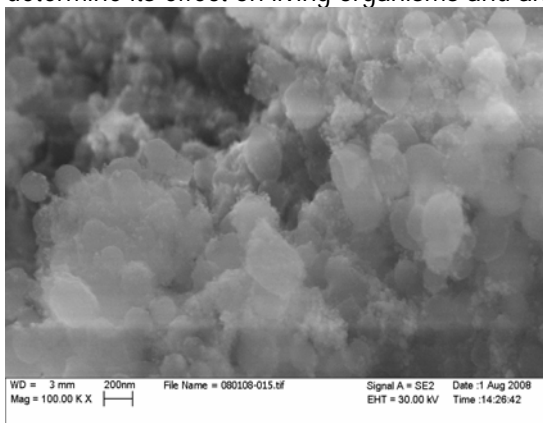


Fig 1. Stober Method: silica-silver (~250 nm)

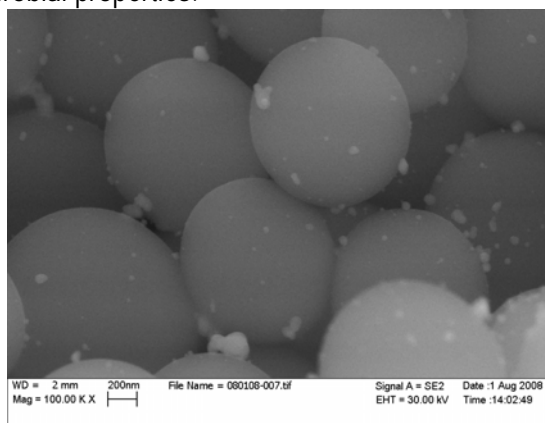


Fig 2. Modified aqueous process (~1 μm)

Lee, Yong-Geun. "Preparation of Highly Monodispersed Hybrid Silica Spheres Using a One-Step Sol-Gel Reaction in Aqueous Solution." *Langmuir: The ACS Journal of Surfaces and Colloids* 23 (2007).

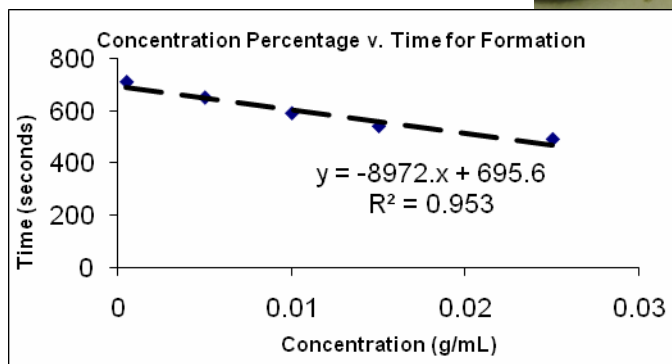
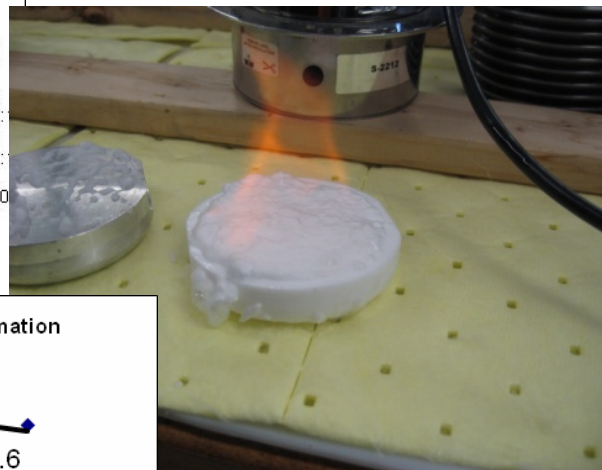
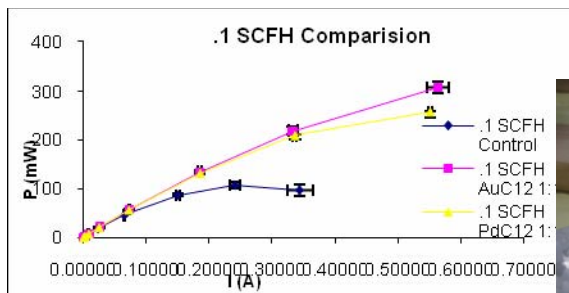
Session II

Alternate Energy Sources

Chairs: Daniel Katz,

Christina Kalarikal

Kenny Kao



Development of a Chemical Mechanism to Stabilize Methane Hydrate for Harnessing Sub-Oceanic Natural Gas Reserves

D. Fourman, Ward Melville High School; D. Katz, Cornell University;

T. Koga, Stony Brook University; J. Jerome, SUNY Suffolk;

M. Rafailovich, Stony Brook University.

Extraction of energy from Methane Hydrates may provide an alternative energy source for today's society. Deposits of Methane Hydrates are located below ocean sediment and underground in permafrost regions [2]. They are present in very large quantities with estimates ranging up to 400 quintillion cubic feet with about 200 quintillion cubic feet in the US alone [1]. This large reserve is approximately 110 times the size of current supplies of natural gas and more than twice as large as gas, oil, and coal reserves combined. Extraction of Methane Hydrates poses environmental risks, including global warming, and underground explosions.

The purpose of our experiments was to assess the effects of several agents (Sodium dodecyl sulfate as a surfactant, Pluronic F108 as a copolymer, and Alconox™ as a surfactant) on the stability and formation of Methane Hydrates. The goal was to create a hydrate that would remain stable at standard temperature and pressure and observe the effects that chemical additives have on hydrate flammability. (Figure 1b). The actions of these chemical agents on hydrate kinetics were examined using a unique laboratory system (Figure 1a) which monitored

temperature and pressure, along with voltage from a photo-sensitive detector that signals the formation of methane hydrate using laser diffraction. We found that in an artificial salt-water environment each of the agents increased the stability of methane hydrate at STP, as compared to the de-ionized water control. Alconox™ had the greatest stabilizing effect (Figure 1c) with Pluronic F108 having the least stabilizing effect. This relationship was true regardless of different concentrations of the agents. A combination of Alconox™ and Pluronic F108 had the greatest effect in stabilizing methane hydrates at STP.

These results show that agents such as surfactants and copolymers can alter the kinetics of methane hydrates and improve their stability at STP. Chemical additives can be utilized to ameliorate the complications traditionally associated with hydrate extraction.

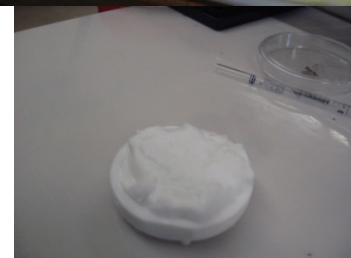
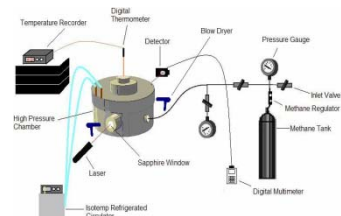


Figure 1a – Methane Hydrate creation Apparatus.
Figure 1b – Hydrate burning\
Figure 1c – A stable Methane Hydrate

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3. "Methane Clathrate." http://en.wikipedia.org/wiki/Methane_hydrate, September 21, 2006.

Creating a Viable Source for Alternative Energy: Optimizing Methane Hydrate Formation, Stability, and Uptake via Natural Organic Matter

Darren Huang,¹ Wenting Cao,² Benjamin Parnes,³ Christina Kalarickal,⁴ Dr. M. Rafailovich,⁴ Dr. C. Lee,⁵ Dr. M. I. Scranton,⁵ Dr. B. Brownawell⁵

¹Herricks HS, New Hyde Park, NY; ²Mission San Jose HS, San Jose, CA; ³Hebrew Academy of Five Towns HS ⁴Stony Brook University, Stony Brook, NY; ⁵Marine Sciences Research Center

As America enters into the 21st century, its economy confronts spiraling gas inflation and an increasing dependence on foreign oil. With the worries of global warming and a diminishing global oil supply, the challenge of a new fuel source is awaiting the next generation. Methane hydrates are gas molecules of methane encased in a crystalline structure formed at extremely high pressures and low temperatures in deep seawater; reserves have the potential to last our society several hundred years. Hydrate retrieval, however, brings forth several complications. The unstable nature found at the seafloor requires a stabilization process to ensure the optimal uptake of the methane gas. Stabilization of methane hydrates, however, would not only make it a viable energy source, but would also ensure safe ventures for ships on the open sea. The most pragmatic manner of stabilization would require a solvent injection of surfactants. Though considerable work has been done in artificial surfactants, we propose both a more cost-effective and environmentally advantageous method in naturally-occurring organic matter as surfactants.¹ Deep seawater is relatively concentrated in lipid, carbohydrate, and protenacious material, which we hypothesize will accelerate hydrate formation time and allow for increased stability at STP conditions by decreasing surface tension and increase area for methane uptake.

Using a high pressure cell, we simulated the conditions of hydrate formation and recorded hydrate formation time, dissociation time, pressure and temperature and laser detection mechanism. By extracting lipids from seawater, we were able to decrease formation time to a mere 490s and dissociation of 76s. Likewise, we utilized gas chromatography to quantitatively assess the amount of methane uptake, and utilizing the Langmuir-Blodgett Trough, we found the effects of lipid concentrations on surface tension in water. Within seawater, we were able to measure the major DOC classes of protein using HPLC, determine lipid classes via GCMS, and carbohydrate concentrations by DOC tests.

We concluded that lipids permitted a significant decrease in formation time and higher methane uptake. However, carbohydrates singularly extended stability time at STP conditions, while specific proteins varied in their effects on hydrate formation. In essence, we were successful in optimizing methane hydrate stability via lipid and carbohydrate addition—indeed, we discovered a novel manner to control hydrate formation in a substantially cost-effective and environmentally-conscious method.

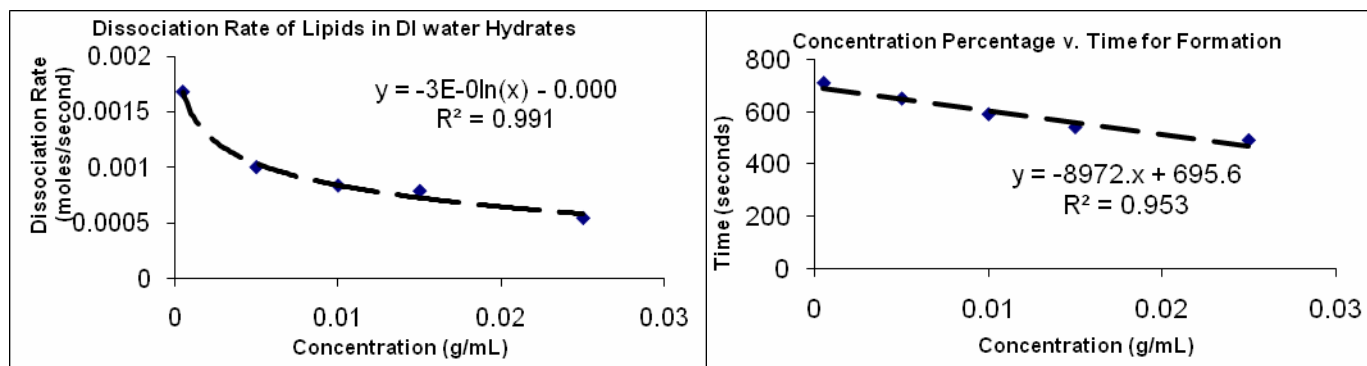


Fig. A. Dissociation rates. A higher percentage of lipids allows a smaller dissociation rate and increased stability.

Fig. B. Hydrate Formation Time. An increase in lipid concentration decreases time for formation.

¹ Ganji, H., Manteghian, M., and Mofrad, H. R., 2007. Effect of mixed compounds on methane hydrate formation and dissociation rates and storage capacity. Fuel Proces. Tech. 88: 891-895.

Nanoparticles as Catalysts in Polymer Electrolyte Membrane (PEM) Fuels Cells and Direct Methanol Fuel Cells

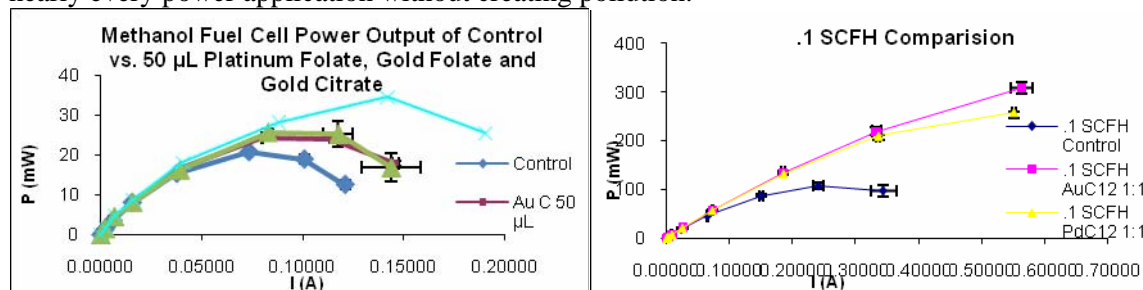
Divya Chhabra¹, Sarah Schnoll² Chun-Kai (Kenny) Kao³, Dr. Miriam Rafailovich⁴

With an energy crisis looming, there is an urgent need for more efficient sources of energy. Because of their low-operating temperature, mobility, high power density and pollution-free operation, fuel cells such as the polymer electrolyte membrane fuel cell (PEMFC) and the direct methanol fuel cell (DMFC) are a promising source of alternative energy. However, they presently generate little power compared to the cost of materials, causing them to be cost-ineffective⁵. Through the implementation of nanoparticles as catalysts, it is possible to increase the fuel cell power output by 441%, and therefore increase the cost efficiency.⁶

We investigated at what metal-thiol ratios the cells generated the most power. After using the two-phase method to synthesize hydrophobic gold and palladium nanoparticles, we used the Langmuir Blodgett Trough to coat hydrophobic Nafion[®], membranes with the nanoparticles. We tested the fuel cell under set resistances, recorded the current and voltage and calculated the power output. Because the DMFC does not have a membrane, we synthesized hydrophilic platinum folate, gold folate, and gold citrate nanoparticles and mixed them into the 3% methanol solution that the cell is designed to run on.⁷

In both cells we found that the cells had higher power outputs when coated with nanoparticles. In the PEMFC, at .1 standard cubic feet of hydrogen gas per hour (SCFH), the gold-coated membrane increased the power output by 216%, the palladium-coated membrane by 164%. At .2 SCFH, the gold-coated membrane increased the power output by 488%, the palladium-coated membrane by 488%. In the DMFC the gold citrate nanoparticles increase the power output by 36 %, the gold folate nanoparticles increase the power output by 82%, and the platinum folate nanoparticles increased the power output by 33%. We also mixed equal amounts platinum folate and gold citrate nanoparticles and the power output was increased by 66%.

In the future, we hope to analyze the air released by the PEMFC to determine which reaction the nanoparticles catalyze in the fuel cell. We will also coat Nafion[®], membranes with varying ratios of gold and palladium to achieve optimal power output. Once the cost of operation can be decreased to \$400/kW (the average cost of a natural gas turbine), fuel cells can be used for nearly every power application without creating pollution.



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⁴ Department of Material Science and Engineering, Stony Brook University, Stony Brook, NY 11794

⁵ United States. Department of Energy. "Future Fuel Cells R&D"

⁶ Kao, Chun-Kai. "Nanoparticle Enhancement of Polymer Electrolyte Membrane Fuel Cell Output"

⁷ Pernodet, Nadine; et al. "Adverse Effects of Citrate/Gold Nanoparticles on Human Dermal Fibroblasts" Small (2006)

The Synthesis of Advanced Silica-Silver Composite Particles For Antimicrobial Purposes

Daniel Chun¹, Georgios Mourdoukoutas¹, Dr. Lourdes Collazo², Dr. Miriam Rafailovich², Sean Moore², Dr. Vladimir Zaitsev²

¹Plainview Old Bethpage JFK High School, Plainview NY

²Dept. of Materials Science and Engineering, Stony Brook University, Stony Brook NY

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Future work will consist of inserting the silica-silver composite particles into a polycarbonate (PC) matrix. Then, cells and bacteria will be plated onto the PC-silica-silver to determine its effect on living organisms and antimicrobial properties.

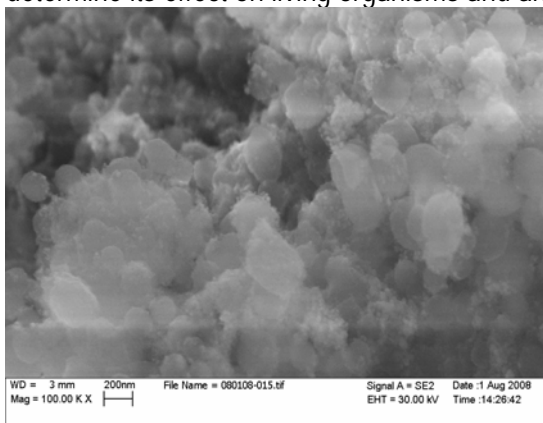


Fig 1. Stober Method: silica-silver (~250 nm)

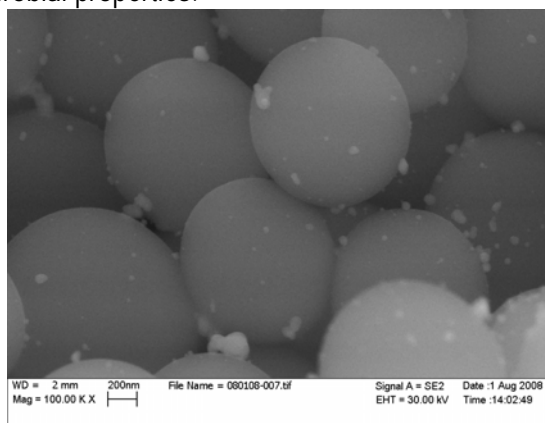


Fig 2. Modified aqueous process (~1 μm)

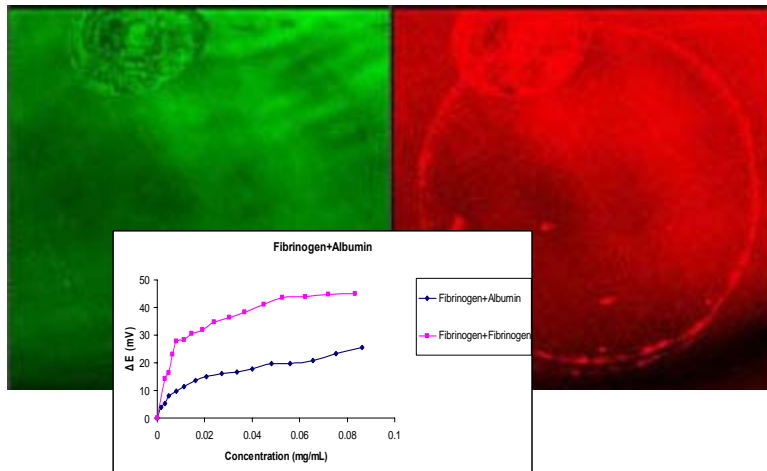
Lee, Yong-Geun. "Preparation of Highly Monodispersed Hybrid Silica Spheres Using a One-Step Sol-Gel Reaction in Aqueous Solution." *Langmuir: The ACS Journal of Surfaces and Colloids* 23 (2007).

Session III

Biomolecule Detection

Chairs: Eliana Pfeffer

Garcia Staff: Yantian Wang



A simple method of discriminating between double-stranded and single-stranded combed DNA on surfaces

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Jennifer Daniel, Stony Brook University, Stony Brook, NY 11790

Julia Budassi, Long Beach High School, Long Beach, NY 11561

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Dr. Miriam Rafailovich, Stony Brook University, Stony Brook, NY 11790

Dr. Jonathan Sokolov, Stony Brook University, Stony Brook, NY 11790

Microarray technology is essential for biomedical analysis such as gene expression analysis, polymorphism or mutation detection, DNA sequencing, and gene discovery [1]. Base pair hybridization of known single-stranded (ss) DNA sequences ("probes") to unknown ("target" strands) is used to determine structure. Conventionally, short probes are used which are then hybridized to long and short target strands. However, long target strands will not effectively hybridize to short probes—they might self hybridize, as an example. Therefore, we are trying to find a way to use longer probes by combing them onto a surface. Using double-stranded (ds) DNA that is combed onto surfaces is disadvantageous because the DNA can come off the surface when denatured, and some of it isn't even denatured. Furthermore, there is no simple method to characterize and ensure that the DNA left on the surface is truly single-stranded and left unbroken. Therefore a better way to create probes would be to controllably deposit long (>50 kbs) ssDNA (produced through heat denaturation or single-sided polymerase chain reaction (PCR)) on a surface and confirm it in-situ as being ssDNA using acridine-orange (AO)—a differential dye that distinguishes between ss and dsDNA under proper dyeing conditions (time dyed, concentration of dye, temperature of dye, type of buffer used, etc).

We optimized the AO staining procedure by using known dsDNA and ssDNA samples diluted to 25 μ g/mL with 2-N-morpholinoethanesulfonic acid (MES) as a calibration for the emission spectrum (intensity versus wavelength, see Figure 1) which would differentiate between the two in an unknown sample, as ds and ssDNA (see Figure 2) fluoresces differently when dyed with AO. Drops of .5 μ L were placed onto a polymethyl-methacrylate (PMMA) surface of about a 170nm thickness, dried at room temperature, soaked in dye diluted to 2.5 μ g/mL in tris-borate-EDTA (TBE) buffer for an hour, and then washed with a TBE solution. Heat denatured ssDNA was tested to ensure that it remained ssDNA throughout deposition.

In the PCR approach, we prepared four samples to be put in the thermocycler with hopes of finding the concentration of DNA and the mixture of primers that yielded the most single stranded DNA. For the process we used buffer, polymerase enzymes, ultrapure water, template DNA (lambda DNA), dNTPs (loose nucleotides), and primers. The concentrations of each of these components remained relatively constant for each sample besides some changes made to find the optimal conditions for producing single stranded DNA. The control was human genomic DNA as a template to compare to the lambda DNA.

In conclusion, we have developed an effective method for distinguishing ssDNA from dsDNA on surfaces, which can be used for optimizing combing of ssDNA. Future work will focus on depositing both heat-denatured and PCR-produced ssDNA under various conditions and determining the best conditions for combing of long, intact molecules.

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[2] Herrick J., Bensimon A. Single molecule analysis of DNA replication (1999) Biochimie, 81 (8-9), pp. 859-871.

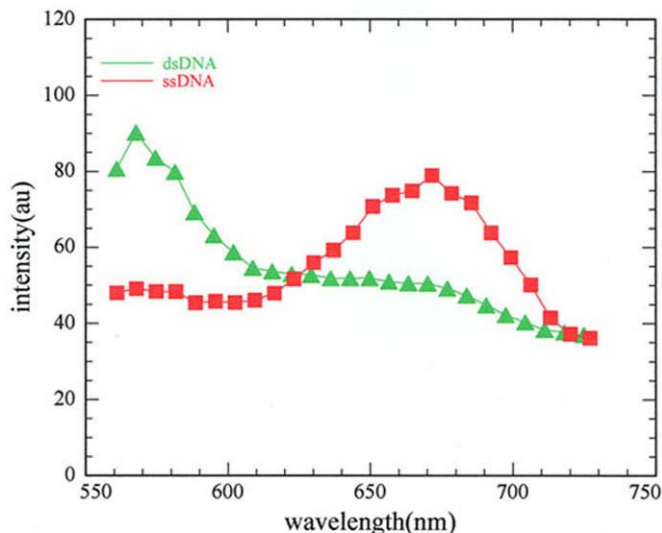


Figure 1. Fluorescence emission spectra of dsDNA and ssDNA deposited on PMMA-coated silicon wafer samples (as described above).

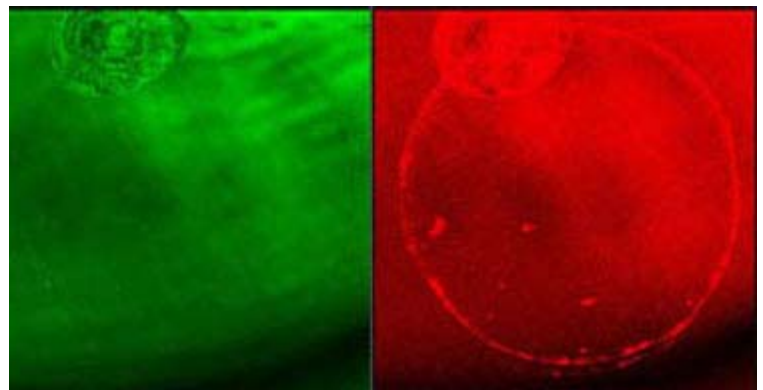


Figure 2. Leica Confocal Microscope image of a ssDNA drop on a PMMA-coated surface. The dimensions of the images are 1.2mm by 1.2mm. The left-most image portrays the DNA's fluorescence from 505 to 560nm, and the rightmost portrays the fluorescence from 605 to 660nm. As there is more fluorescence on the "red" side, the sample is single-stranded.

Molecular Imprinting of a Biosensor to Recognize Biomarkers for Early Cancer Detection

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²Yale University, New Haven, CT

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Molecular imprinting is an inexpensive method for the fabrication of thin films with cavities that have an affinity for a target molecule. Successful molecular imprinting of proteins has, until recently, been very difficult.¹ Proteins are important to imprint, because, if done successfully, detection methods of these proteins in the body could be medically useful.

Molecular imprinting begins with templating a gold-plated silicon wafer with fibrinogen molecules into a Thiol (11-mercapto-1-undecanol surfactant) matrix. Through the principles of molecular imprinting, the adsorption of the target molecule after washing with de-ionized water creates cavities, which are complimentary to the shape of that target molecule. Gold was chosen as the templating surface because of its conductive properties, which enables the potentiometer to detect the electrical signal given off by the protein when it adsorbs to the surface of the thin film. To test the selectivity of the sensor, first an albumen solution was introduced to test whether different molecular shapes can be adsorbed into the cavities created by the fibrinogen molecules. To further test the selectivity of the Thiol matrix a fibrin solution was tested (figure 1).

Fibrinogen is a known protein that is involved in the first steps of wound healing. When one is wounded, the body naturally lowers the wounds pH from 7.4 to 4. The change in pH signals the Fibrinogen molecules to collect at the wound site.

The next step in wound healing is the addition of Thrombin, this protein cleaves the A and B knobs (figure 3) turning Fibrinogen to Fibrin. The fibrin now is able to form fibers creating a scab, therefore stopping the wound from bleeding. If the fibrinogen-imprinted Thiol matrix is able to differentiate between the fibrinogen and fibrin then the biosensor is more selective than previously believed. The results (figure 2) indicate that

Fibrin and Fibrinogen give off opposite charges, indicating differentiability and high efficiency.

The presence of cancer causes the over-production of specific proteins; these proteins are known as cancer markers. Due to the absence of an efficient chemical detection method, a physical detection method, called a digital rectal exam (DRE), an extremely uncomfortable procedure, is used. Using molecular imprinting, one would be able to detect prostate cancer without the use of this uncomfortable physical detection method. It would possibly be a more effective way to sense the cancer markers, because the biosensor is so sensitive and specific, as proven by results in figure 2.

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¹Recent developments in the molecular imprinting of proteins, David E. Hansen

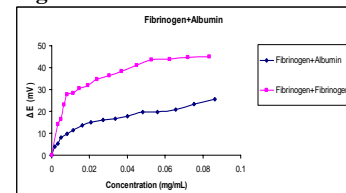
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²Fibrinogen and Fibrin." [Sigma-Aldrich.com](http://www.sigmaaldrich.com). Sigma Aldrich. 12 Aug 2008

<http://www.sigmaaldrich.com/Area_of_Interest/Biochemicals/Enzyme_Explorer/Analytical_Enzymes/Fibrinogen_and_Fibrin.html>.

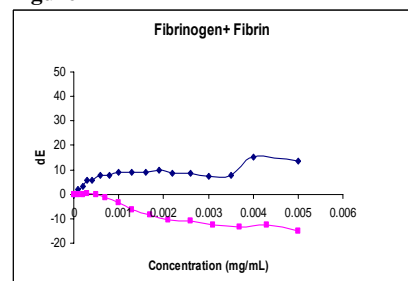
Tien, H. Ti. "Self-assembled lipid bilayers as a smart material for nanotechnology." *Materials Science and Engineering C* 3(1995): 7-12.

Figure 1



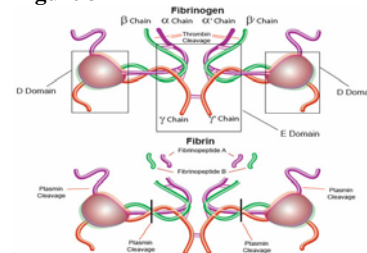
The addition of Albumin to a Fibrinogen imprinted chip did not produce as great of a response as the Fibrinogen to a Fibrinogen imprinted chip.

Figure 2



The addition of Fibrin to a fibrinogen imprinted chip gives off an opposite charge from that a Fibrinogen added to a fibrinogen imprinted chip.

Figure 3



This difference between Fibrinogen and Fibrin is very small. When Thrombin is added to Fibrinogen, the A and B nubs are cleaved and bonded to D domain of other Fibrin molecules to form fibers.²

Session IV

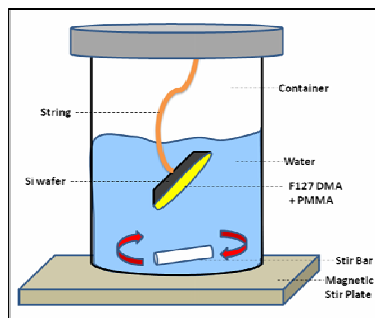
Drug delivery systems

Chair: Robert Winston

Garcia Staff: Stancy Joseph,

Divya Bhatnagar,

Jack Lombardi



Fabricating temperature sensitive N-isopropylacrylamide gels for use as topical delivery systems

Cruz Granados, Long Beach High School, Long Beach, NY

This project in its present form planned to engineer a thermo-sensitive N-isopropylacrylamide gel that could be used in topical delivery systems. N-isopropylacrylamide gels are a type of hydrogel that swell at low temperatures and shrink at temperatures above 32°C. Gels with concentrations 1.5 and 1.0 molar were prepared using ammonium persulfate as an initiator, Methylene-bisacrylamide as a crosslinker and tetramethylethylenediamine as an accelerator. These gels were then washed for seven days with deionized water to remove any impurities from the gel.

After the washing with deionized water the rheology of the gels was obtained to observe the modulus of the gel and how the gel responds to varying stressors such as temperature. Another part of this research was to observe the swelling and deswelling kinetics of the N-isopropylacrylamide gel. This was done by analyzing the absorbency of deionized water with a spectrophotometer. A third part of the research was to perform a mass/length change experiment to observe the mass and length change that The research conducted can be used to develop this gel so that it is able to transport drugs and other substances that need to be delivered topically. In the future I would like to load this gel with a protein or other drug and observe the release rate.



Fig. 1: NIPA gels as prepared in 20 mL vials. The higher molar concentration are more opaque than the lower molar concentrations.



Fig. 2: The 1.0 mol/L gel with the least opacity.

Ju, X (2006 October 16). Effects of internal microstructures of poly (N-isopropylacrylamide) hydrogels on thermo-responsive volume phase-transition and controlled-release characteristics.

Smart Materials and Structures, 15, Retrieved July 28, 2008, from <http://cat.inist.fr/?aModele=afficheN&cpsid=18314520>

Engineering and Rheological Modeling of Hydrogels for Controlled Drug Delivery via Folate-Coated Platinum Nanoparticles

Naveen Murali¹, Elias Lebovits², Daniel Peng³, Stancy Joseph⁴, Miriam Rafailovich⁴

¹Staples High School, Westport, CT

²Ramaz Upper School, New York, NY

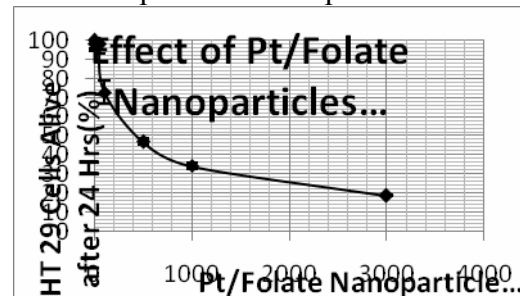
³Monta Vista High School, Cupertino, CA

⁴SUNY Stony Brook

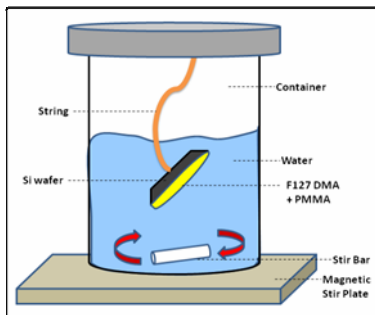
Hydrogels have gained attention for their use in biomedical drug release due to their protected transport of nutrients and ability to regulate the release of drugs. In particular, the tri-block copolymer surfactant Pluronic® F127, PEO₉₉-PPO₆₉-PEO₉₉, is being studied for its mechanical and physical properties. The unique thermoreversible quality of Pluronic F127 allows it to form a liquid at cold temperatures due to the water solubility of both the PEO and PPO blocks in cold environments. The spatial transformations of the polymer's micelles cause F127 to develop into a hydrogel in warm environments and permit drug loading and drug release by diffusion.¹ The polymer has been cross-linked into a swelled, hydrogel state by modification with dimethacrylate (DMA) followed by exposure to UV light after the addition of a photoinitiator. Cross-linking Pluronic F127 can suspend the polymer in its hydrogel state while permitting the thermoreversible response of the micelles for drug release and loading.

At the same time, cancerous cells display a tendency to over-express folate receptors on their cell members because of a need for folate in order for the cells to replicate and stay healthy.² Our research shows that platinum nanoparticles, when coated with folic acid, can trick cancer cells into absorbing the toxic platinum particles which destroy the cells' cytoskeletons. By loading our drug delivery hydrogel system with the folate-coated platinum nanoparticles, we have devised a means for the controlled release of the toxic nanoparticles in response to a temperature stimulus from the hydrogel to attack cancerous cells. We conducted a MTT assay to determine the optimal concentration of folate coated particles that would be least invasive to healthy cells while still useful for cancer therapy.

To increase the release rate of the nanoparticles from the hydrogel, we attached the polymer to a thermo-electric (TE) module which operates by the Peltier effect to convert thermal differentials into an electric voltage. By creating an artificial



cold or hot environment, the TE module can stimulate the drug release from the hydrogel by moderating the opening and closing of the micelles. We tested the attachment of cross-linked F127 to thin films of PMMA spun in concentrations of 20, 30, 40, and 50 mg/ml on Si wafers to model the hydrogel attachment to the TE module.



The rheological properties of the hydrogel were studied for personalized drug administration in patients with diabetes and hypertension. The σ' , elastic modulus, and σ'' , viscous modulus, of the polymer were tested in modified glucose and NaCl polymer solutions using a rheometer.

¹ Satish CS, Satish KP, Shivakumar HG. "Hydrogels as controlled drug delivery systems: Synthesis, crosslinking, water and drug transport mechanism." *Indian Journal of Pharmaceutical Sciences* 2006; 68:133-40

² Dai, Hongjie. "Carbon nanotubes as multifunctional biological." *PNAS* 102.33 (2005): 11600-11605.

Session V

Cell surface interactions

Chair: Alex Ramek,

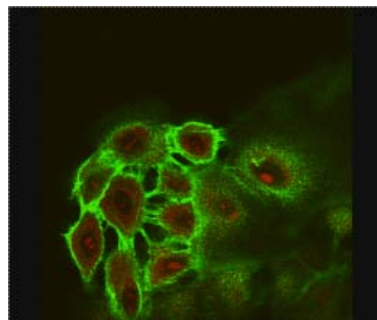
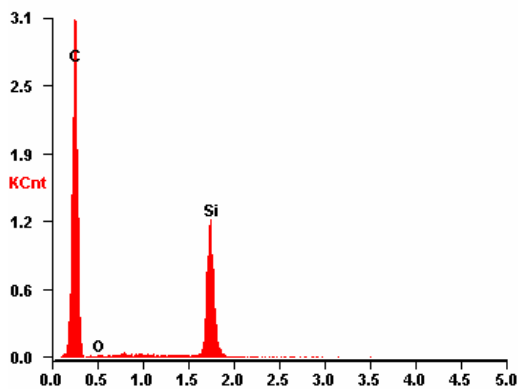
Garcia Staff: Ying Liu,

Tatsiana Mironava,

Jason Yang,

Chung Chueh (Simon) Chang

Wanji (Ruby) Yen



An Analysis of Polymer Substrates as Inducing Surfaces: Novel Implications for the Optimal Control of Stem Cell Differentiation

Michael Tischler¹, Simon Chang², Ruby Yen³

¹*Jericho High School*

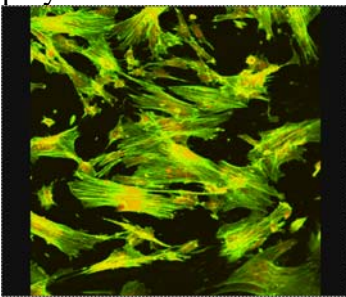
²*Stony Brook University, Department of Materials Science and Engineering*

³*Stony Brook University, Department of Biomedical Engineering*

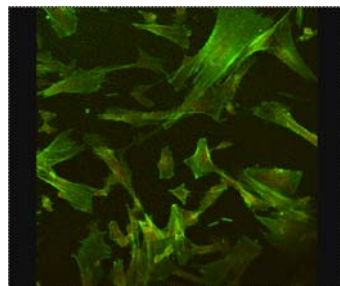
In contemporary society, stem cells are hailed for their potential to serve as remedial agents for diseases. From leukemia to Parkinson's disease, stem cells are thought to have the potential to cure various diseases through differentiated cell replacement therapy¹. However, due to the controversy over the use of embryonic stem cells, there has been an increasing importance put on maximizing the efficiency and abilities of adult stem cells. In our study, we utilized Dental Pulp Stem Cells (DPSC), a multi-potent adult stem cell, and sought to engineer an optimal surface for these DPSCs to proliferate and differentiate on.

First, we sought to determine an optimal polymer substrate for cell proliferation and differentiation. We utilized glass, a polybutadiene/polystyrene copolymer, and ethylene vinyl acetate, as our polymer surfaces, and cells were plated on each. Each sample was then subjected to confocal microscopy, through which we were able to examine the morphology of the cells on each sample. Furthermore, we conducted a 10-day cell growth curve, which enabled us to see which surface was best for cell proliferation. Additionally, we utilized Atomic Force Microscopy (AFM) to measure the rigidity of our polymer substrates. We measured the polymer substrates alone and hypothesized that the thin films, which were the most rigid samples, would grow the most rigid cells. Furthermore, if the non-induced AFM readings for the DPSCs become more rigid as our experiment goes on, we will have found an effective surface that is capable of self-inducing stem cell differentiation.

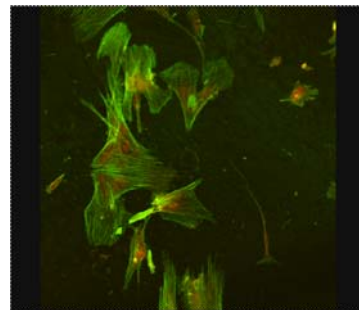
We hope that our data from the Confocal Microscopy is consistent with that of the cell curve in that for both experiments, the same surface is optimal for cell growth. Furthermore, we hope to find a strong, positive correlation between the rigidity of each polymer substrates and the cells which are left to differentiate on them.



Control



**Thin Film
PB/PS**



Thin Film EVA

THE INFLUENCE OF POLYBUTADIENE ON THE PROLIFERATION AND MECHANICS OF KERATINOCYTES IN THE ABSENCE OF 3T3S

Jackie Belizar, Miller Place High School

Reena Glaser, Smithtown High School West

Matthew Hung, Smithtown High School West

Mentor: Dr. Marcia Simon and Simon, Stony Brook University

REU: Ruby, Stony Brook University

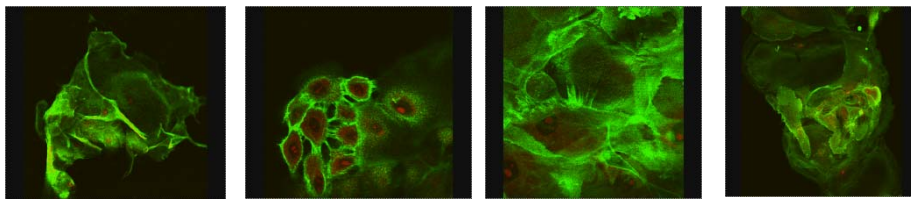
We decided to conduct our experiment as a result of previous observations made of keratinocytes proliferating at rates greater than plastic medium on polybutadiene in the absence of 3T3 embryonic fibroblasts and at rates that are similar to surfaces containing 3T3s. It was observed that the thin PB polymer allowed for greater confluence than the thicker surface. To initiate the experimental procedures that compared to the original design, we prepared two Polybutadiene (PB) solutions with toluene as the solvent. The concentration of two PB solutions was 2mg/ml (200Å) and a 20mg/ml (2000Å). These solutions would be used in the spinning of thin films for cell culturing. We plated neonatal foreskin keratinocytes with and without mouse 3T3 embryonic fibroblasts at two different densities of 2,000 cells and 10,000 cells. Our set up was a wafer, 200Å, 2000Å, glass and plastic for two periods of cell counting (7 day, 10 day) and Confocal Microscope imaging.

The significance of the original observation relates to the use of scaffolds for wound healing and skin regeneration in the human body. The PB polymer is a natural synthetic rubber that can be easily generated in the lab and inserted into the body. There is no danger of rejection of this surface from the body; however, the utilization of 3T3s derived from embryonic mice is foreign and have the possibility of being rejected in a human subject. If we were capable of regenerating skin tissue with only polybutadiene and keratinocytes, we would eliminate this obstacle and alleviate the implications that arise with inserting foreign materials into the bodies of burn recovery patients.¹

Our data portrayed the most colonization on the surfaces containing 3T3 embryonic fibroblasts. The surface without these cells that exhibited a large percentage of confluence was the plastic followed by the thick polybutadiene. In addition, growth on the thick PB was observed to be much greater as that of the thin PB. These results were unexpected and varied strongly from prior observations. These results proved that low density plating of keratinocytes will not result in cell growth that can be compared to colonization on surfaces with 3T3s. In addition, the cells grew more consistently on the plastic rather than on the polybutadiene. Nevertheless, the cells were able to proliferate on the PB. (Figures 1a,b,c,d)

In the future, we are going to continue our study of keratinocytes and their growth on polybutadiene in order to determine the effect of this surface on the its morphology and its mechanics. Also we going to study the difference between neonatal foreskin keratinocytes against adult mature keratinocytes D039 using the Atomic Force Microscope.

1. Simon, Marcia. "The epidermal cornified envelope and its precursors." [The keratinocyte handbook](#). By E. B. Lane and Fiona M. Watt. Ed. Irene M. Leigh. Cambridge, UK: Cambridge UP, 1994. 275-92.



Figures 1a,b,c,d show Wafer, Thin, Thick&Glass without present of 3T3s

Layer-by-Layer Assembly of a Bilayer Tissue Through the Harvesting of Cell Sheets On Photosensitive Polymer Substrates

Susie Ahn Herricks High School

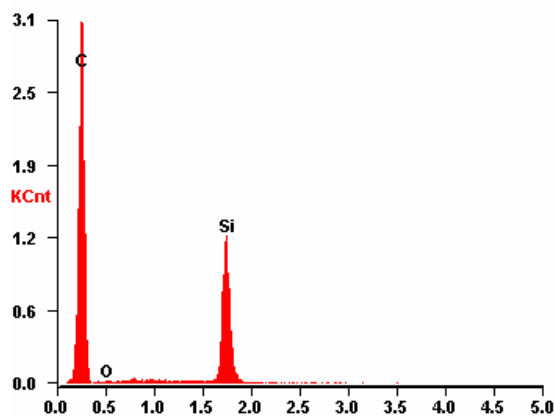
Tiya Nandi Jericho High School

Tatsiana Mironava, Miriam Rafailovich, Lourdes Collazo, Chunhua Li, Stony Brook University

Monica Apostol, Estee Lauder Laboratories

Multifunctional, multilayered tissues similar to those that exist naturally in the human body can be assembled by culturing thin layers of various cell types, harvesting the layers individually, and depositing the layers in a well-defined manner onto a porous, biocompatible scaffold to develop into transplantable tissues. Enzymes such as trypsin-EDTA are generally used to detach adherent cell layers from solid substrates. However, in cases where very thin and fragile cell sheets must be transferred, such enzymes may not be suitable. Our investigation involves the utilization of a photosensitive triblock copolymer as a substrate for the non-enzymatic harvesting of cell sheets. These cell sheets will then be assembled into a bilayer tissue.

After cleaving Si wafers into approximate 1cm x 1cm samples and spin-coating them with the photosensitive polymer, the wafers were annealed for 24 hours and subsequently plated with two different cell lines. Currently, we have plated human dermal fibroblasts (CF-29s), mouse calvarias (MCs), neonatal dermal fibroblasts, and human mammary epithelial cells (MCF-10As). After 3-4 days of growth in an incubator, the samples were exposed to UVC light for one hour. Hypothetically, the photosensitive thin film reacts to the UV light, causing the cell layer to detach and float to the surface of the medium. Then, that cell layer is transferred onto a cell layer of another type. However, we have only been able to loosen the UV-exposed cell layer enough to transplant small portions of it to another cell sheet. Despite the moderate success of the photosensitive polymer, the bilayer tissues, when examined using confocal microscopy, indicated that the different cell layers were healthy and proliferating quite well in bilayer (Fig. 1). This implies that PMMA, which is thought to lift off and attach to the cell layer during UV exposure, has been evaporated and is nonexistent in the cell layers. To confirm this inference, a PS film of 500 nm was floated onto a wafer spin-coated with the photosensitive polymer and exposed to UV light. The PS film was then floated onto a clean silicon wafer and examined through scanning electron microscopy (SEM). The negligible oxygen levels indicate that PMMA is not present on the film (Fig. 2). It has also been confirmed that liftoff of cell layers has not occurred due to dewetting in the films, which has been attributed to the thinness of the films, overly long annealing times, and inadequate cleaning of the Si wafers. In the coming weeks, new sample sets will be prepared with shorter annealing times and a preliminary cleaning procedure. These samples will be plated with dermal fibroblasts and another cell line and exposed to UV light. In this new experimental set, we expect that the performance of the photosensitive polymer will be successful in the harvesting of entire cell layers. If the photosensitive polymer proves to be an effective method for the harvesting of cell sheets, we hope to develop organized bilayer tissues and eventually, fully transplantable multilayer tissues.



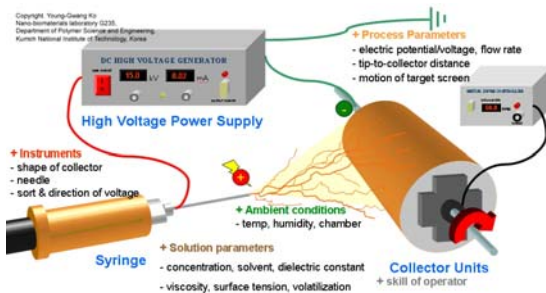
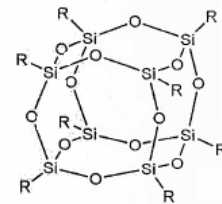
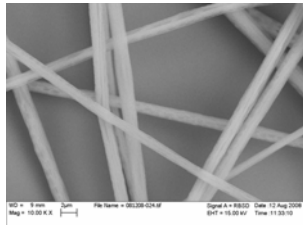
Session VI

Properties of Electrospun Fibers

Chair: Yusung John Lim

Garcia Staff: Ying Liu,

Chunhua Li



Enhancement of Electrospun Fibers and Polymer Thin Films with Polyhedral Oligomeric Silsesquioxanes

Neil Patel, Herricks High School, Jeremy Joven, Valley Stream Central High School

Yusung John Lim, Massachusetts Institute of Technology

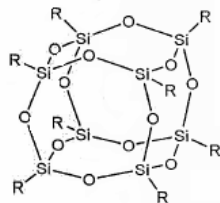
Robert De La Cruz, Miriam Rafailovich, John Jerome
Department of Material Sciences and Engineering, Stony Brook University

Electrospun fibers have a wide range of applications. Superfine fibers with diameters ranging from 10 μm to 10 nm can be produced with applications in filtration, composite materials, catalyst supports, tissue engineering, and drug delivery.¹ The goal of the project is to incorporate polyhedral oligomeric silsesquioxanes (POSS) into electrospun fibers. POSS has both inorganic polymer and organic polymer properties. It has a precise three dimensional structure, shown in Figure 1, with a silsesquioxane cage, and eight R groups allow for the reinforcement of polymer segments and coils at the molecular level. POSS has been shown to enhance thermal and mechanical properties.²

The purpose of this project is to electrospin fibers containing polyhedral oligomeric silsesquioxanes and to analyze the mechanical properties and surface morphology of these fibers. It is believed that the POSS nanoparticles should enhance the strength of electrospun fibers. During the course of experimentation, solutions of polymethyl methacrylate (PMMA) and polystyrene (PS) in chloroform, tetrahydrofuran (THF), and dimethyl formamide (DMF) were created with varying concentrations from 20% to 30% polymer. These solutions were pumped through a syringe as a voltage of 10 kV was applied between the metal needle and the collector plate containing silicon wafers. The jet is elongated by the electric field, and solvent evaporation results in the formation of polymer fibers which deposit on the collector.³

The electrospun fibers are then analyzed using scanning electron microscopy (SEM) and atomic force microscopy. SEM pictures show that the fibers with POSS have a greater degree of porosity than fibers without POSS as shown in Figure 2. The presence of POSS in PS fibers can be confirmed by using Energy Dispersive Analysis of X-Rays (EDAX) with the SEM to confirm the presence of oxygen. Atomic force microscopy will be used to analyze the surface morphology and physical properties of the POSS nanofibers. An important measure will be the modulus of the fibers which will allow the strength of the fibers to be quantified.

Further research will be conducted to evaluate the effects of different concentrations of POSS on the morphology of electrospun fibers. In addition to such investigations, studies will be performed to discover the effects of POSS on thin films. As the military, in particular the US Air Force, becomes more technologically dependent, there comes an increasing demand for a new generation of lighter weight, higher performance polymeric materials. Work on POSS coated silicon wafers may shed some light on such a coveted necessity.



1. International Journal of Nanotechnology
and Nanoscience
Volume 1, Number 1, 2006
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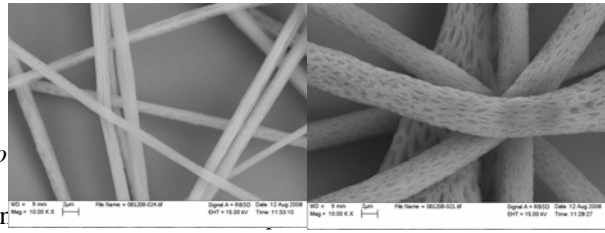


Figure 2: SEM Pictures of PS fibers with (right) and without (left) POSS.

¹ Ji-Huan He, Yu-C
5(3), 253-262, 200

² Sherly R. Largo,

³ M. H. He, Structure of POSS Nanoparticle
Increments for POSS Polymers in Solution. Air Force Research
Laboratory, Institute of Chemical Engineers Journal, Volume 53, Issue 1
(p 51-55) 2006.

Effect of Polymer and Solvent Blends on Electro-spinnability and the Physical Properties of Electrospun Fibers

Melody Tan, Valley Stream Central HS, Jake Bryant, Ward Melville HS

John Lim, Massachusetts Institute of Technology

John Jerome, Miriam Rafailovich, Robert De La Cruz

Department of Material Sciences and Engineering, Stony Brook University

Electrospinning is a process which uses a high-voltage source to create polymeric fibers of diameters in the micro or nanoscale range. These fibers can be used in a wide range of applications such as protective shields in speciality fabrics, composite reinforcement, and structures for nano-electronic machines.¹ By blending different polymers of assorted molecular weights and various solvents, the resulting properties can be manipulated to be applied in new fields of technology.

In our experiment thus far, we have made solutions using Polystyrene (Mw = 280k) and Polymethylmethacrylate (Mw = 120k and 15k) blends dissolved in several solvents such as Dimethylformamide, Tetrahydrofuran, Methyl ethyl ketone (Butanone), and Toluene. These solutions were electrospun onto silicon wafers previously spuncast with the respective solution. The fibers created were then analyzed with the Scanning Electron Microscope (SEM) for physical properties such as diameter, roughness, pores due to differences in solvent vapor pressures, and beading patterns.

Electro-spinnability is affected by the viscosity of the solutions. Solutions with higher molecular weights are more viscous than solutions with lower molecular weights, and therefore need a lower concentration in order to be electrospun. We have observed that fibers from Tetrahydrofuran solutions are more brittle than those of Dimethylformamide solutions. Toluene has also been found to be an unfavorable candidate for electrospinning due to its low dielectric constant.²

As we continue our research, we plan to experiment with more polymers and solvents and also varying concentrations of the blends. We also plan to view the fibers with the Transmission Electron Microscope (TEM), which will give us a cross sectional image and allow for analysis of the internal composition of the fibers.

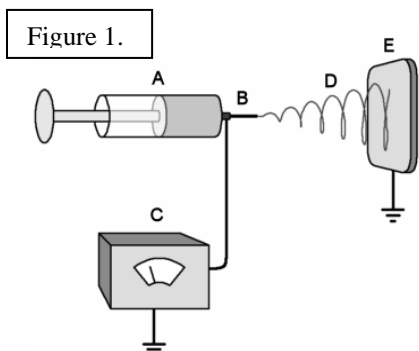


Figure 1. A simplified electrospinning schematic.³

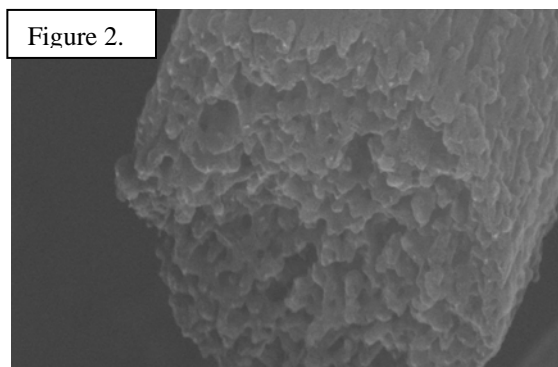


Figure 2. Scanning Electron Microscopy (SEM) image of a cross section of an electrospun fiber of 7.5% Polymethylmethacrylate, 22.5% Polystyrene, and 70% Dimethylformamide solution.

¹ Frenot, Audrey, and Ioannis S. Chronakis. "Polymer nanofibers assembled by electrospinning." *Current Opinion in Colloid & Interface Science* 8 (2003): 64-75.

² "Dielectric Constants of Materials". Clipper Controls. 8/10/08 <http://www.clippercontrols.com/info/dielectric_constants.html#T>.

³ "Neotherix - novel scaffolds for tissue repair". Neotherix. 8/10/08 <<http://www.neotherix.com/technology.php>>.

Session VI

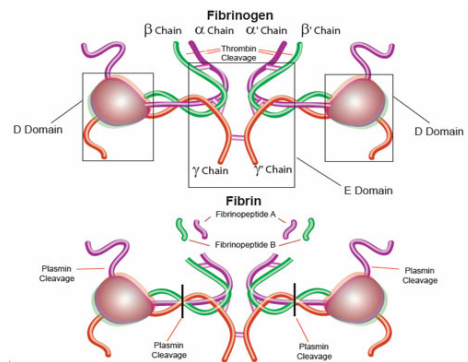
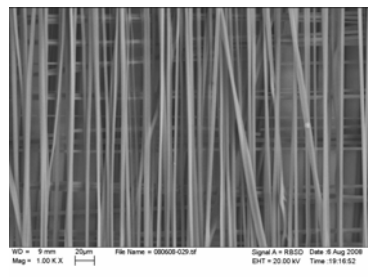
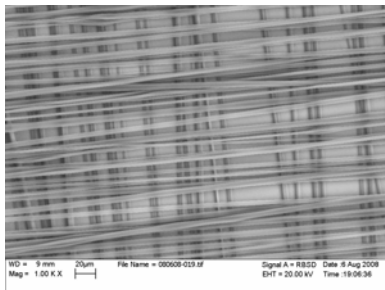
Fibrillar Scaffolds

Chair: Alex Ramek

Garcia Staff: Ying Liu,

Jaseung Koo

Jason Yang



Effects of Electrospun PMMA/TiO₂ Fibers on Cells and DNA

Michelle Bayefsky, Ramaz High School

Dr. Miriam Rafailovich, Lourdes Collazo, Jason Yang

Department of Materials Science and Engineering, Stony Brook University

Nanoparticles are of great scientific interest because their size results in unique properties, such as a high surface area to volume ratio. However, their size may also result in new dangers that do not apply to bulk substances. For example, gold/citrate nanoparticles have been found to penetrate human dermal fibroblasts and significantly alter cells' abilities to grow, spread, adhere and form extracellular matrix (Pernodet et al 2006).

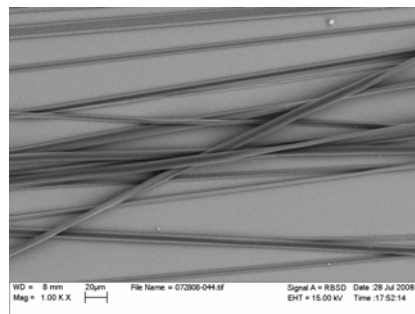
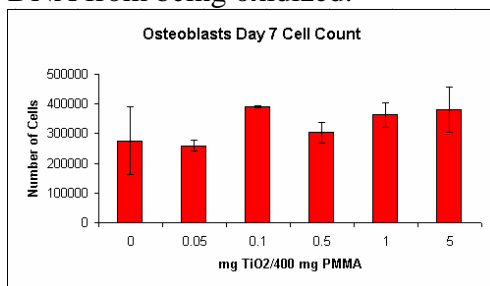
Titanium dioxide nanoparticles are widely used in paints, cosmetics and sunscreen because they are odorless, white and able to absorb UV light. However, studies have shown that like gold/citrate nanoparticles, TiO₂ nanoparticles have harmful effects on cells. Studies have also shown that TiO₂ is a potent photocatalyst and when the nanoparticles come in contact with UV light, they oxidize nearby bioparticles such as DNA.

We proposed to reduce the deleterious effects of TiO₂ nanoparticles on cells by encapsulating them in PMMA nanofibers.

Solutions with increasing concentrations of TiO₂ nanoparticles in PMMA and chloroform were electrospun and analyzed using SEM and TGA. SEM images showed that nanoparticles were in fact within the PMMA fibers and TGA showed that the increasing concentrations of TiO₂ resulted in increasing amounts of TiO₂ nanoparticles within the electrospun fibers.

The effects of the TiO₂ nanoparticles within the electrospun fibers were then tested on osteoblasts and dermal fibroblasts. The osteoblasts and dermal fibroblasts were plated on the fibers and the effects were measured by cells counts and viewing under the confocal microscope. Counts for the osteoblasts on day 7 showed that the cells were growing as well at the highest concentration of TiO₂ as they were for the control, which were PMMA fibers without TiO₂. The dermal fibroblasts appeared healthy with concentrations of 0 mg TiO₂, 0.1 mg TiO₂ and 5 mg TiO₂ and unhealthy with concentrations of 0.05 mg TiO₂, 0.5 mg TiO₂ and 1 mg TiO₂, possibly due to contamination of the incubator.

We also aimed to reduce the negative effects of TiO₂ nanoparticle catalysis on DNA through the same method of encapsulating through electrospinning. 1 mg of fiber from each of concentration of TiO₂ was combined with DNA, exposed to UV light for one hour, and run through an agarose electrophoresis gel. Results show that breakage of the DNA was reduced with an increasing concentration of TiO₂ in the fiber. This can be explained by the possibility that the TiO₂ is absorbing the UV light while the fiber is trapping the resulting free radicals and preventing the DNA from being oxidized.



Dermal Fibroblast Cell Migration on 3-Dimensional Electrospun PMMA Fibrous Scaffolds

Sarajane Gross¹, Elias Goodman¹, Alicia Franco², Ying Liu³, Dr. Clark⁴, Miriam Rafailovich³

¹ North Shore Hebrew Academy High School, Great Neck, NY

² SUNY Stony Brook, Dept. of Biochemistry, Stony Brook, NY

³ SUNY Stony Brook, Dept. of Material Science and Engineering, Stony Brook, NY

⁴ SUNY Stony Brook, Dept. of Biomedical Engineering, Stony Brook, NY

The adequate reconstitution of human soft tissue wounds requires the coordinated interaction of endothelial cells and fibroblasts during the proliferation phase of healing.^{1a} Tissue Engineering seeks to improve biological function through the use of engineered scaffolds which allow for effective migration and proliferation of cells. The purpose of this project is to better mimic the natural ECM of dermal fibroblasts by creating a 3-dimensional electrospun scaffold and to study how cell migration occurs in 3-dimensions, a more natural environment for the cells, vs. the cell migration previously studied in 2-dimensions. This project would also like to attempt to control the direction in which the cells are migrating so that a more efficient method of wound healing could be accomplished. Hypotheses include that cells will follow the alignment of the fibers and will not change directions until cell density becomes too high in that specific area. Cells will interact with one another along parallel fibers and form a pattern in order to distance themselves from one another. Finally, cell migration distance should have been longer for the 2-layer scaffold because on the 3-layer scaffold, it was expected that the cells would migrate down more so than migrating across.

For the purpose of our research, thin films were required, on which fibers were spun. Solutions of PMMA dissolved in toluene were created for our thin films on both silicon wafers and transparent glass cover slips. A thickness of approximately 1,000 Å was measured via ellipsometry. With thin films prepared, more solutions for the process of electrospinning were made. Four gram solutions generated at different concentrations produced different fiber diameters. A solution consisting of 30% PMMA, 50% THF and 50% DMF measured fibers to be about 1 µm. thick. A different solution of 20% PMMA and 80% chloroform made 8 µm. thick fibers. With all mixtures ready, various "patterns" of electrospun scaffolds were prepared. These included alignment in one direction, cross-layered alignment in two and three directions (figures #1, #2), random orientation and a unique set-up of fibers aligned on one layer and then cross-layered in a second direction on just half of the wafer. All samples were annealed at a temperature higher than the glass transition temperature of PMMA (114°C), to evaporate the organic solvent and ensure that the fibers were securely adhered to the wafer and/or glass. Prior to cell plating, both optical microscopy and SEM were utilized to capture pictures of the assembled scaffolds.

CF 31 dermal fibroblasts were plated onto the electrospun scaffolds that had been coated with fibronectin. Cell counts were taken before staining procedures. Their nuclei were then stained red with Propidium Iodide (PI) and the actin stained green with Alexa Fluor. Confocal microscopy was utilized (figure #3).

Results from experimentation are analytic for the most part, with little to no actual numerical data. Cell interaction was observed and hypotheses formulated. Cells successfully migrated to the third layer on the 3D scaffolds, indicating that 3D scaffolds are plausible and should be studied in the future for advancement in the field. It was also noted through various captured videos and confocal microscopy images that the cells on different layers do not interfere with one another and can move smoothly without interaction on the different levels. When calculated, it was shown that there was no great difference in cell migration distance between the 2 and 3-layer scaffold. Merely through examination, it was verified that cells can in fact change directions when following the fibers. Future work includes measuring cell density on the x-lattice as well as the energy required for the change of direction and verifying if cells can migrate up as well as down.

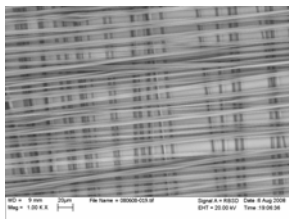


Fig.1
2-layer scaffold (SEM)

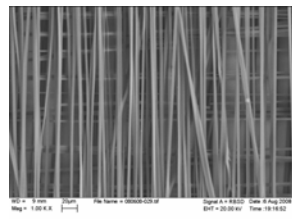


Fig.2
3-layer scaffold (SEM)

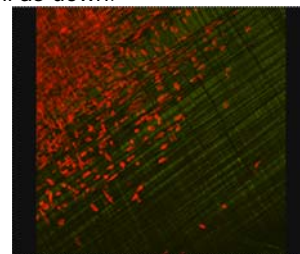


Fig. 3
2-layer scaffold
(Confocal)

^{1a} Oberringer, M., C. Meins, M. Bubel, T. Pohlemann. "In vitro wounding: effects of hypoxia and transforming growth factor beta1 on proliferation, migration and myofibroblastic differentiation in an endothelial cell-fibroblast co-culture model.." *J Mol Histol.* 39(1)(2007): 37-47.

Mechanism of fibrinogen fiber formation on Poly(methyl methacrylate) (PMMA) fibers

Jisha Thomas *New Hyde Park Memorial High School*

Alex Ramek *Harvard University*

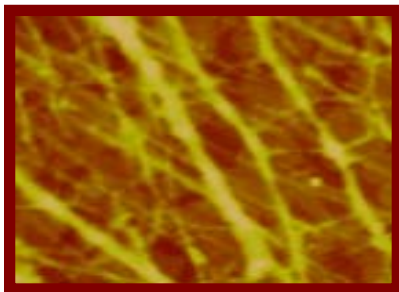
Jaseung Koo *Stony Brook University*

Miriam Rafailovich

Fibrinogen is a protein formed in the liver that plays a key role in promoting thromboses or blood clotting by causing platelets to aggregate inside blood vessels. When fibrinogen is converted into fibrin by the cleaving of thrombin, the fibrin matrix "gives the clot shape, strength, structure, flexibility and stability"¹. However, high levels of fibrinogen and clot formation can cause thrombosis which is "the formation of blood clots in blood vessels"² which leads to strokes and heart attacks. On the other hand, low levels of fiber formation can interfere or halt wound healing. This investigation sought to understand the mechanism of fibrinogen fiber formation on PMMA fiber surface. If fibers form on the PMMA fiber surface, this polymer can potentially be used as a scaffold to induce clotting.

In order to initiate this project, we created twelve silicon wafers; six were coated with PMMA while the other 6 were coated with P4VP. In previous research, it has been understood that fibrinogen fibers do not form on P4VP in concentrations above 9%. However, fibrinogen fibers do form on the PMMA surface so the P4VP will enable us to study the PMMA fiber and fibrinogen interaction without any surface interactions. Next, out of the 12 wafers, we had to electrospin 4 PMMA and 4 PVP silicon wafers in order to form aligned PMMA fibers on both (see fig.1). In order to electrospin, I prepared 2 solutions consisting of 1.6 grams of PMMA with 4.32 mL of chloroform reagent. After electrospinning and annealing the wafers for 10 hours, we made fibrinogen solution to put on 2 of the PMMA wafers with the PMMA fiber and 2 of the P4VP wafers with PMMA fibers. We will incubate these samples for 3 hours. Our controls are 2 PMMA silicon wafers and 2 P4VP silicon wafers without any fibers.

All of these samples will be studied and fibers will be observed using the atomic force microscope. We hope to observe both the surface with fiber interaction and the fiber to fiber interactions. For further research, we hope to culture dermofibroblast cells on the fibers and observe growth and movement. If successful, the results of this project may be a critical resource in the medical field of wound healing.



¹Lord ST. Fibrinogen and Fibrin: scaffold proteins in hemostasis. *Current Opinion in Hematology* 2007; 14:236-241.

²Definition of thrombosis. [MedlineNet](http://www.medterms.com/script/main/art.asp?articlekey=25023). Retrieved August 13, 2008, from <http://www.medterms.com/script/main/art.asp?articlekey=25023>.

Characterizing Interactions between Fibrinogen Dimmers during Fiber self-assembly

Brandon Hsu¹, Jaseung Koo², Dennis Galanakis², Miriam Rafailovich²

¹Monta Vista High School, Cupertino, CA

²SUNY Stony Brook; Stony Brook, NY

Recently, it has been discovered that fibrinogen can self-assemble into fibers on a hydrophobic surface. However, when talking about wound healing, it is usually fibrin that forms fibers to seal the wound. Minor differences in the structure of fibrin and fibrinogen cause drastic changes in their biological functions. Both fibrinogen and fibrin are dimmers which contain three pairs of protein chains, A α , B β , and γ . The dimmers are individually divided into three different regions of high protein density, a central E region and two outer D regions. The D regions of each dimer also have a specific conformation which allows there to be two different pockets, E_a and E_b, along with α C chains extending from its side. In a typical blood clotting situation, thrombin would cleave the fibrinopeptides at the dimer's N-terminus region to produce a transformed fibrin product. After the fibrinopeptides are cut, the "A-nub" (amino acid sequence G-P-R) and "B-nub" (amino acid sequence G-H-P-R) are exposed to bind with their respective E_a and E_b pockets. These interactions, together with those of contiguous chains, create a strong fiber interaction.

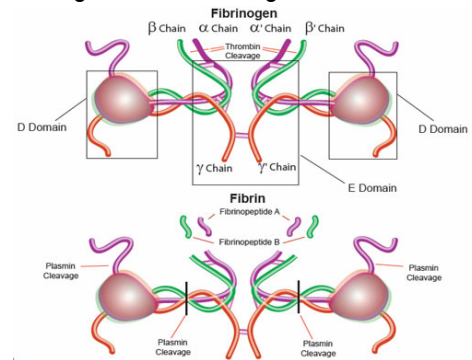


Figure 01. Structure of Fibrinogen and Fibrin
<http://www.sigmaldrich.com/img/assets/27040/fibrinogen-cleave.png>

On the other hand, even though fibrinogen dimmers do not have their fibrinopeptides removed, fibers can still form. Based on Dr. Dennis Galanakis's hypothesis, although the pockets and "nubs" lack a complete interaction, the mere fact that the "nubs" are close to the pockets allows for a partial interaction to take place. In preparation to test the role of specific interactions in a fiber, antibodies were incubated with a fibrinogen solution on a silicon wafer with a MTE monolayer. The antibodies used include GPR, GHPR, Anti-B, and Anti-A. The addition of GPR inhibits the "A-nub" from binding into the E_a site. In a fibrin dimer, the E_a and "A-nub" displays the strongest interactions¹. Without it, the α C chains, one of the stronger bonds, interacts to form a branching, network pattern (Fig. 02). Another test was with the antibody GHPR. Even though the GHPR blocks the E_b site, the bond between the "B-nub" and E_b site is relatively insignificant compared to that of the "A-nub" and E_a site. As seen in Figure 03, although the E_b site is blocked, a straight, un-branched fiber is formed. When the Anti-B antibody is added, just like the GHPR results, the stronger A:a interaction is present, and thus thick, long fibers are formed (Figure 04). Lastly, for fibers incubated with Anti-A antibodies, branched fibers were formed because the "A-nub" and E_a sites were not able to bond with each other. (Figure 05). For future experiments, time-dependent and concentration-dependent tests will be performed to find out how the fibrinogen dimmers bind as a function of time and how the saturation of fibrinogen dimmers affects the formation of fibrinogen.

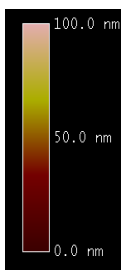


Figure 02. AFM image-Fibrinogen with GPR
 20 μ m by 20 μ m
 Z scale: 100 nm

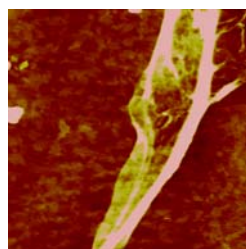


Figure 03. AFM image-Fibrinogen with GHPR
 30 μ m by 30 μ m
 Z scale: 100 nm

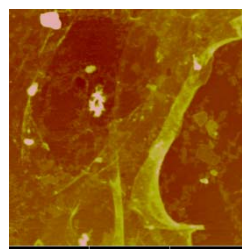


Figure 04. AFM image-Fibrinogen with Anti-B
 10 μ m by 10 μ m
 Z scale: 50 nm

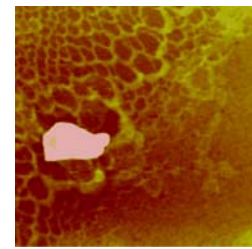
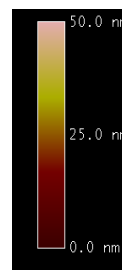


Figure 05. AFM image-Fibrinogen with Anti-A
 10 μ m by 10 μ m
 Z scale: 50 nm

¹Lord, Susan. "Fibrinogen and fibrin: scaffold proteins in homeostasis." *Current Opinion in Hematology* 14(2007): 236-241.

Self-Assembly of Fibrinogen Clots: A Study of Biomaterials in Contact with Blood

Pooja Rambhia¹, Sara Snow², Alex Ramek³, Adam Fields⁴, Jaseung Koo⁵, Dennis Galanakis⁵, Miriam Rafailovich⁵

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Fiber formation plays a critical role in the process of wound healing, and is also the driving force behind blood clotting. Conversely, inhibition of clotting on blood-contact biomaterials is crucial for preventing deleterious thromboses and improving patient prognoses. In the human body, vascular stents are crucial in the process of correcting atherosclerosis; however, research has shown that current polymer-coatings on these blood-contact biomaterials induce fiber formation, thus leading to clotting. Our research was premised on probing surfaces with various chemistry to understand if surface interactions alone could influence the spontaneous formation of a clot. We were successful in finding a polymer that can be used as a coating on a bare-metal stent that would inhibit fibrillogenesis, thus optimizing blood contact biomaterials.

A time-dependent study on fiber formation without the presence of thrombin on various polymer surfaces was performed to determine the effects of prolonged exposure to the fibrinogen. Atomic force microscopy (AFM) was employed to image the fibers, which showed that polystyrene with percentages of phenol above 9% inhibits the formation of fibers while polystyrene, and sulfonated polystyrene promoted fiber formation. Presently, polymer-coated vascular stents are being incubated with fibrinogen to observe fiber formation and to determine the efficacy of these current biomaterials. Bare metal stents will then be coated with polystyrene covinyl phenol 13%, using the langmuir-blogett trough, and then incubated with fibrinogen to observe whether or not fiber formation will be inhibited. This study could potentially lead to development of a novel polymer-coating on vascular stents that inhibits clot formation, thus optimizing blood-contact biomaterials.

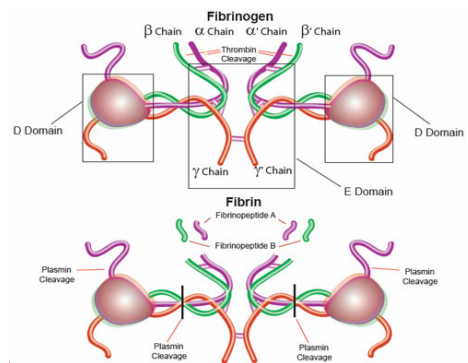
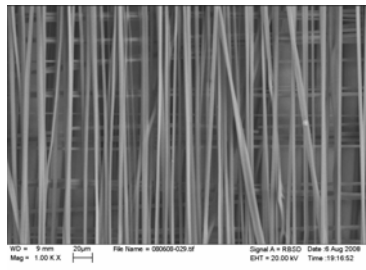
The time-dependent study on fiber formation also exhibited that polystyrene and sulfonated polystyrene, with varying levels of sulfonation, induced fibrillogenesis. This finding has novel implications in wound healing. Presently, cell migration tests are being conducted to observe the potential growth of dermal fibroblasts, cells that are crucial in the wound healing process, along newly formed fibers. The movement of the dermal fibroblasts towards these newly formed fibers would indicate that this clotting mechanism can allow for effective wound healing.

Session VIII

Nanoparticle Toxicity

Chair: Nicole Elstein
Daniel Gross

Garcia Staff: Tatsiana Mironava,
Chung Chueh (Simon) Chang
Wanji (Ruby) Yen



Comparative Study of the Effects of Nanoparticle Toxicities on Normal and Cancerous Cells

Sowmya Sundaresh, *Hicksville High School*

Rachel Mu, Show Lin, Dr. Miriam Rafailovich*

Department of Materials Science and Engineering, Stony Brook University

Nicole Elstein, *Lafayette College*

Nanotechnology is a developing field in various areas in which much research is being performed. It has been a recent concern that certain nanoparticles are toxic to some cells may due to effects of the particles penetrating into cells. Previous research has shown that gold citrate nanoparticles produce adverse affects when human dermal fibroblasts were exposed to them. For example, the particles damaged the actin of the cell and altered protein adsorption, which in turn decreased cell area and damaged cell growth.¹ The use of coatings, such as folate, may alter the way in which nanoparticles interact with normal and cancerous cells.²

The purpose of the experiment conducted is to determine whether certain nanoparticles could be used as a possible anti cancer therapy by testing the effects on normal and cancerous cells. Also, the effect of presoaking the nanoparticles in Fetal Bovine Serum (FBS) prior to exposure to the cells is tested to see if presoaking the nanoparticles will induce or restrict nanoparticle toxicity. The two cell systems that were exposed to these nanoparticles were normal and cancerous breast and keratinocytes.

So far in progress, keratinocytes were exposed to gold citrate nanoparticles of 15nm and 40nm sizes and normal breast cells were exposed to titanium dioxide (TiO₂) along with zinc oxide (ZnO) nanoparticles, respectively. In addition, platinum folate nanoparticles were made to test its effects on keratinocytes and squamous cell carcinoma. The cells were imaged under the confocal microscope using Alexa Fluor 488 and propidium iodide.

As seen in previous research, platinum folate nanoparticles target cancerous breast cells in comparison to non-cancerous breast cells due to the folate coating; however confocal images had not been taken to depict the visual effect. The confocal images taken during the current experiment show that the nanoparticles changed the cell shape and damage the actin, but the cancer cells were affected to a greater extent (Figure 1 and 2). A cell growth curve is being developed to quantify cell proliferation of keratinocytes due to the exposure of both sizes of the gold citrate nanoparticles; currently, the results show that there is not much of a difference between the 15nm and 40nm particles. Nevertheless, the factor of presoaking the gold citrate nanoparticles in FBS does inhibit the toxic effects of these nanoparticles. Subsequently, the effect of the titanium dioxide and zinc oxide nanoparticles on normal breast cancer cells shows that zinc oxide is more toxic to the cells than titanium dioxide (Figure 3 and 4), even at smaller concentrations, similar to their effects on keratinocytes and squamous cell carcinoma from previous experiments.

In the near future, more experiments will be performed to determine whether platinum folate, gold citrate, titanium dioxide, and zinc oxide nanoparticles are all possible candidates to become a potential anti cancer therapeutic drug through observing more cell counts and confocal images. Furthermore, protein adsorption from each type of cell will be tested using platinum folate, gold citrate, titanium dioxide, and zinc oxide nanoparticles using a thermogravimetric analysis (TGA).

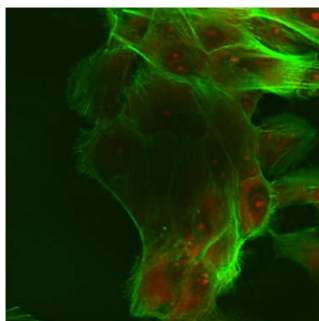


Figure 1. Normal breast cells exposed to platinum folate nanoparticles at 63x

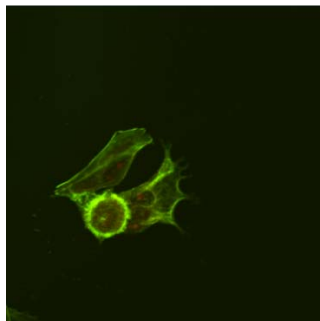


Figure 2. Cancerous breast cells exposed to platinum folate nanoparticles at 63x

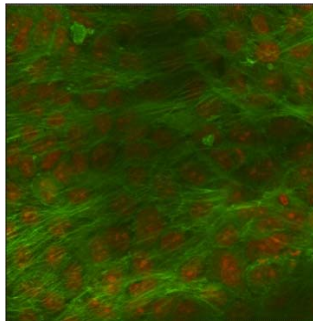


Figure 3. Normal breast cells exposed to TiO₂ at 0.02 mg/mL at day 6

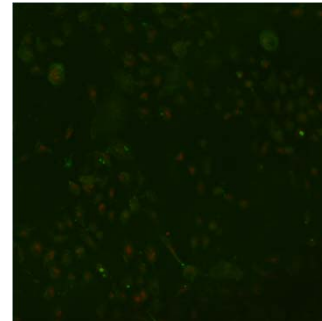


Figure 4. Normal breast cells exposed to ZnO at 0.02 mg/mL at day 6

¹ Pernodet, Nadine; et al. *Adverse Effects of Citrate/Gold Nanoparticles on Human Dermal Fibroblasts*. Small (2006) 766-733

² Lu, Yingjuan; Sega, Emanuela; Leamon, Christopher; Low, Philip. *Folate receptor-targeted immunotherapy of cancer: mechanism and therapeutic potential*. Advanced Drug Delivery Reviews 2004, 1161-1176

Colloidal Gold Nanoparticle Penetration in MC3T3-E1 Osteoblasts: Effect of Particle Size on Cell Proliferation and Structural Morphology *in vitro*

Samuel Kim, *Syosset High School*

Lourdes Collazo, Tatiana Mironava, Miriam Rafailovich*

Department of Materials Science and Engineering, Stony Brook University

Colloidal gold (Au) nanoparticles are known to be one of the lead candidates in the field of nanotechnology. Due to their unique properties shown to trigger significant responses in living cells, Au nanoparticles have widely been used to study how changes in their physical characteristics may influence cell proliferation, morphological structure, and spreading.¹ The present article investigates the effects of particle size on the permeation of Au nanoparticles through mouse calvarial (MC)3T3-E1 osteoblasts and their ability to induce major adverse effects on cell viability.

MC3T3-E1 cultures and Au nanoparticles of 15 nm and 45 nm were prepared. On the first, fourth, and sixth days after the nanoparticles were added to the MC3T3-E1 cultures, cells were counted with the hemacytometer for quantitative analysis. Qualitative analysis of the MC3T3-E1 structure and viability influenced by the permeation of Au nanoparticles was performed with both the confocal and phase-contrast microscopes. Cell count numbers for each variable were analyzed using the Chi-Squared test and the concept of standard deviation.

It was found that the 15 nm-sized Au nanoparticles had the greatest inhibitory effect on the cellular functions of MC3T3-E1 osteoblasts, evidenced by the relatively low cell counts on each specific day. Furthermore, the actin fibers were observed to be strained and much thinner than usual, most likely due to the rupture of the plasma membrane and consequent penetration of the nanoparticles into the cell body (Figure 1). The 45-nm sized Au nanoparticles generated higher cell counts, thereby showing that large nanoparticles may have less adverse effects on cell activity than the results induced by smaller nanoparticles. Since no nanoparticles were present in the control group, it had the greatest cell counts for all three days. Furthermore, actin fibers appeared to be healthy and proliferative (Figure 2). The average number of cells per 1 mL for the 15 nm group, 45 nm group, and control group on Day 6 were calculated to be 3.55×10^5 , 5.70×10^5 , and 2.25×10^6 , respectively. Thus, it can be seen that decreasing the dimensions of Au nanoparticles may result in major antagonistic effects on living cells.²

A consideration for future work would be to examine the effects of different sizes of Au nanoparticles on cancerous rat osteosarcoma (ROS) osteoblasts to determine how Au nanoparticles interact with the cancerous cell line for biomedical applications.

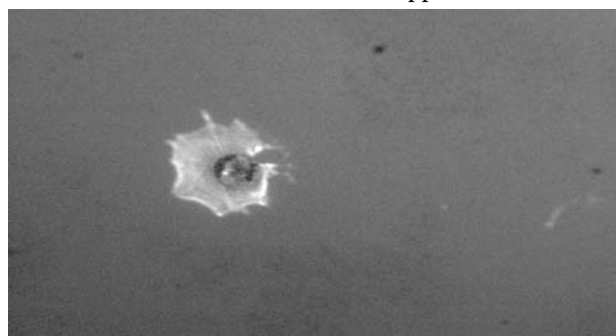


Figure 1: Hg (Mercury) Lamp image of ruptured MC3T3-E1 osteoblast and cluttered Au nanoparticles inside cell body.

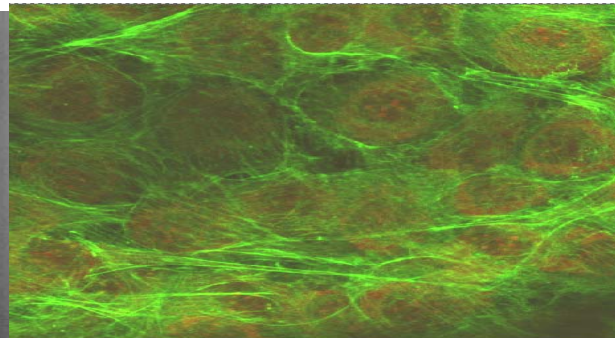


Figure 2: Confocal microscope image of MC3T3-E1 osteoblasts. Actin fibers are shown in green and nuclei are shown in dark red.

¹ Pernodet, Nadine, Xiaohua Fang, Yuan Sun, Asya Bakhtina, Aditi Ramakrishnan, Jonathan Sokolov, Abraham Ulman, and Miriam Rafailovich. "Adverse Effects of Citrate/Gold Nanoparticles on Human Dermal Fibroblasts." *Nanoparticle effects* 2 (2006): 766-73.

² Sonavane, Ganeshchandra, Keishiro Tomoda, Akira Sano, Hiroyuki Ohshima, Hiroshi Terada, and Kimiko Makino. "In vitro permeation of gold nanoparticles through rat skin and rat intestine: Effect of particle size." *Colloids and Surfaces B: Biointerfaces* 5 (2008): 1-10.

Methods of photocatalytic activity reduction of titanium and zinc oxides nanoparticles: control of reactive oxygen species

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With a rising concern for skin cancer, it has become a common societal practice to wear sunscreens to protect our skin from ultraviolet (UV) light. However, it is known that the main components of sunscreens, titanium dioxide and zinc oxide, are photocatalysts. This means that when exposed to UV light, these particles produce free radicals, or reactive oxygen species (ROS), which attack various biomolecules¹. In the past, micron-sized particles have been analyzed for their impact on DNA; however, these micron-sized particles have become obsolete in sunscreens due to their opaque and pasty white appearance². Therefore, we want to study micron-sized particles and compare their photocatalytic activity with nanoparticles.

In an attempt to minimize the impact of the free radicals we decided to coat the titanium dioxide particles with a biocompatible polymer, polyhydroxyethyl methacrylate (pHEMA). This polymer will prevent the release of the free radicals onto biomolecules and entire cells, thereby reducing their known harmful effects.

We have succeeded in coating our titanium dioxide particles by exposing particle-monomer aqueous mixtures to UV light. The free radicals caused the monomer to polymerize into pHEMA and coat the particles. We measured the mass percent of our coating relative to the nanoparticles with the use of thermogravimetric analysis (TGA). Our TGA results indicate that the polymer content was up to 6 mass percent.

We exposed suspensions of uncoated titanium dioxide mixed with GTP to UV light at 300nm. These suspensions consisted of nanoparticles and micron-sized particles in three different types of media, each with a different pH: distilled water (pH 5.92), phosphate buffered solution (PBS, pH 6.7) and deionized water (pH 6.9). The percent of GTP degradation from 0 minutes to 120 minutes was then measured with the use of a UV-VIS spectrophotometer.

Our most interesting results to date involve the dependence of GTP degradation rate on the media pH used. For the uncoated micron-sized particles, we found that as media pH decreases from 7 (neutral) to below 6 (acidic), the GTP degradation rate increases (Fig 1). Change in the degradation rate with one particular type of nanoparticle was not significant. Our findings on pH dependence indicate that titanium dioxide induced damage to cells can depend on tissue condition, such as inflammation.

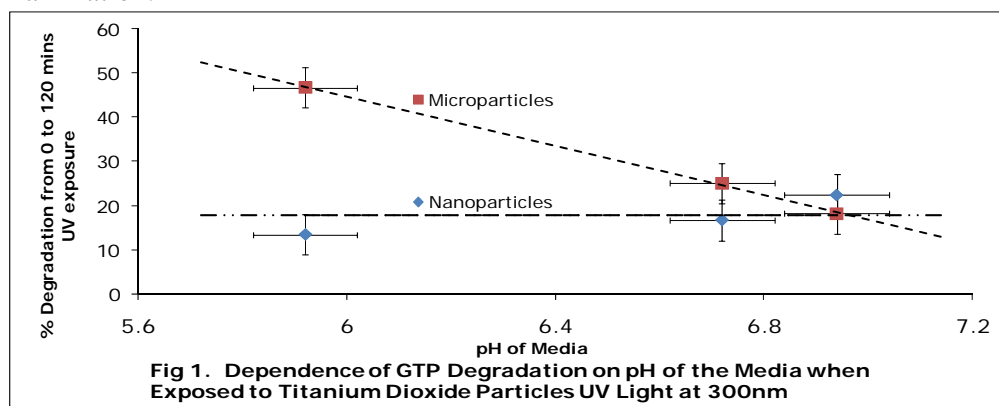


Fig 1. Dependence of GTP Degradation on pH of the Media when Exposed to Titanium Dioxide Particles UV Light at 300nm

¹ Brezova, V., Gabcova, S., Dana, D., & Stasko, A. (2005, February 3). Reactive oxygen species produced upon photoexcitation of sunscreens containing titanium dioxide (an EPR study). *Journal of Photochemistry and Photobiology*, 79, 121-134. Retrieved July/, 2008. doi:10.1016/j.photobiol.2004.12.006

²Zaitseva, I. V. Stability of sunscreen to UV radiation and reduction of photocatalytic activity of titanium dioxide. *Intel Science Talent Search 2005*.

Gold Nanoparticles: Protein Adsorption and Nanoparticle Uptake

Sally Park, Plainview Old-Bethpage JFK High School
Jibrán Durán, Bayside High School

Tatiana Miranova, Stony Brook University

Dr. Miriam Rafailovich, Department of Materials Science and Engineering, Stony Brook University

Nanoparticles are an ideal source of probing cells, which are usually 10 μ m, due to their small size as well as their dependence on size to for physical and chemical properties. [1] In particular, gold nanoparticles have been targeted for research due to their potential utilization in cancer diagnosis and treatment. Gold nanoparticles are toxic to the human cells, but their toxicity differs according to size of the particle. [2] Furthermore, tissue penetration by gold nanoparticles is related to the size of the particle. [3] Although significant amount of research has been performed, much is still unknown about gold nanoparticles and their effect on human cells. This study plans to determine whether protein adsorption is enhanced by presoaking gold nanoparticles in protein (Fetal Bovine Serum) and to find a correlation between apoptosis (cell suicide) and nanoparticle uptake by human dermal fibroblast cells.

To determine the effect of presoaking gold nanoparticles in FBS on protein adsorption, human dermal fibroblasts from CF-29 (Caucasian Female, 29 years) were plated and 250,000 cells were placed in each flask labeled with 15nm presoaked in FBS, 15nm not presoaked + FBS, 45 nm presoaked in FBS, 45nm not presoaked + FBS. Gold nanoparticles were presoaked in FBS for 24 hours and after 3 days of cell culturing, nanoparticles were added to the cells. Then, cells were prepared for counting. Next, a Thermogravimetric analysis (TGA) was completed in order to observe whether presoaking nanoparticles in FBS protein had a significant affect on adsorption. Not enough data was collected from the test to determine whether a significant affect was present or not.

In the second experiment, much of the same procedure will be followed except different nanoparticle concentrations will be added to cells after 24 hours of plating. Furthermore, the flasks will be labeled 15 nm 100 μ L/1mL, 15nm 150 μ L/1mL, 15nm 200 μ L/1mL, 45nm 100 μ L/1mL, 45nm 150 μ L/1mL, and 45nm 200 μ L/1mL. TGA will also be performed on these samples. Ideal results would illustrate apoptosis increasing as size and concentration of nanoparticles increase.

Future work involves repeating each experiment a minimum of three times each in order to make them more valid and accurate. Also, we hope to utilize different proteins with varying lengths to see the differences in the nanoparticles' coating and its effects on the cells.

Confocal Microscopy – Toxicity of Nanoparticles

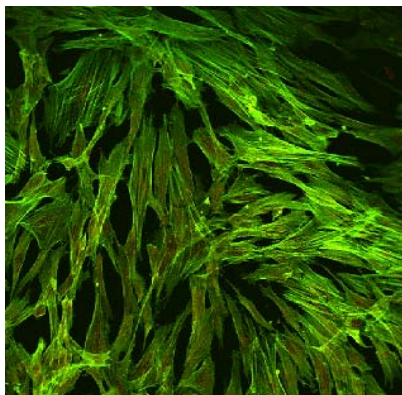


Fig. 1: Control (no nanoparticles)

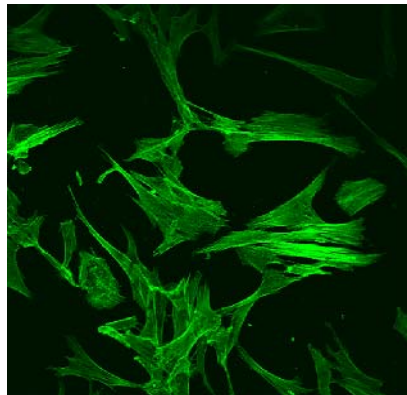


Fig. 2: 94.84 mg/ml 15nm AuNP

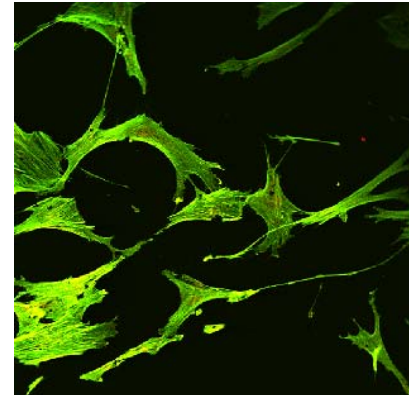


Fig. 3: 13.03 mg/ml 45nm AuNP

**pictures obtained from Tatiana Mironava (mentor)

[1] Salata, OV.(2004). Applications of nanoparticles in biology and medicine. *Journal of Nanobiotechnology*.

[2] Pitsillides, et al.(2003). Selective Targeting with Light-Absorbing Microparticles and Nanoparticles. *Biophysical Journal*, 4023-4032.

[3] Sokolov, et al. (2003) Real-Time Vital Optical Imaging of Precancer Using Anti-Epidermal Growth Factor Receptor Antibodies Conjugated to Gold Nanoparticles. *Cancer Research*.

The Effects of TiO₂ and ZnO Nanoparticles on Chemically-Induced Dental Pulp Stem Cells and MC-3T3 Osteoblasts

Rida Malick and Paul Masih Das, Lawrence High School
Nicholas Mulchan, Plainview-Old Bethpage John F. Kennedy High School
Simon Chang, Ruby Yen, and Miriam Rafailovich, Stony Brook University
Vladimir Jurukovski, Suffolk Community College

Titanium dioxide and zinc oxide nanoparticles are found in many skin care products and sunscreens. These nanoparticles are also prominent in the dental application area, found in toothpaste and temporary dental filling materials. However, titanium dioxide and zinc oxide nanoparticles have been shown to cause adverse effects, including oxidative stress-mediated toxicity and DNA damage¹, on different cell types. These nanoparticles have been classified as “possibly” carcinogenic to humans and further research needs to be done to validate this concern.

This project includes testing nanoparticles on two types of cells: MC-3T3 osteoblasts and dental pulp stem cells. MC-3T3 osteoblasts are used to grow keratinocytes, found in the epidermis of the skin. Dental pulp stem cells have two principal properties: they are self-renewable and have the ability to differentiate into many cell types². This project is focusing on the differentiation and biomineralization of dental pulp stem cells into osteoblasts, cells responsible for bone formation.

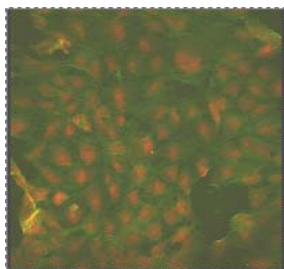
Nanoparticles were tested on MC-3T3 osteoblasts first. The cells were exposed to titanium dioxide and zinc oxide nanoparticles at three different concentrations: 0.01, 0.1, and 1.0 mg/ml. Some MC-3T3 cells were also grown without nanoparticles for comparison. Cells were counted on the second, fifth, seventh, and ninth day after being exposure to nanoparticles. A cell growth curve was then formulated to determine how the nanoparticles affected the proliferation of the MC-3T3 osteoblasts. Cells were also imaged with the confocal microscope after six days. Before imaging, cells were stained with alexa fluor phalloidin, which made actin fibers green, and propidium iodide, which made nuclei red.

It was found that nanoparticles generally had negative effects on the cells. As the concentration of nanoparticles increased, more harm was done to the cells, shown in damage to actin fibers. This prevents the cells from reproducing as well. Cells with nanoparticle concentrations of 1.0 mg/ml died after five days and, therefore, could not be imaged. See Figures 1 and 2 below.

Data shows titanium dioxide and zinc oxide nanoparticle toxicity to MC-3T3 osteoblasts. However, more trials need to be done to confirm the results. Dental pulp stem cells are currently being exposed to nanoparticles and confocal microscopy is to be done on the cells. If titanium dioxide and zinc oxide nanoparticles do have negative effects on the dental pulp stem cells, then new precautions need to be taken with dental products containing these nanoparticles.

Figure 1: TiO₂ Confocal Images

0.01 mg/ml 20X



0.1 mg/ml 20X

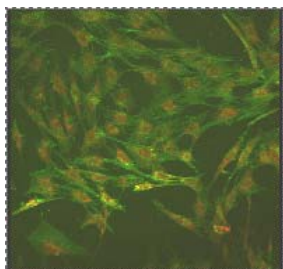
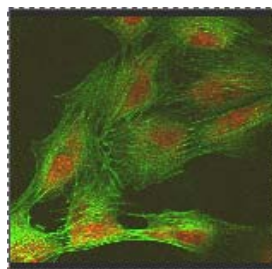
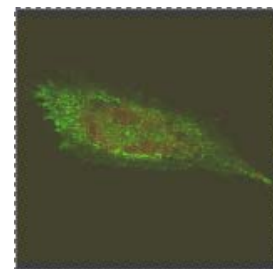


Figure 2: ZnO Confocal Images

0.01 mg/ml 63X



0.01 mg/ml 63X



Figures 1 and 2: As the concentrations of nanoparticles increased, the cell count decreased and more damage was done to the cells.

¹Chung, Hai Won et. al (2008) Titanium Dioxide Nanoparticles Trigger p53-Mediated Damage Response in Peripheral Blood Lymphocytes *Environmental and Molecular Mutagenesis* 49:399-405

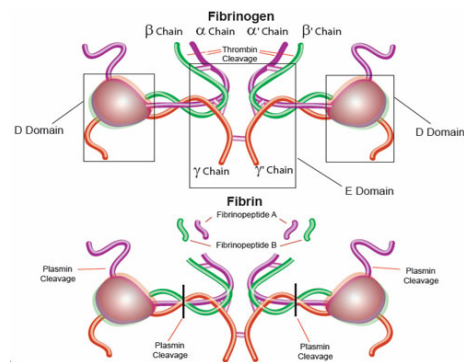
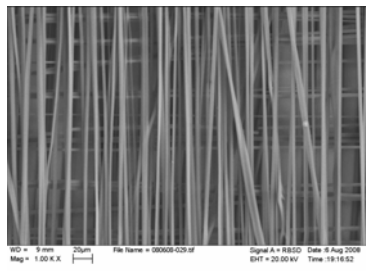
²Gronthos, S. et. al. (2002) Stem Cell Properties of Human Dental Pulp Stem Cells *J Dent Res* 81(8):531-535

Session IX

Nanoparticle Toxicity

Chair: Kate Dorst
Aryeh Sokolov

Garcia Staff: Yitzi Meng,
Chung Chueh (Simon) Chang
Wanji (Ruby) Yen
Lourdes Collazo





RESEARCH SCHOLARS PROGRAM 2008

This Program is sponsored in part
by the National Science Foundation



Biom mineralization on Sulfonated Polystyrene Surfaces for Bone Tissue Engineering

Lara Fourman¹, Lourdes Collazo², Miriam Rafailovich², Yizhi Meng³, Kate Dorst², Aryeh Sokolov⁴

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²Department of Material Science & Engineering, Stony Brook University, Stony Brook, NY

³Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY

⁴Queens College, Queens, NY

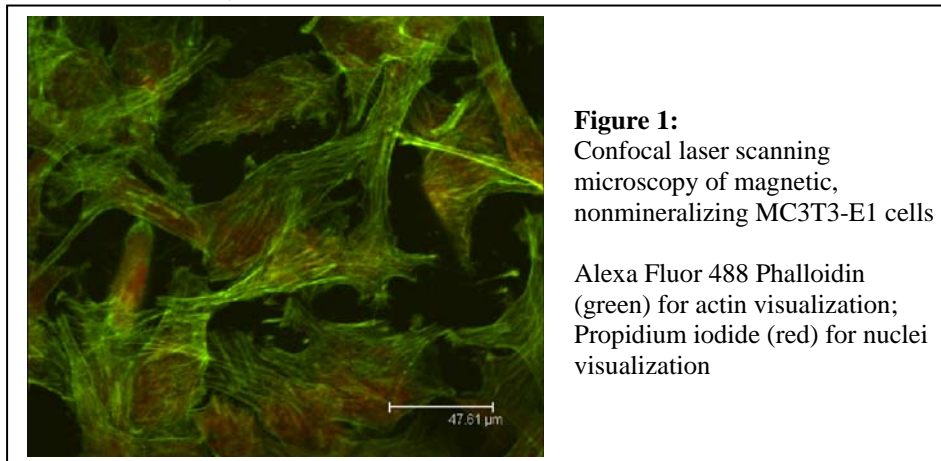
Understanding biomineralization in the extracellular matrix (ECM) of osteoblasts can elucidate bone formation and lead to the development of a successfully engineered bone tissue scaffold.¹ This study aims to investigate biomineralization on sulfonated polystyrene surfaces plated with MC3T3-E1, mouse osteoblastic, cells, and how such mineralization is affected by the presence of magnetic fields. It also examines how fibronectin, which can self-organize on a charged surface², is affected by changes in surface resistivity and magnetic fields.

Before characterizing mineralization of osteoblasts and their ECM, a substrate that induces protein adsorption and network formation must be synthesized for ECM development. It was recently demonstrated that fibronectin, an essential protein in the cell ECM, can be induced to self-assemble into a fibrillar network on a densely charged surface, such as sulfonated polystyrene (SPS). The morphologic characteristics of these fibronectin fibrillar structures greatly resemble those assembled on cell surfaces *in vitro*.²

Unlike Pernodet et al.², who changed the charge of the SPS, my research tests how fibronectin formation is affected by the resistivity of the silicon wafer and presence of a magnetic field. After four days of incubation, the fibronectin surfaces will be examined under the optical microscope and atomic force microscope (AFM). The AFM can image and measure the thickness of the adsorbed protein layer. The bicinchoninic acid (BCA) protein assay reagent will also be used to determine the quantity of adsorbed protein on the silicon surfaces. Subburaman et al. has demonstrated that these self-assembled fibronectin networks can undergo calcium carbonate mineralization.³

After synthesizing this SPS surface, the mineralization properties of MC3T3-E1 cells can be investigated. It has been shown that bone mineralization can be enhanced by electric stimulation. Menéndez reasons that, in the presence of a magnetic field, ionic calcium (Ca^{2+}) movement would increase in frequency and energy of collisions between ionic particles and the cell membrane, along with being directed in only one direction. This Ca^{2+} movement is one of the main actions that would explain this enhanced bone mineralization under magnetic field exposure.⁴

Cells were maintained in two types of media, one that induces mineralization and one that does not. Some cells were also exposed to magnetic fields to observe if they would mineralize better under field conditions. The experimental procedure for cell culture is based on the process explained in Meng et al.¹ Cell counts, confocal laser scanning microscopy (Fig. 1), atomic force microscopy, and scanning electron microscopy will be performed in order to understand how biomineralization occurs in the ECM of bone cells. Understanding this process will take us one step closer to creating an engineered bone tissue scaffold to accelerate bone formation and regeneration.



¹Meng, Y., Y.X. Qin, E. DiMasi, X. BA, M. Rafailovich, and N. Pernodet. "Biom mineralization of a Self-Assembled Extracellular Matrix for Bone Tissue Engineering." *Tissue Engineering* 14 (in press).

²Pernodet, N., M. Rafailovich, J. Sokolov, D. Xu, N.L. Yang, and K. McLeod. "Fibronectin fibrillogenesis on sulfonated polystyrene surfaces." *Journal of Biomedical Materials Research* 64A (2003): 684-692.

³Subburaman, K., N. Pernodet, S.Y. Kwak, E. DiMasi, S. Ge, V. Zaitsev, X. Ba, N.L. Yang, and M. Rafailovich. "Templated biomineralization on self-assembled protein fibers." *Proceedings of the National Academy of Sciences* 103 (2006): 14672-14677.

⁴Menéndez, R.G. "Three molecular mechanisms to explain some biological effects of electromagnetic fields and hypogravity." *Medical Hypotheses* 52 (1999): 239-245.

Organization and Biomineralization of Osteoblasts on Superparamagnetic Nanocomposites for Bone Tissue Engineering

Sanchita Singal, *Herricks High School*;

Aryeh Sokolov, *CUNY Queens College*; Nicole Brenner, Lourdes Collazo and Dr. Miriam Rafailovich, *Department of Materials Science and Engineering, Stony Brook University*.

The initiation of bone formation to an intended orientation is a potentially viable clinical remedy for bone disorders¹. Additionally, electromagnetic fields are promising biomedical agents for mending bone fractures and misalignment by enhancing osteoblast activity (De Mattei *et al.*, 1999) and promoting the net flux and uptake of calcium (Ozawa *et al.*, 1998).² Therefore, this investigation aims to further promote the beneficial effects of magnetic fields via a superparamagnetic polymer. In the presence of an external magnetic field, this polymer possesses the ability to stimulate osteoblast cell growth and regulate bone cell alignment, serving as a biocompatible prosthetic technology with the potential to proliferate and regenerate bone cells.

Poly (methyl methacrylate), or PMMA, is an FDA-approved substance for internal use and is currently used as bone cement³. Thus, PMMA was used in the creation of the superparamagnetic polymer—a 90%-10% blend of PMMA-Cloisite20A-Iron—and a 90%-10% polymer blend of PMMA-Cloisite20A Clay as well. Clay was used because it contains an abundance of materials that support cell growth, and the iron induces magnetism within the polymer⁴. Moreover, in order to increase homogeneity, the samples were brabended and molded at 160° C. The magnetic moment of each of the samples was tested

using a Vibrating Sample Magnetometer, the results of which indicated that the PMMA-Cloisite 20A Clay had a high magnetic moment per emu/mole and exhibited superparamagnetic properties (Figure 1).

Furthermore, cell counts and confocal images were taken on specific days over a one-week period to examine the effects of the three different substrates on osteoblasts. Figure 2 shows the PMMA-Cloisite20A-Iron sample exhibiting the greatest proliferation and an initial form of alignment after seven days of incubation.

At present, a twenty-eight day cell run consisting of cell counting, confocal imaging, and scanning electron microscopy is currently underway. During this trial, the osteoblasts will be exposed to induced and non-induced media in order to determine the most favorable environment for these cells to biomineralize in, thereby allowing them to stiffen into bone tissue. Tests to characterize the quantities and type of proteins released by the osteoblasts will be conducted and the biocompatible viability of the nanocomposite will be further explored.

This work was supported by the Simons Foundation, Garcia MRSEC Polymers at Engineered Surfaces, and the National Science Foundation (NSF).

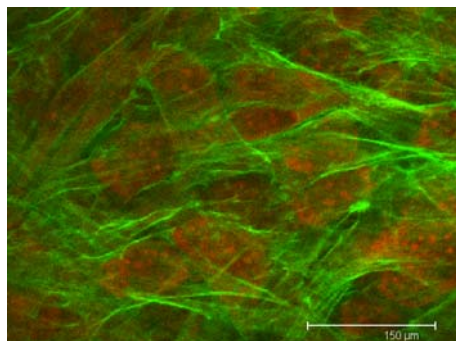
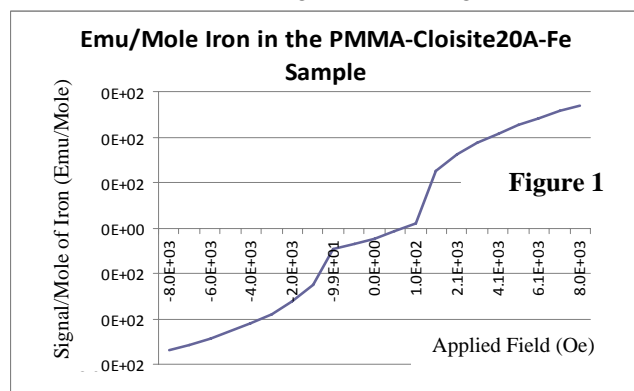


Figure 2. Confocal Microscope Image of the PMMA-Cloisite20A-Fe sample at 63x Magnification. The green dye (Alexa Fluor 488) is indicative of the cell's actin filaments while the red dye (Propidium Iodide) stains for the cell's nucleus.



¹ H. Kotani *et al.* (2002). Strong Static Magnetic Field Stimulates bone Formation to a Definite Orientation In Vitro and In Vivo. *Journal of Bone and Mineral Research*. Volume 17: 1814-1821.

² Y. Yamamoto *et al.* (2003.). Effects of Static Magnetic Fields on Bone Formation in Rat Osteoblast Cultures. *J Dent Res* 82(12): 962-966.

³ HJ Kock *et al.* (2008). In Vitro Studies on Various PMMA Bone Cements: A First Comparison of New Materials for Arthroplasty. *Z Orthop Unfall*. 146(1):108-113.

⁴ Pyun, J. and Krzysztof Matyjaszewski. Synthesis of Nanocomposite Organic/Inorganic Hybrid Materials Using Controlled/"Living" Radical Polymerizations. *Chemical Materials*. 13 (2001): 3436-3448.

The Effects of a Sulfonated Polystyrene Thin Film Scaffold on Human Dental Pulp Stem Cells: Reproduction, Proliferation, and Differentiation

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² Locust Valley High School, 99 Horse Hollow Rd, Locust Valley, New York

³ Stony Brook University

Dental Pulp Stem Cells (DPSCs) are multipotent stem cells known to be able to differentiate into a few types of specialized cells, including osteoblasts, odontoblasts, and endotheliocytes^[1]. As in all fields of tissue engineering, a suitable scaffold that allows optimal growth, reproduction, and proliferation is needed in order for the DPSCs to thrive and differentiate into these cell types. Differentiation occurs as cells respond to the different chemical and geometric properties of these various scaffolds^[3]. Previous studies have shown that porous surfaces with microconcavities are the most effective in inducing osteoblastic differentiation and bone formation (mineralization)^[3].

This study analyzes the growth and differentiation of DPSCs on a thin film scaffolding of the polymer sulfonated polystyrene (SPS). It is hoped and hypothesized that the presence of this polymer scaffold will be a favorable environment for the cells to biomineralize, an early part of the bone-forming process and the differentiation into osteoblasts, even without the presence of chemical agents that induce biomineralization.

DPSCs were plated on 1 cm² pieces of silicon wafer (1, 0, 0) that had been spin-coated with a solution of SPS in dimethylformamide (10 mg/mL), in addition to glass slides, which served as a control. The DPSCs were given either induced medium or non-induced medium. After 2, 5, 7, and 9 days, cells that had been grown on SPS and glass slides, both with non-induced medium, were counted to produce a growth curve using a hemacytometer. After 1, 2, 3, and 4 weeks of growth, cells were analyzed using Atomic Force Microscopy (AFM), Confocal Microscopy, the Ocular Microscope, and Scanning Electron Microscopy (SEM). The AFM was used to determine the modulus or rigidity of our cells and to see the extracellular matrix on the surface of the polymer. The confocal, ocular microscope, and SEM served to analyze the cell bodies and any biomineralization crystals that may have formed.

Below are several images as viewed under the ocular microscope after one week of growth at a magnification of 50x. Figure 1 shows a crystal that was found on the SPS in non-induced medium sample after the cells had died and the sample was dry. Figure 2 shows a crystal that was found on SPS in induced medium. Both images show crystals of what we hope to be calcium phosphate, evidence of biomineralization. Crystals were present on the SPS sample even without the inducing agents, which confirms that SPS can induce biomineralization and differentiation. Further imaging and measurements will confirm this theory.



Figure 1 – crystal on SPS, non-induced

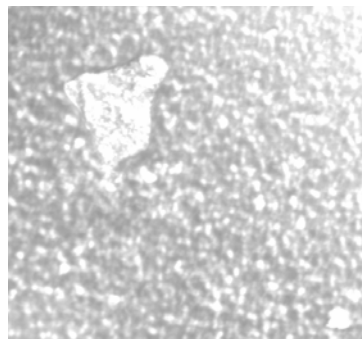


Figure 2 – crystal on SPS, induced

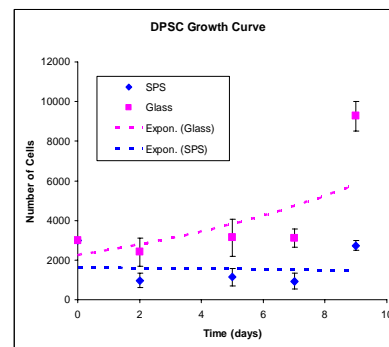


Figure 3 – 9 Day Growth Curve

In addition, Figure 3, above, shows a growth curve. It can be concluded that overall, the sulfonated polystyrene surface stunted cell growth.

We hope to continue our observations on these cells with the AFM, confocal microscope, and SEM to further analyze the biomineralization crystals, and to make more cell counts to give our growth curve more accuracy. In addition, future studies will be conducted on the biomineralization of these cells. We hope to find optimal properties of a sulfonated polystyrene thin film scaffold that will allow for osteoblastic differentiation and eventually, bone formation.

[1]D'Aquino, R., A. Graziano, M. Sampaolesi, G. Laino, G. Pirozzi, A. De Rosa, and G. Papaccio. "Human postnatal dental pulp cells co-differentiate into." *Cell Death and Differentiation* (2007): 1162-171.

[2]El-Backly, Rania M., Ahmed G. Massoud, Azza M. El-Badry, Raef A. Sherif, and Mona K. Marei. "Regeneration of dentine/pulp-like tissue using a dental pulp." *Australian Endodontic Journal* (2008): 52-67.

[3]Graziano, Antonio, Riccardo D'Aquino, Maria Gabriella Cusella-De Angelis, Francesco De Francesco, Antonio Giordano, Gregorio Laino, Adriano Piatelli, Tonino Traini, Alfredo De Rosa, and Gianpaolo Papaccio. "Scaffold's Surface Geometry." *Journal of Cellular Physiology* (2008): 166-72.

[4]Sloan, A. J., and A. J. Smith. "Stem cells and the dental pulp: potential roles in dentine." 2007. 1 Aug. 2008.

[5]Zhang, Weibo, X. Frank Walboomers, Toin H. Van Kuppevelt, Willeke F. Daamen, Philippe A. Van Dame, Zhuan Bian, and John A. Jansen. "In vivo evaluation of human dental pulp stem cells." *Journal of Tissue Engineering and Regenerative Medicine* (2008): 117-25.

[6]Zhang, Weibo, X. Frank Walboomers, Toin H. Van Kuppevelt, Willeke F. Daamen, Zhuan Bian, and John A. Jansen. "The performance of human dental pulp stem cells on different." 11 June 2006. 1 Aug. 2008 <www.sciencedirect.com>.

The effect of collagen on biomineralization through its interaction with fibronectin and elastin

Ariella Kristal¹, Xiaolan Ba², Miriam Rafailovich²

¹*Solomon Schechter High School of Long Island, Glen Cove, New York 11542*

²*Dept. of Materials Science and Engineering, Stony Brook University, Stony Brook, New York 11794*

Biomineralization is the process by which living organisms build inorganic mineral-based structures¹. In the human body, biomineralization is essential to bone formation. By understanding the fundamental mechanics of this process, we will be able to engineer scaffolds to regenerate defective bone tissue. It has been shown that fibronectin and elastin will self-assemble on sulfonated polystyrene (SPS), forming structures similar to the extracellular matrix (ECM),¹ and will induce biomineralization when introduced to a metastable calcium phosphate solution.² We investigated how type I collagen, the most abundant protein in the human body and a main component of the ECM, interacts with fibronectin and elastin during the mineralization process. We examined the in vitro mineralization process of collagen, collagen and fibronectin, and collagen and elastin on an SPS surface through several procedures: Atomic force microscopy (AFM) was used to measure the surface morphology, shear modulation force microscopy (SMFM) was used to characterize the modulus both on and off the fibers, and scanning electron microscopy (SEM) was used to reveal crystal nucleation on the protein matrices. Early-stage mineralization was monitored and revealed that fiber height increased the greatest amount in a three hour time period on the collagen-fibronectin sample. The idea that fibronectin increases the rate of mineralization was corroborated with data from SMFM which revealed the greatest modulus increase. After seven days, SEM images showed that collagen didn't template any crystals on the surface (Figure 1), amorphous calcium-containing minerals covered the fibers on collagen matrix combined with fibronectin (Figure 2), and nano-size spherical crystals formed on the fiber crossing of the collagen-elastin matrix (Figure 3). We will continue to study the changing surface morphology, and modulus characterization, and crystal nucleation.

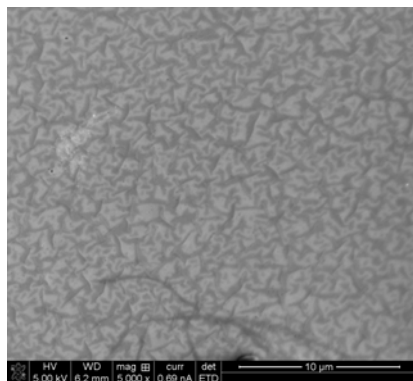


Figure 1: Collagen Control – Day 7

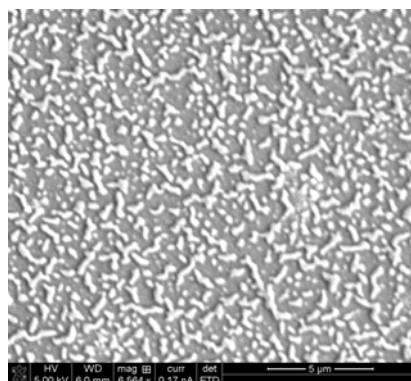


Figure 2: Collagen-Fibronectin – Day 7

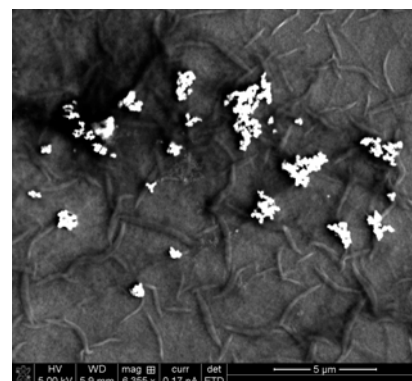


Figure 3: Collagen-Elastin – Day 7


¹ K. Subburaman, N. Pernodet, S. Y. Kwak, et al. (2006) *Proc Natl Acad Sci USA* 103 (40):14672-77

² X. Ba, Y. Meng, Y. Huang, et al. (2008) *Key Engineering Materials* 361-363: 427-430

Summer Scholar Program Schedule of Activities

EVERY DAY STARTS WITH A GROUP MEETING IN HEAVY ENGINEERING!

CHECK SCHEDULE DAILY!

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
Week of 6/26	<p>30</p> <p>10:00 AM Group Meeting <i>Welcome; Attendance; Phone Numbers: young Scholars; IDs and Parking, Lourdes Collazo</i> <i>Welcome; Lab Rules & Regulations; Communication,</i></p> <p>10:30 AM "Skin Bank" Marcia Simon</p> <p>11:30AM "Competitions" Allen Sachs</p> <p>12:00 PM Lunch</p> <p>1:30 PM "Mitigating Global Warming with Renewable Fuels" <i>Devinder Mahajan</i></p> <p>2:30 PM ID Cards and Campus Tours</p>	<p>1</p> <p>10:00 AM Group Meeting <i>Lab Notebooks, Miriam Rafailovich</i></p> <p>10:30 AM "Energy Jobs" Jim Smith</p> <p>11:30AM "Patents & I.P." Donna Tumminello</p> <p>12:00PM Lunch</p> <p>1:30 PM EH&S Training Wang Center</p> <p>2:30PM ID Cards & Campus Tours</p>	<p>2</p> <p>10:00 AM Group Meeting</p> <p>10:30 AM <i>Thinking Outside the Box: The Fun and Challenge of Doing Transitional Research,</i> Srinivas Pentylala</p> <p>11:30 AM <i>Distribution of Lab Boxes, Storage, Etc</i></p> <p>12:30 PM Lunch</p> <p>1:30 PM "Chemical Disposal" Vladimir Zaitsev</p> <p>3:00PM Safety Quiz</p>	<p>3</p> <p>10:00 AM Group Meeting <i>Drug Delivery and Hydrogels,</i> Daniel Cohen</p> <p>10:30 AM "Learning Science Database.; Godlind Johnson; "Excel Tutorial" Vladimir Zaitsev</p> <p>12:30 PM Lunch</p> <p>1:30 PM <i>Early Dismissal</i></p>	<p>4</p> <p>HAPPY 4TH OF JULY</p> 
Week of 7/7	<p>7</p> <p>10:00 AM Group Meeting</p> <p>10:30 AM "Biomaterialization - nature's nanomaterials, studied by synchrotron x-rays" Elaine Dimas</p> <p>11:30AM Lab Tours</p> <p>12:00PM Lunch</p> <p>1:30 PM "Laser experiment & Elypsometer" Dr Chunhua Li</p>	<p>8</p> <p>10:00 AM Group Meeting</p> <p>10:30 AM "Polymers" Dilip Gersappe</p> <p>11:30AM "Spin-Casting Nanocomposite Films." Steve Schwarz</p> <p>12:00PM Lunch</p> <p>1:30 PM Spinning</p>	<p>9</p> <p>10:00 AM Group Meeting</p> <p>10:30 "Diatoms & Coccoliths in the Ocean: From Biomineralization to Global Warming" Cindy Lee</p> <p>11:30AM "Statistics" Miriam Rafailovich</p> <p>12:00PM Lunch</p> <p>1:30 PM Spinning</p>	<p>10</p> <p>10:00 AM Group Meeting</p> <p>10:30 Israel as a playground for exotic viruses Dr. Yehuda Stram,</p> <p>11:30AM Spinning</p> <p>12:00PM Lunch</p> <p>1:30 PM Spinning</p>	<p>11</p> <p>10:00 AM Group Meeting</p> <p><i>Differentiation of Stem Cells Driven by the Mechanics of the Substrate "</i> Vladimir Jurokovski</p> <p>12:00 AM Lunch</p> <p>12:45 PM <i>Journal Club</i></p>
Week of 7/14	<p>14</p> <p>10:00 AM Group Meeting</p> <p>10:30 AM "Statistics" Miriam Rafailovich</p> <p>11:30AM "Introduction into Electron Microscopy research in CMPMS Department of BNL" Steve Volkov</p> <p>12:30 PM Lunch</p> <p>1:15 PM <i>Journal Club & Project Distribution</i></p>	<p>15</p> <p>10:00 AM Group Meeting</p> <p>10:30 AM "The Brave New World of Genetic Engineering" Michael Hadjiargyrou</p> <p>11:30 PM "What to theorists do for a living?" Dilip Gersappe</p> <p>12:30 PM Lunch</p> <p>1:30 PM project distribution, start work</p>	<p>16</p> <p>10:00 AM General Meeting</p> <p>10:30 AM "Bioengineering" Mahn-won kim</p> <p>11:30 AM "DNA" Jonathan Sokolov</p> <p>12:30 PM Lunch</p> <p>1:30 PM project distribution, start work</p>	<p>17</p> <p>10:00 General Meeting</p> <p>10:30 AM Allen Sachs "Research Ethics"</p> <p>11:30 PM start work</p> <p>12:30 PM Lunch</p> <p>1:30 PM ,start work</p>	<p>18</p> <p>10:00 AM General Meeting</p> <p>10:30AM Student Presentations</p> <p>12:00 PM Journal Club</p>
Week of 7/21	<p>21</p> <p>10:00 AM Group Meeting</p> <p>10:30 AM "Statistics" Miriam Rafailovich</p> <p>11:30 PM lab work</p> <p>12:30 PM Lunch</p> <p>1:30 PM lab work</p>	<p>22</p> <p>10:00 AM General Meeting</p> <p>10:30AM Brookhaven National lab tour</p>	<p>23</p> <p>10:00 AM General Meeting</p> <p>11:30 PM lab work</p> <p>12:30 PM Lunch</p> <p>1:30 PM lab work</p>	<p>24</p> <p>10:00 AM General Meeting</p> <p>11:30 PM lab work</p> <p>12:30 PM Lunch</p> <p>1:30 PM lab work</p>	<p>25</p> <p>10:00 AM General Meeting</p> <p>10:30AM Student Presentations</p> <p>12:00 PM Journal Club</p>

<p>Week of 7/28</p>	<p>28</p> <p>10:00 AM General Meeting Collect statistics homework assignment</p> <p>11:00 AM Work in Labs</p>	<p>29</p> <p>10:00 AM General Meeting Assignment of Presenters for Friday</p> <p>11:00 AM Work in Labs</p>	<p>30</p> <p>10:00 AM General Meeting <i>Meeting, Lab, and Equipment Rules</i></p> <p>11:00 AM Work in Labs</p>	<p>31</p> <p>10:00 AM General Meeting</p> <p>11:00 AM Night Fishing</p>	<p>1</p> <p>10:00 AM General Meeting</p> <p>11:00 AM Work in Labs</p> <p>Journal Club</p>
<p>Week of 8/04</p>	<p>4</p> <p>10:00 AM General Meeting</p> <p>11:00 AM Work in Labs</p>	<p>5</p> <p>10:00 AM General Meeting</p> <p>11:00 AM Work in Labs</p>	<p>6</p> <p>10:00 AM General Meeting</p> <p>11:00 AM Work in Labs</p>	<p>7</p> <p>10:00 AM General Meeting</p> <p>11:00 AM Work in Labs</p>	<p>8</p> <p>10:00 AM General Meeting</p> <p>11:00 AM Work In Labs</p>
<p>Week of 8/8</p>	<p>11</p> <p>10:00 AM General Meeting</p> <p>11:00 AM Work in labs Prepare for Symposium</p>	<p>12</p> <p>10:00 AM General Meeting</p> <p>11:00 AM Work in labs Prepare for Symposium</p>	<p>13</p> <p>10:00 AM General Meeting</p> <p>11:00 AM Work in labs Prepare for Symposium</p>	<p>14</p> <p>10:00 AM General Meeting</p> <p>11:00 AM Work in labs Prepare for Symposium</p>	<p>15</p> <p>End of Summer Research Presentations SAC Ballroom A 10 am- 3pm</p>

ACKNOWLEDGEMENTS

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