

Tuesday Afternoon Poster Sessions, December 13, 2016

Biomaterial Surfaces & Interfaces

Room Mauka - Session BI-TuP

Biomaterial Surfaces & Interfaces Poster Session

BI-TuP1 A Real Time Observation of Nano/Bio Interface of Protein Nanoparticles Using Graphene Liquid Cell Electron Microscopy, *Tolou Shokuhfar*, University of Illinois at Chicago, USA

Investigation of crystalline structure and chemistry of biomaterials including biopolymers, bacteria cultures and proteins has always been a great interest for both materials and biomedical communities. The difficulties associated with the imaging of biological structures can be listed as follows: 1) If the imaging is conventional in Scanning / Transmission Electron Microscope (S/TEM), all liquid phase will be evaporated in the vacuum environment when inserted into the microscope, otherwise room temperature fixation, dehydration, infiltration, fixation, embedding and staining should be applied, but in this case, the sample will go through several chemical and thermal processes which involves loss of liquid phase and artificial contrast improvement through preferential staining so that the sample will not be in its native state. 2) If cryogenic imaging is utilized, the sample will be frozen, so all these steps mentioned in 1 will be eliminated. By lowering diffusion rates at liquid nitrogen temperatures, the electron beam induced sample damage will be less, which is good for imaging, but the other energy activated processes, which again depend on the electron beam-sample interaction, are not possible for these frozen samples to occur. 3) In order to see the dynamic processes, *in situ* fluid cell holders can be used. Even though electron beam related dynamic processes can be visualized with these holders, the sample thickness will be too much including the two silicon nitride windows and the liquid sandwiched in between the windows. 4) To characterize the chemistry and crystal structure knowledge at the native state with the highest resolution, the material needs to be thin and should be sealed/sandwiched in between two single layers of graphene sheets forming graphene liquid cell (GLC), so that the Selected Area Electron Diffraction (SAED) and Electron Energy Loss Spectroscopy (EELS) studies can be carried out in the Cs corrected S/TEMs with the highest resolution available in terms of the crystal structure and chemistry, respectively. We have shown even individual iron ions can be detected in the liquid state when released from the ferritin structures encapsulated in GLC and which proves the necessity of using GLC for the achievement of this sort of resolution [1-2].

[1] T Shokuhfar *et al.*: High resolution electron microscopy and spectroscopy of ferritin in biocompatible liquid cells and graphene sandwiches, *Advanced Materials*, 26, 3410, pp. 3410-3414.

[2] The authors acknowledge funding from the National Science Foundation- CAREER award- Grant No- DMR- 1350734.

BI-TuP2 Combined Effect of Antimicrobial Coatings, Gamma Radiation and Negative Air Ionization with Ozone on *Listeria Innocua*, *Escherichia coli* and mesophilic bacteria on ready-to-eat cauliflower florets, *Monique Lacroix*, INRS-Institut Armand-Frappier, Canada

The objective of this study was to evaluate the effect of an antimicrobial bioactive edible coating on the microbiological quality of ready-to-eat cauliflowers. Combined treatments using antimicrobial coating in combination with a low γ -radiation dose or negative air ionization (NAI) with ozone on the microbiological quality of ready-to-eat cauliflowers was also evaluated. The antimicrobial coating was based on a microemulsion of citrus and lemongrass extract in mixture. The microemulsion was also mixed with a polymeric formulation based on maltodextrin and methylcellulose.

Results showed that each treatment alone was effective on *Listeria innocua*, *Escherichia coli* and mesophilic bacteria.

The antimicrobial coating was able to reduce by 2 Log CFU/gr, the level of *E. coli* and *L. innocua* and by 1.5 log CFU/gr, the level of total mesophilic bacteria. The bioactive coating acts also in synergy with γ -radiation, inducing no bacterial growth of *L. innocua* and *E. coli*, as well as a control of the growth of mesophilic bacteria during 7 days of storage. However, the use of NAI + ozone did not act in synergy with the antimicrobial coating to reduce the level of the pathogens under study. However, storage of coated vegetables under NAI + ozone atmosphere would be a good technique to reduce and control bacterial growth during storage to prevent cross-contamination.

BI-TuP3 Antibacterial Nano-film Fabrication for Ophthalmic Application, *Minwook Chang*, Dongguk university, Republic of Korea; *J. Hong*, Chungang university, Republic of Korea

Super-hydrophilic coatings have been extensively studied because of their diverse applications, especially for anti-bacteria films. Anti-bacterial coatings in biomedical devices need to be durable and bio-compatible, but super-hydrophilic films are commonly very fragile due to a porous structure, which is essential for super-hydrophilic functionality, chemical contamination, and thermal stability. To overcome these drawbacks of antibacterial coatings, we introduced polymeric silsesquioxane into the nano-coating, resulting in superior thermal stability and matrix structure based on siloxane groups. Layer-by-layer assembly was used as a multilayer fabrication method to exquisitely control morphology, thickness, and functionality of the nano-coating, and fabricate suitable structures for super-hydrophilic films through simple dipping and washing steps. Antimicrobial and nanoindentation tests were carried out to demonstrate the successful enhancement in the antibacterial and mechanical properties of the nano-coatings.

BI-TuP4 Micro/Nano-Bioactive Structure of Titanium Implant Surface, *Zhoucheng Wang*, Xiamen University, China

Multi-level micro/nano-structure on titanium surface was constructed by Al₂O₃ sandblasting, acid etching in H₂SO₄ and HCl solution, and then anodizing in HF solution at a constant potential. The biological activities of different treated titanium samples were observed in vitro experiment. Scanning electron microscopy (SEM), [app:ds:Energy] [app:ds:Disperse] [app:ds:Spectroscopy] ([app:ds:EDS]) and X-ray diffraction (XRD) were employed to characterize the morphology, composition and crystalline phase of the different treated titanium samples. The results showed the multi-level micro/nano-composite structure in which approximately consisting of 10~20 μ m diameter hollow by sandblast, 5~8 μ m diameter pit by acid etching and 10 nm diameter nanotexture by anodizing. The results of vitro experiments showed that the multi-level micro/nano-composite structure had better biological activity than the control group. After modification and heating treatment of the surface, the multi-level micro/nano-composite structure sample showed [app:ds:excellent] hydrophilic property and biological activity.

BI-TuP6 Assembly of Proteins and Oriented Purple Membrane on Functionalized Carbon Nanomembranes, *Natalie Frese*, Bielefeld University, Germany; *D. Rhinow*, Max Planck Institute of Biophysics, Germany; *A. Turchanin*, Friedrich Schiller University, Germany; *N. Hampp*, Philipps University, Germany; *A. Götzhäuser*, Bielefeld University, Germany

This presentation is about hybrid structures comprising carbon nanomembrane (CNM) as a functional substrate and oriented assembled purple membranes (PMs). CNMs are monomolecular cross-linked layers of aromatic amphiphilic molecules with lateral dimensions of several square centimeters and a thickness of about 1 nm. PM from Halobacterium salinarum is a membrane consisting of bacteriorhodopsin (BR), which is a light-driven proton pump, and lipids.

CNM has already been successfully tested as a substrate for electron cryo-microscopy of PM. To realize the oriented assembly of PM patches on CNM, we used a PM mutant, which has histidine (HIS) tags selectively on one side of the membrane and a nitroliotriacetic acid (NTA) terminated NBPT-CNM. The functionalized CNM has also been tested with different HIS-tagged proteins.

BI-TuP7 Detection Fecal Occult Blood for Early Colorectal Cancer by TOF-SIMS, *C.C. Yu*, Vanung University, Taiwan, Republic of China; *W.J. Lin*, *S.M. Wang*, Central Police University, Taiwan, Republic of China; *Fu-Der Mai*, Taipei Medical University, Taiwan, Republic of China

Due to a dramatic change in both the lifestyle and eating habits, a steady increase in risk of gastrointestinal diseases every year. Early detections and treatments can reduce the mortality caused by gastrointestinal diseases. Fecal occult blood (FOB) is a small amount of blood in the stool which is invisible to human eye. If blood is detected in FOB, the cause may be gastrointestinal disorders, such as colorectal polyps, colorectal cancer and, etc. Fecal occult blood test (FOBT) is a rapid and non-invasiveness method for early diagnostics about gastrointestinal diseases. In this study, we present an alternative method for FOBT other than the traditional methods by using the time-of-flight secondary ion mass spectrometry (TOF-SIMS). TOF-SIMS can analyze the molecular composition of the sample surface with a simple pre-treatment or even without any pre-treatment. Furthermore this method can avoid the potential pitfalls found in the traditional FOBT, such as the false positive results due to the interfere of

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food and drugs in the chemical method and the false negative responses due to the hemoglobin degradation during the storage in the immunization method. Preliminary results shown that there are significant differences at $m/z = 86$ and $m/z = 184$ for the stool samples with FOB. Currently we test different pre-treatments to achieve consistency and accuracy of the experimental results.

BI-TuP8 Preparation of Water-Based Cationic Polyurethane Dispersion for Antibacterial Applications, *G.H. Wu, Cheng-Tien Hsieh*, National Taiwan University, Taiwan, Republic of China; *S. Hsu*, National Taiwan University, Taiwan, Republic of China

Waterborne biodegradable cationic polyurethane (WCPU) nanoparticle dispersion was synthesized by reaction of polycaprolactone, isophorone diisocyanate, and N-methyldiethanolamine under 75 °C and vigorous stirring under acidic condition. The nanoparticles dispersed in the aqueous medium were uniform with an average size of ~80 nm and a zeta potential of ~60 mV. The WCPU nanoparticle dispersion may be cast into films. The contact angle of the films was ~67° and the zeta potential was ~16 mV. The WCPU nanoparticles demonstrated excellent antibacterial activity against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) (100% inhibition with a contact time of 3 h). Meanwhile, the antibacterial ratio of WCPU films to *E. coli* and *S. aureus* reached 100% after 24 h of contact. Moreover, The WCPU nanoparticles could be used as a transfection reagent without significant toxicity for concentrations less than 1000 mg/mL and showed the ability to condensate plasmid DNA. The transfection efficiency for HEK293T cells was ~60% after 48 h of transfection. Moreover, the WCPU dispersion can be made into microspheres conveniently. The WCPU dispersion prepared in the study has potential antibacterial and biomedical applications.

BI-TuP9 Hydrophilic Itaconic Acid Based Material Coating on Silicone Implants Surface for Inhibition of Capsular Contracture, *M. Birajdar*, Chung Ang University; *YB. Choy*, Seoul National University, Korea; *S. Lee*, CHA; *Hansoo Park*, Chung Ang University

The capsular contracture has been a serious problem for silicone implants, leading to surgical removal or surgical replacement. Recently, the silicone implants were modified with textured surface and coated with biomimetic materials to overcome this complication. In this work, we modified the surface of silicone implants with highly hydrophilic itaconic acid (IA) based materials having antibacterial properties and investigated their effect on inhibition of capsular contracture. Various formulation including IA alone, Oligo IA, IA-gelatin polymers, gelatin particles containing IA were tested. The results confirmed the coating of silicone surface with thin layers of IA based materials with concentration dependent manner. It was also found that the contact angle was decreased in all groups, confirming the increase in hydrophilicity. In vitro cell adhesion and protein adsorption were also tested on the modified silicone and compared to the unmodified silicone. These surface modification of IA based materials could be used as a potential alternative to inhibit the formation of capsular contracture.

BI-TuP10 Photoacoustic Microscopic Imaging with Bone Targeted Near Infrared Fluorophore, *TaeJoong Eom, H.D. Lee*, Gwangju Institute of Science and Technology (GIST), Republic of Korea

In order to obtain 3D bone structure images, we should regard x-ray dose for CT imaging or ultrasound image confusing from other organs. By using a bone-specific near infrared (NIR) fluorophore functionalized by phosphonate groups, we demonstrate photoacoustic microscopy (PAM) system for in-vivo bone imaging with out regarding x-ray dose. The NIR light has certain key advantages for biomedical imaging, including relatively ultra low tissue absorption with red blood cell, reduced scatter, and minimal autofluorescence. Especially, the phosphonated NIR fluorophore targets bone tissue with high specificity and this property is inherent to the chemical structure of the fluorophore. Since the heptamethine indocyanine structure has absorption/emission spectrum at ~780/ ~800 nm and the quantum yield of fluorophore is around 10-20 %, the NIR fluorophore can be applied for PA imaging system. The most of surplus optical energy is transferred to the thermal energy which can generate acoustic signal. For the NIR pulse excitation, we applied a high power pulsed laser, which has output power of 600 mW, pulse duration of 1 ns, and repetition rate of 10 kHz at the center wavelength of 780 nm. The NIR fluorophores were administered intravenously into nude mouse to obtain in-vivo PA bone imaging. The foot and tail bone structures of mouse image were successfully obtained by scanning a PAM system with a single ultrasound transducer.

BI-TuP11 Histological Analysis of Bone Regeneration with Hydroxyapatite Isolated from Two Natural Sources (Gallus Domesticus and Sciaenops Ocellatus) in Bone Defects Induced in Tibiae of Rabbits, *D.I. Balleza-Ovalle*, Universidad Autónoma de Tamaulipas, México; *H. Hernández-Cocolezzi*, Benemérita Universidad Autónoma de Puebla, México; *J.H. Luna-Domínguez, C.A. Luna-Lara, H. Tellez-Jimenez*, Universidad Autónoma de Tamaulipas, México; *E. Águila-Almanza*, Benemérita Universidad Autónoma de Puebla, México

In the field of dentistry, biomaterials that meet the requirements for optimal bone tissue formation have play a vital role for the treatment of bone reabsorption caused by periodontal disease, dental extractions or periapical lesions. Hydroxyapatite (HA) is a biocompatible nonabsorbable material chemically similar to the mineral component of bones and hard tissues, therefore, it can be used as a scaffold. HA can be isolated from natural sources by the thermal calcination method. The aim of this study is to assess the osteoconductive effect of hydroxyapatite isolated from two natural sources (*Gallus domesticus* and *Sciaenops ocellatus*) by histological analysis applied in bone defects induced in tibiae of rabbits over a period of four weeks. In this experimental study, five healthy adult male rabbits of New Zealand strain, weighing approximately 3.5 kilograms, which were kept in cages according to the Mexican Official Standard NOM 062 Z00-1999 for the use of laboratory animals were used. Four defects with a size of 4 mm in diameter and 6 mm of deepness were made in the proximal metaphyseal planodiáfiso of both tibiae and HA was applied to the defect according to the grouping. After four weeks of the surgery, both tibiae were recovered in blocks containing all the graft area for histological analysis. Results: In the hydroxyapatite groups the new bone growth involved an area of 78.53% (*Sciaenops ocellatus*) and 72.23% (*Gallus domesticus*). The control group involved 15.97%. Conclusion: After four weeks, HA groups shown to be osteoinductive agents and they allowed the growth of bone tissue to a higher growth rate and bone of higher quality than in the control group.

BI-TuP12 Cell based Tissue Engineering using Hydrogel Materials, *Kangwon Lee*, Seoul National University, Republic of Korea

Stem cell and growth-factor based therapies hold tremendous promise, but clinical effectiveness has been limited by transplanted cell death, efficacy of growth factors and limited control over cell fate *in vivo*. Macroporous biomaterials have been used in partially circumvent these problems by improving transplanted stem cell survival and controlling phenotype by providing molecular cues. In this presentation, we demonstrated strategies for bone repair. Mechanotransduction pathways have been harnessed to control stem cell behavior by manipulating the elasticity of both porous and non-porous materials. Here, we developed injectable, void-forming hydrogels in which critical biomaterial properties controlling stem cell behavior, including elasticity, could be decoupled from pore formation and cell confinement. Upon controlling of matrix elasticity, bone regeneration could be regulated and optimized. Next, CPC is promising for dental and craniofacial applications due to its ability to be injected or filled into complex-shaped bone defects and molded for esthetics, and its resorbability and replacement by new bone. The objective of this strategy was to investigate bone regeneration via novel macroporous CPC containing absorbable fibers, alginate hydrogel microbeads and growth factors in critical-sized cranial defects in rats. Macroporous CPC scaffolds containing progen, fibers and microbeads with growth factors were investigated in rat cranial defects for the first time and had new bone up to 2-fold that of traditional CPC control at 4 weeks, and 3-fold that of traditional CPC at 24 weeks, and hence may be useful for dental, craniofacial

BI-TuP13 Collagen Fibrils Imaging in Air and in Liquid Using Atomic Force Microscope-Based Fast Nanomechanical Mode, *B. Kim, Mina Hong, G. Pascual, K. Lee*, Park Systems Corporation

Collagen is a protein that provides structure in various connective tissues in animals and can be found in ligaments, tendons, and skin. The characterization of collagen's mechanical properties at nanoscale can potentially reveal significant insights into the causes of macroscale phenomena such as the elasticity of skin and its degradation as we age. One tool that has been used to acquire nanoscale data of collagen is the atomic force microscope (AFM). Conventional AFM techniques based on force-volume spectroscopy have been used to analyze the topography and mechanical properties of collagen. However, these techniques are extremely time-consuming—acquisition of a quantifiable elasticity map can take hours to complete. A new AFM-based nanomechanical mode has been developed to address this drawback and can perform the same task significantly faster without sacrificing resolution. Our investigation revealed

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that our sample collagen bundles had diameters ranging from 60 to 600 nm and an average elastic modulus of about 1.9 GPa, a value in agreement with other reported research. The total time taken to acquire this data was measured in minutes as opposed to hours.

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