Biomaterial Surfaces & Interfaces

Room Naupaka Salon 1-3 - Session BI-TuP

Biomaterial Interfaces Poster Session

Moderator: David Castner, University of Washington

BI-TuP1 Inhibiting Upstream Motility of *Pseudomonas Aeruginosa via* Nanopillared Surface Structuring*, Rachel Rosenzweig,* *V.K. Ly, K. Perinbam, A. Siryaporn, A.F. Yee,* University of California, Irvine

Bacteria often populate environments where fluid flow is present such as the lungs of mammals, vasculatures of plants, industrial transportation fuel lines, and medical devices. *Pseudomonas aeruginosa* is an opportunistic biofilm forming bacterium that exhibits the ability to twitch upstream when surface attached. The upstream movement is facilitated by the retraction and extension of their type iv pili mechanosensor ATPase motors, pilT and pilU, when encountering shear stress. Such motility modalities of *P. aeruginosa* lead to bacterial surface adherence, colonization, and infectious biofilm formation. Here, upstream motility inhibition and surface detachment of *P. aeruginosa* were accomplished on polymeric biomaterial structures with arrays of nanopillared geometries.

Nanopillared surface structures were fabricated using thermal nanoimprint lithography on a synthetic polymer, poly(methyl methacrylate) (PMMA), commonly used in medical devices. The arrays of nanopillars range in periodicities from 200nm, 300nm, 500nm to 600 nm. Upstream motility direction, displacement, velocity, and detachment of wild-type *P. aeruginosa* expressing GFP were monitored in microfluidic flow channels with flat or nanopillared bottom surfaces and quantified using fluorescence microscopy. The cell motility inhibition and detachment under shear stress were observed to have a nanopillar surface area dependence most likely due to decrease in surface mechanosensing capabilities of the type iv pili. This bacteria-nanostructured surface interface phenomenon allows us to tailor surfaces with specific nanopillared geometries for structurally controlling cell motility and detachment under fluid flow. The disruption of surface attached biofilm forming bacterial upstream movement is crucial in preventing harmful infection from contaminated medical devices such as catheters and has broad application in industrial fuel line dependent transportation.

BI-TuP2 Effect of Preheating Treatments on Interfacial Reaction between Dental Porcelain and Low Magnetic Susceptibility Zr–14Nb Alloy*, Atsushi Takaichi,* Tokyo Medical and Dental University, Japan*; Y. Kajima,* Tohoku University, Japan*; H. Doi, T. Hanawa, N. Wakabayashi,* Tokyo Medical and Dental University, Japan

[Objective]

In this study, we focus on using the Zr-14Nb alloy for porcelain-fused-to-metal (PFM) restoration in dental prosthetics, owing to their good mechanical properties and biocompatibility, as well as the low magnetic susceptibility. The interface between the alloy and the porcelain is of critical value in ensuring the long-term integrity of the PFM restoration, thus we investigated the changes at the ceramo-metal interface induced by preheating treatments.

[Methods]

Cylindrical cast specimens of the Zr–14Nb alloy were prepared. After sandblasting with Al_2O_3, the Zr–14Nb samples were subjected to a preheat treatment at 700 °C for 5, 10, or 20 min and those without treatment were taken as control samples. Dental porcelain was veneered on them; then, their bond strength (MPa) was evaluated by performing shear bond tests (n = 8/group) and the results were analyzed using ANOVA and Tukey's tests (p = 0.05). The surface characteristics of the preheated Zr–14Nb specimens were evaluated by scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS), laser microscope, and X-ray diffractometry (XRD). The elemental distribution on the interface between the Zr–14Nb alloy and the porcelain was determined by SEM-EDS. MR images were obtained using 3.0 T MR scanners (MAGNETOM Spectra 3T), and artifacts volume from the specimens were quantified by constructing 3D image.

[Results and Discussion]

The samples subjected to the heat treatment for 5 min showed the highest mean bond strength (43.7±5.9 MPa). On the preheated sample groups, white oxide layers, which were predominantly composed of monoclinic zirconia, were formed, exhibiting a greater roughness than control samples; besides, on the interface of the metal–ceramics, a greater diffusion range of Nb was observed than that found on the control samples, which could contribute to increase the bond strength between porcelain and Zr-Nb alloy. On the other hand, the bond strengths of the samples subjected to 20 min preheating treatment were the lowest (33.6±3.2 MPa), which may be ascribed to the formation of a brittle thick oxide layer under excessive heat treatment.

[Conclusion]

The suitable preheat treatment performed on the Zr–14Nb substrates contributed to the increase in the surface roughness and the diffusion of Nb, which enhanced the micro-retention and chemical bonding and improved the bond strength of Zr–14Nb and porcelain. The Zr-14Nb alloy is a promising candidate for fixed dental prosthesis, as long as the appropriate treatment conditions are adopted.

BI-TuP3 Surface Characteristics and Corrosion Behavior of CoCrMo Alloys Fabricated by Selective Laser Melting after Various Heat Treatments*, Yuka Kajima,* Tohoku University, Japan*; A. Takaichi, T. Oishi, N. Kittikundecha, Y. Tsutsumi,* Tokyo Medical and Dental University, Japan*; N. Nomura,* Tohoku University, Japan*; N. Wakabayashi, T. Hanawa,* Tokyo Medical and Dental University, Japan*; A. Kawasaki,* Tohoku University, Japan

[Objective] Selective laser melting (SLM) has attracted significant attention as an advanced method for fabricating biomedical devices. SLM-manufactured parts easily accumulate large amounts of residual stress due to rapid heating and cooling. Thus, they require a post-fabrication heat treatment to relieve the residual stress. However, the heating process inevitably changes the microstructure of the alloys, which may affect their corrosion behavior. The objective of this study was to assess the morphological characteristics and corrosion properties of SLMed CoCrMo alloys following various heat treatments.

[Methods] Block specimens were prepared using an SLM machine equipped with a fiber laser (EOSINT M280) and commercially available CoCrMo alloy powders (MP1). Specimens were heated to 750 or 1150 °C and held at this temperature for 1 or 6 h in a furnace in an Ar atmosphere. Specific section cuts of XY and YZ planes were used for analyzing microstructures and corrosion resistance. Microstructures were investigated via scanning electron microscopy (SEM), field-emission transmission electron microscopy (FE-TEM), field-emission electron probe microanalysis (FE-EPMA), electron backscattered diffraction (EBSD), and X-ray diffraction (XRD). Additionally, anodic polarization was performed with a potentiostat (HABF-501A) with a function generator (HB-111).

[Results and Discussion] The SEM images showed that fine precipitates were formed within the grains and at the grain boundaries in the specimens heated to 750 °C. On the other hand, after heating to 1150 °C, coarse precipitates, identified as $M_{23}C_6$ by TEM and EPMA analysis, grew along the grain boundaries. Both γ and ε phases formed in all heat-treated specimens, and the volume fraction of the ε phase decreased with increasing heat-treatment temperature and time. In the samples heated to 750 °C, the microstructures exhibit the epitaxial growth of columnar grains with a < 001 > fiber texture along the build direction as well as the as-built state. In samples heated to 1150 °C, defect-free equiaxed grains with random orientations were found, indicating that recrystallization occurred. Considering the anodic polarization curves, the heat treatment process did not greatly affect the corrosion resistance of the SLMed specimens; resistances of all heated samples were comparable to traditional cast samples, with those heated to 750 °C exhibiting the highest corrosion potential. The enhanced corrosion resistance of SLMed CoCrMo alloys provides further support for their use in medical applications.

BI-TuP4 Analysis of Drug Coated Polymer Stents Studied by XPS and Ar_n^+ Sputter Profiling*, David Surman,* Kratos Analytical Inc.*; J. Counsell,* Kratos Analytical Ltd., UK

Cardiovascular interventional therapy with stents has emerged as one of the most effective treatment methods for coronary heart disease, however, thrombosis and hyperplasia are the usual pathological responses to the implantation of foreign devices into the body. Originally stents were made of steel although these have now been superseded by polymer based materials. Recent developments have introduced a new range of stents made from bio-resorbable polymers, however problems such as thrombosis and hyperplasia still remain. To suppress this immune response and that of overgrowth and subsequent restenosis anti-inflammatory drugs are now loaded onto the surface of stent implants.

In this presentation we investigate the surface of drug loaded polymer stents using X-ray photoelectron spectroscopy (XPS) and sputter depth profiling using Ar_n^+ clusters. The stents studied are made of polylactic acid (PLA) dosed with an anti-inflammatory drug with a molecular structure of $C_{51}H_xNO_{13}$. XPS yields quantitative information regarding drug distribution

which is shown to be higher on the abluminal (outer) than the luminal (inner) surfaces of the stent. Combining Argon cluster sputtering with XPS enables the distribution of the drug into the stent structure to also characterised.

Conventional methods to study the effects of aging and drug mobility in stents involve immersion in a buffer solution for varying periods of time. Subsequent analysis of the solution with HPLC determines the extent of drug dissolution from the stent. Although this approach is accurate in determining the amount of drug dissolved, it is still unknown how much drug remains and how it is distributed. This is addressed in this study where the drug distribution for stents immersed in PBS buffer solution for 1-3 months was determined by Ar_n^+ cluster depth profiling of the stents themselves. These results were used to determine the effects on simulated ageing and the propensity for the drug to migrate into the solution with time.

BI-TuP5 Anchored Protease-Activatable Polymersomes for Molecular Diagnostics of Cancer Cells, ***Jong-Woo Lim,*** Yonsei University, Republic of Korea*; H.-O. Kim,* Korea University, Republic of Korea*; J. Choi,* Yonsei University, Republic of Korea*; H. Lee,* Korea Basic Science Institue, Republic of Korea*; H.Y. Son, J. Kim, G. Park, H. Chun,* Yonsei University, Republic of Korea*; D. Song,* Korea University, Republic of Korea*; Y.-M. Huh, S. Haam,* Yonsei University, Republic of Korea

Real-time quantitative and qualitative analyses of metastasis-associated proteases are critical for precise diagnosis and novel therapeutic treatment of advanced cancers. However, conventional methods based on DNA, peptides, and proteins require sophisticated chemistry and additional processes to expose detection moieties, and they lack elements of temporal control, which limit their applicability. We designed unique protease-activatable polymersomes (PeptiSomes) for high sensitivity, in situ quantitative analysis of activating membrane-type 1 matrix metalloproteinases (MT1-MMP, MMP14). To do this, we first synthesized an amphiphilic block polymer–peptide and a copolypeptide based on mPEG -b-pLeu and MT1-peptide-b-pLeu, respectively. Amphiphilic self-assembled PeptiSomes in water were capable of disassembling and releasing the encapsulated self-quenched fluorescence dye (calcein) via enzymatic activation by MT1-MMP. Our PeptiSome system may potentially prevent the initiation and progression of cancer metastasis. Furthermore, the PeptiSome approach described here is likely to facilitate the development of rapid protease assay techniques and further extend the role of proteases as metastasis indicators and therapeutic targets.

BI-TuP6 Study on Meta-material Structure in Oil Repellent Bile Duct Stent, ***Tomoki Nishino,*** Ritsumeikan University, Japan*; H. Tanigawa,* The Research Organization of Science and Technology, Japan*; A. Sekiguchi,* Litho Tech Japan Corporation, Japan*; K. Aikawa,* Saitama Medical University, Japan

The bile duct is a tubular pathway that drains the bile made in the liver into the duodenum. In bile duct cancer, biliary atresia or the like, the bile duct narrows, the flow of bile is inhibited and it becomes difficult for the duodenum to flow from gall bladder to the duodenum. For this reason, bile flows back to the liver, causing a disorder that causes jaundice. Natural progression will result in liver failure, possibly leading to death. As a treatment method, in order to open the obstruction of the bile duct, the endoscope is used to indwell the stent in the bile duct to ensure discharge of the bile into the duodenum. By placing the stent with a metal tube or a resin tube having a mesh-like structure, a path through which bile normally flows is secured.

However, since bile is a viscous liquid containing oil, it is known that the tube clogs by adhesion or gelling in the bile duct stent. Currently, no effective technology has been studied for this problem. When the tube is clogged, there is only a countermeasure therapy to replace the biliary stent by reoperation. If it is possible to reduce clogging of the stent, it is possible to reduce the number of reoperation, which not only reduces the burden on the patient but also leads to a reduction in the burden on the medical field including doctors.

This study is a technology development to reduce clogging of the biliary stent. We introduce a biliary stent combining metamaterial technology that realizes oil repellency and antifouling property which is not realized on the surface of the material prepared. When considering the oil repellency function for oil containing fluid such as bile, we considered the snail shell structure with nano hydrophilic effect to be effective. On the shell surface of the snail, there is a concavo-convex structure of 200 nm to 400 nm, and it is running a dirt by making a thin water film.

The film having a metamaterial structure was produced by a semiconductor fine processing technique. When the produced film was evaluated for oil repellency, it was confirmed that good oil repellency in water was obtained. Therefore, in order to evaluate a bile duct stent with a metamaterial structure, bile ducts were placed in pigs for 7 days. The inner surface of the usual bile duct tube resulted in bile sticking and a lot of contamination, but the bile duct tube of the meta-material structure was a result that the bile was repelled and there was no stain. We present that bile duct with oil repellency and antifouling property is effective as new metamaterial technology.

BI-TuP7 The Blood Cell-nanoparticle Interface: Functional Cellular Responses, Mechanisms of Interaction and Signaling pathways, *C. Brommesson, N. Abrikossova, P. Eriksson, Z. Hu, K. Uvdal,* ***Andreas Skallberg,*** Linköping University, Sweden

The use of nanomaterials in biomedical applications create a large need for studies elucidating potential harmful effects of the materials in living systems. Nanomaterials specifically aimed for *in vivo* applications are sure to encounter all components of blood, directly or indirectly, and increased knowledge of underlying mechanisms at the blood cell-nano interface will be valuable in the further development of these materials.

We have specifically investigated blood platelet and neutrophil granulocyte interaction with several types on nanoparticles. These blood cells are rapidly responding, and potent cells involved the immune response and following inflammatory processes. Using well-defined and characterized NPs we have focused on clarifying the induced functional cellular responses, interaction mechanisms and involved signaling pathways. Our results demonstrate uptake of polymeric (Pdots) and Cerium/Gadolinium based NPs in neutrophils and show that both active and passive uptake processes contribute to the internalization of these particles. In connection, cellular mechanisms underlying our previously described antioxidative properties of cerium containing NPs[1] are further investigated and identified. Nanoparticle induced platelet aggregation and release of inflammatory mediators is also shown herein to be valuable for evaluating NP- blood compatibility.

[1] *P. Eriksson el al. Cerium oxide nanoparticles with antioxidant capabilities and gadolinium integration for MRI contrast enhancement. Scientific Reports 2018; 8:6999*

BI-TuP8 Developing a pH Responsive Hydrogel as an Alternative for Colonoscopy Preparation, ***Phuong Nguyen,*** University of New Mexico*; S. Mounho,* University of Texas at Austin, USA*; D. Cuylear, H. Canavan,* University of New Mexico

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States. The most reliable screening method of CRC is a colonoscopy which requires a 4-Liter poly(ethylene glycol) electrolyte lavage solution (PEG-ELS) for preparation. ~40% of patients are non-compliant to their colonoscopy schedules, with many patients who abstain reporting refusal due to significant discomfort associated with this preparation. Furthermore, there are distinct gender differences in the tolerance of PEG-ELS in male and female populations. We hypothesize the differences in clinic are a result of cytotoxicity effects of PEG. PEG is approved by the FDA for use in medical devices, and has been recognized for many years as a biocompatible/bioinert polymer but few studies have truly studied the short-term and long-term effects of high concentrations of PEG on multiple cell lines. We have developed a pH responsive hydrogel to control the release of PEG – reducing adverse effects associated with colonoscopy preparations. The hydrogels have been characterized using NMR, and XPS to ensure chemical identity, rheometry to assess the stiffness/robustness of the hydrogels in varying environments, and SEM and other techniques to confirm uniformity of size. Biocompatibility testing of exposure to increasing PEG concentrations over a period of 3 hours, 6 hours, 12 hours, 24 hours, and 48 hours shows PEG is biocompatible to intestinal human cell lines in short intervals and low concentrations. Furthermore, at low concentrations PEG increases cell growth and viability as seen in previous studies. At higher concentrations, however, PEG is cytotoxic to cells. Although it would be difficult to get to toxic levels of PEG in the body in a single dose, current uses of PEG in which large, non-uniform, quantities are ingested in a short time frame should be re-evaluated due to possible adverse cumulative effects due to the cytotoxicity effects seen *in vitro.* Further directions of this work will evaluate the pH responsiveness of our hydrogel formulation to deliver PEG *in vitro* and *in vivo,* and assessment of the cellular response to the hydrogels using mammalian cells specific to the gastrointestinal system of humans, as well as imaging analysis to envision their penetration.

BI-TuP9 Atmospheric Pressure Mass Spectrometric Imaging of Live Tissue Specimen using Electrospray assisted CW Laser Desorption and Ionization Source, ***Jae Young Kim***, Daegu Gyeongbuk Institute of Science & Technology; *S.Y. Lee, M.H. Shin,* Daegu Gyeongbuk Institute of Science & Technology, Korea; *D.W. Moon,* Daegu Gyeongbuk Institute of Science & Technology, Republic of Korea

Although atmospheric pressure mass spectrometry (AP-MS) is a promising analytic technique for biological samples because of its ambient analytic process and no or minimal sample pretreatment, still many challenges must be overcome to acquire MS data from them. Because mass spectrometry is a quantitative technique for the measurement of the masses of the charged molecules that comprise a sample of material, small but actual parts should be severed from the sample. Thus, desorption and ionization methods for small spot sampling to the biological sample are the most important technique to minimize the sample damage and to obtain high resolution MS imaging. Currently many ambient desorption/ionization sources except lasers are working in ionized gaseous state or spray state of charged droplet, reducing the size of source devices is limited.

We use electrospray ionization source and visible continuous wave (CW) lasers as AP-sampling/ionization sources and develop/combine these electrospray and lasers for the purpose of mass spectrometry applications. The energetic light generated by CW lasers focuses on a very small spot of the sample through the objective lens. At same time, the electrospray device forms the charged droplet particles on the sample. Regarding desorption and ionization procedures in open air, CW lasers mainly desorb actual parts from the sample and electrospays mainly ionize these small substances.

An inverted optical microscope was used as a sampling stage and the additional pumping system including ion transfer tubing, chamber, and dry pump known as air flow assisted ion transfer equipment was installed with the MS inlet. Therefore an AP-MS system is developed and preliminary MS data are achieved with a help of gold nanoparticles. With an addition of the two-dimensional programmed scanning stage to this ambient MS system, many bio-molecular mass spectrometric imaging were obtained from the mouse hippocampal tissues. High spatial resolution MS imaging with a pixel size of four micrometer can be secured at a sample moving velocity of 30 µm /s. MS imaging of bio-molecules including monoacylglycerols, cholesterols, fragments of sphingolipids and glycerophospholipids has been obtained from mouse hippocampal tissues.

BI-TuP10 Improvement of Cell Imaging by Graphene Encapsulation in ToF-SIMS Method, ***Sun Young Lee***, Daegu Gyeongbuk Institute of Science & Technology, Korea; *H.J. Lim, J.Y. Kim,* Daegu Gyeongbuk Institute of Science & Technology; *D.W. Moon,* Daegu Gyeongbuk Institute of Science & Technology, Republic of Korea

For last decades, ToF-SIMS has been in use in bio sample imaging and has resulted in some important bio issue discoveries. SIMS analysis requires ultrahigh vacuum environment and analysis in atmospheric not allowed. Its ultra-high vacuum based operation necessitates proper sample preparations ranging from simple ones like washing and drying to relatively sophisticated as frozen hydration.[1] Therefore, any of them can neither represent the biologically native state nor preserve membrane integrity. So we have developed a new sample preparation using wet cells covered by single layer graphene for hydrated and native state bio sample.

Graphene acts as a gas impermeable honeycomb mesh on cells preventing solution evaporation and keeping cells wet even under ultra-high vacuum. Besides, the high electrical conductivity of graphene compensates the charging effects during analysis. ToF-SIMS images in this study were obtained using ToF-SIMS 5 (ION-TOF GmbH) equipped with liquid metal ion gun (LMIG) [2,3] and analyzed both positive and negative ion modes and about 1000 specimen-related spectra were obtained from A549 lung carcinoma cell. Thus, we obtain various lipid SIMS images from cell membrane through a graphene layer without cracking.

Major limitations of applying mass spectrometry imaging techniques such as SIMS to biomedical researches are due to harsh bio-sample preparation including freezing, matrix addition and drying. The possibility that secondary molecular ions can be sputtered through a single layer graphene from wet cells will open innovative applications of mass spectrometry imaging of wet cells to various biomedical research areas.

[1] K. Schaepe, J. Kokesch-Himmelreich, et al, Biointerphases 10.1 (2015): 019016

[2] Vanbellingen, Q. P.; Elie, N.; Eller, M. J.; Della-Negra, S.; Touboul, D.; Brunelle, A. Rapid Commun. Mass Spectrom, 29, 1187-1195 (2015)

[3] Shon, H. K.; Yoon, S.; Moon, J. H.; Lee, T. G. Biointerphases, 11, 02A321 (2016)

BI-TuP11 Behavior of Shewanella Oneidensis MR-1 in a Sulfur and Zinc-Rich Medium and its Applications for Biosensing and Biomaterials, ***James Rees***, *S. Sawyer, Y. Gorby,* Rensselaer Polytechnic Institute

An increasing focus of current microbiology research is the fact that some strains of bacteria, called dissimilatory metal-reducing bacteria (DMRB), are capable of utilizing certain metallic ions as terminal electron acceptors in their metabolic processes. One strain in particular, Shewanella oneidensis MR-1, can reduce ions of iron, lead, arsenic, and uranium, among others. Under anaerobic conditions it has also been shown to reduce sulfur compounds, nitrates, and chromates. The cultivation of DMRB under controlled conditions therefore has significant implications for the low-energy, room-temperature synthesis of metal sulfide and/or metal oxide semiconductors. Furthermore, Shewanella and other DMRB can form biofilms that interact electronically with solid-phase minerals in their environment. For this reason there exists a potential to grow DMRB directly into porous substrates in order to create biosensors that are capable of producing electrical signals that provide information about metal ion concentration in water as well as a range of other water quality variables.

I will highlight my recent work exploring the behavior of Shewanella oneidensis MR-1, in a medium rich in both zinc and thiosulfate ions. I have grown Shewanella bacteria in a three-electrode system and used a potentiostat to hold the system at a fixed DC voltage during cultivation while also measuring current output. After completing the cultivation step, I have used cyclic voltammetry and electrochemical impedance spectroscopy to characterize the DC and AC current-voltage dynamics of the system, which can reveal the reduction-oxidation activity of key bacterial proteins. In the second experiment, I have grown Shewanella bacteria under both aerobic and anaerobic conditions in media rich in zinc and thiosulfate ions and used scanning electron microscopy and energy dispersive microscopy to characterize the minerals that precipitate within the batches. I compare results from a minimal medium containing only the zinc and thiosulfate sources to a more traditional Shewanella medium containing various vitamins, minerals and other nutrients to support growth. I also compare the inoculated batches to sterile control batches containing no bacteria in order to infer the effect that the bacteria have on mineralization in their environment. Finally, I use confocal microscopy to explore the fluorescence behavior of the precipitates generated in both inoculated and sterile batches.

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