

## Biomaterial Surfaces & Interfaces

### Room Naupaka Salon 1-3 - Session BI-TuP

#### Biomaterial Interfaces Poster Session

**BI-TuP-1 Cell-Mimetic Biosensors for Detecting Avian Influenza Virus Through the Viral-Fusion Mechanism, Jong-Woo Lim**, College of Veterinary Medicine, Research Institute for Veterinary Science, Seoul National University, Republic of Korea; *G. Park, C. Park*, Department of Chemical and Biomolecular Engineering, Yonsei University, Republic of Korea; *M. Yeom*, College of Veterinary Medicine, Research Institute for Veterinary Science, Seoul National University, Republic of Korea; *S. Lee*, Department of Chemical and Biomolecular Engineering, Yonsei University, Republic of Korea; *K. Lyoo*, College of Veterinary Medicine, Jeonbuk National University, Republic of Korea; *S. Haam*, Department of Chemical and Biomolecular Engineering, Yonsei University, Republic of Korea; *D. Song*, College of Veterinary Medicine, Research Institute for Veterinary Science, Seoul National University, Republic of Korea

Avian influenza virus (AIV) is a major respiratory disease of poultry, especially High pathogenic AIV (HPAIV) presents high morbidity and mortality. To control the spread of HPAIV, it is very important to detect HPAIV at the early stages for taking timely countermeasures such as quarantine and isolation. In this study, we developed cell mimicking nanoparticles (CMPs) for rapid detection of HPAIV as well as low pathogenic AIV (LPAIV) via a viral fusion mechanism. CMPs were polymeric nanoparticles constructed with sialic acid and FRET dye pairs, exposing FRET off signal responding to membrane fusion with AIV activated by enzymatic cleavage at endosomal condition. CMPs exhibited highly sensitive detection of various types of LPAIVs and HPAIVs in the biological environment. The developed detection system utilizing the viral infection pathway can be a potent diagnostic assay for LPAIV and HPAIV, contributing to minimizing the economic loss of the viral outbreaks.

**BI-TuP-2 Using Cinnamaldehyde Plasma Treatment to Develop an Antioxidant Coating, Ashley N. Keobounnam, M. Hawker**, California State University, Fresno

When a burn injury occurs, the heat damage caused by said burn increases the permeability of the microvasculature. This increased permeability leads to the leakage of plasma into the interstitial spaces of cells, which can lead to hypovolemic shock. One approach to reduce the likelihood of hypovolemic shock is to reduce microvascular permeability. Oxidants are an agonist that increase post-burn permeability through increasing the concentration of reactive oxygen species, ultimately leading to oxidative stress. Microvascular permeability remains in an increased state so long as the rate of reactive oxygen species generation is greater than the body's rate of detoxifying. One strategy to inhibit the effects of oxidative stress is to introduce antioxidants directly to the burn site. Current studies regarding burn wounds focus on the healing process after the initial injury. However, methods to stabilize the patient before significant plasma leakage occurs remain relatively unexplored.

This work focused on developing a burn wound patch with an antioxidant coating towards the goal of reducing microvascular permeability. We opted to use a plasma enhanced chemical vapor deposition (PECVD) strategy to deposit an antioxidant coating on a model polymer substrate (chitosan). Specifically, we utilized cinnamaldehyde - an organic compound with antioxidant activity - as a plasma precursor. Chitosan substrates were modified using optimized plasma parameters, where both pulsing and continuous plasma power conditions were explored. In previous studies, continuous PECVD films exhibited limited monomer functional group retention, whereas pulsed PECVD films exhibited greater monomer functional group retention. Antioxidant activity relies heavily on chemical structure, therefore, we explored how plasma power conditions (continuous vs pulsed) impact antioxidant effectiveness. Surface wettability was analyzed using water contact angle goniometry. X-ray photoelectron spectroscopy was used to characterize surface chemistry. Additionally, radical scavenging activity of PECVD treated materials was evaluated using a 2,2-diphenyl-1-picrylhydrazyl assay. Overall, this work opens new directions for antioxidant therapy in burns.

**BI-TuP-3 Modifying Commercially-Available Wound Dressing Materials with Continuous and Pulsed 1,8-Cineole Plasma, Mia Rose Kayaian, M. Hawker**, California State University, Fresno

Hospital-acquired infections (HAIs) are one of the primary causes of death. According to Medicare data, HAIs are estimated to cost the United States anywhere from 28.1 to 96.8 billion dollars yearly. Most HAIs begin with bacterial attachment to the wound. If left untreated, the infection site

can become a chronic wound. The clinical standard for existing chronic wounds are oral and topical antibiotics, but this can quickly become problematic due to the possible development of antibiotic resistance. As an alternative, designing a wound dressing to actively kill bacteria on contact would be optimal for chronic wound treatment. Plasma-enhanced chemical vapor deposition (PECVD) using 1,8-cineole as the plasma feed gas has been shown to actively kill bacteria on glass slide surfaces. Yet, 1,8-cineole-based PECVD strategies have not been extended to wound dressing materials.

The goal of the present study is to use 1,8-cineole PECVD to modify commercially-available wound dressing materials: hydrofibers and hydro polymers. Our strategy was to deposit a film with antibacterial properties using plasma treatment. By adjusting plasma parameters, it is possible to control the coating chemistry. However, no attempt has been made to compare pulsing vs. continuous-wave deposition. In other PECVD systems, pulsing has enabled the plasma precursor's functional groups to be maintained within the coating. Since the functional groups give 1,8-cineole its antibacterial properties, we anticipated that pulsing would enable the functional groups to be maintained upon PECVD. This study utilized 20 W peak power and a 20 minute application time for both continuous and pulsed deposition. The pulsed deposition used 10%, 25%, and 50% duty cycles. Water contact angle goniometry was used to evaluate changes in surface wettability of the material before and after plasma treatment. Additionally, x-ray photoelectron spectroscopy was used to quantify the elemental composition of plasma-treated and control surfaces. Zone of inhibition testing was performed to evaluate antibacterial properties against both gram-positive and gram-negative bacteria. Overall, 1,8-cineole PECVD strategies were effectively applied to wound dressing materials. This work represents progress towards addressing the need to directly target chronic wound infectious sites.

**BI-TuP-4 Fractional Analysis Process of Surface-Adsorbed Proteins Using Sds-Page, Naofumi Ohtsu**, Kitami Institute of Technology, Japan

When a biomaterial is implanted in a human body, an immediate event occurring on its surface is competitive adsorption of proteins. Such surface adsorbed proteins predominate cell attachment, proliferation, and differentiation that relate with material's biocompatibility. On the other hand, tissue fluids comprise many protein species, of which electrostatic properties are different by their structure. Compositions of the adsorbed proteins thus depend on the material's surface property. Fractional analysis of surface-adsorbed protein would thus provide significant clue for understanding the tissue-material interfacial reaction. However, separation of the surface-adsorbed proteins is difficult task; conversely, the proteins dissolved in a solution is capable to be separated by an electrophoresis, easily. Based on these, in the present study, we attempted to perform the fractional analysis of surface-adsorbed proteins through the extraction into a solution and subsequent separation by electrophoresis.

A prescribe amounts of bovine serum albumin (BSA) and lysozyme (LSZ) as model proteins were dissolved into a phosphate buffered saline (PBS) solution. Initially, a titanium (Ti) plate widely used for medical implants was immersed into the protein-contained PBS solution for 60 min at 310 K, aiming to adsorb BSA and LSZ on its surface. Next, the proteins adsorbed on the surface was extracted into a distilled water or a sodium dodecyl sulfate (SDS) solution of various concentrations. The extraction was conducted by immersing the Ti substrate into such solutions filled in a glass beaker, along with an ultrasonication for 10 min. Thereafter, the extracted proteins were separated by polyacrylamide gel electrophoresis (PAGE). Concentration of each separated protein was determined from the corresponding band intensity in PAGE gel.

When using a distilled water as the extraction solution, the proteins could not be detected in PAGE gel because almost proteins were re-adsorbed onto an inner-wall of glass beaker. Such re-adsorption was prevented when using the SDS solution exceeding 0.5 %, and actually, over 85% of adsorbed protein could be extracted. Additionally, when measuring the band intensity in the gel, we could confirm the linear correlations between the concentrations and the band intensity, in the range from 0.5 to 5 µg·mL. Based on these, we conducted fractional determination of surface-adsorbed BSA and LSZ though the analysis process above. The results demonstrated that the ratio of adsorbed BSA and LSZ was varied with the mixture ratio of these proteins, evidencing the validity of the suggested fractional analytical process.

**BI-TuP-5 Advanced Surface Analysis of Porous Bioactive Polymer Coatings on a TiAl6V4 Substrate Prepared by Supercritical Foaming for Orthopedic Applications, Katja Andrina Kravanja, M. Finšgar, Ž. Knez, M. Marevci Knez, University of Maribor, Slovenia**

Ti-based alloys are increasingly used as medical implants due to the combination of excellent biocompatibility, high strength, and corrosion resistance. However, their biological inertness and persistent postoperative complications such as inflammation and infection necessitate the development of bioactive coatings for orthopedic implants that enable localized, controlled release of active ingredients (AIs) at the implant site for improved osteointegration [1]. Formulation of AIs with supercritical fluids is one of the leading and well-established strategies to produce high-quality products by incorporating environmentally friendly and economically feasible properties. Supercritical (SC) foaming yields porous polymer matrices with a large specific surface area, allowing encapsulation and tailored release of AIs [2, 3].

This work aimed to develop and characterize porous bioactive coatings on TiAl6V4 surface made of biodegradable and biocompatible polymers using SC foaming technique to achieve controlled release of model synthetic and natural AIs with anti-inflammatory and antibiotic properties. The chemical structure, interactions, and morphology of the bare and coated substrates were characterized by advanced surface analysis, particularly tandem time-of-flight secondary ion mass spectrometry (ToF-SIMS), X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and 3D profilometry. Chemical information about the surface of multicomponent coatings was evaluated by determining molecular-specific signals for the coating components. The in-depth molecular distribution was determined by gas cluster ion beam (GCIB) 3D imaging by ToF-SIMS. In addition, the elemental composition, elemental environment, and depth profiling using GCIB for effective sputtering were acquired by XPS measurements. The topography of the newly prepared coatings and the agglomeration of the AIs were determined by AFM, while the large-scale study of the coatings and substrate roughness is presented by 3D profilometry. The results demonstrate that the fabricated coatings provide a good foundation for future pharmacokinetic and cellular research.

1. Kravanja, K.A. and M. Finšgar, *A review of techniques for the application of bioactive coatings on metal-based implants to achieve controlled release of active ingredients*. Materials & Design, 2022: p. 110653.
2. Knez, Ž., et al., *Industrial applications of supercritical fluids: A review*. Energy, 2014. **77**: p. 235-243.
3. Čolnik, M., et al., *Biodegradable polymers, current trends of research and their applications, a review*. Chemical Industry and Chemical Engineering Quarterly, 2020. **26**(4): p. 401-418.

**BI-TuP-6 PEG-b-PLA-NHS based Self Assembled Vaccine Platform as an Adjuvant-free Influenza Virus Vaccine, Jaehyun Hwang, Chonnam National University, Republic of Korea; G. Park, Yonsei University, Republic of Korea; J. Lim, Seoul National University, Republic of Korea; E. Ga, S. Moon, Chonnam National University, Republic of Korea; C. Park, Yonsei University, Republic of Korea; H. Kim, Kangwon National University, Republic of Korea; D. Song, Seoul National University, Republic of Korea; S. Haam, Yonsei University, Republic of Korea; W. Na, Chonnam National University, Republic of Korea**

Vaccines have been considered most effective tool to defend against viruses that can cause disease. However, some current vaccines are still suboptimal due to obstacles such as a risk of side effect and low immunogenicity. In the present work, we developed a self-assembled vaccine (SAV) platform based on antigen conjugated with an amphiphilic block copolymer, mPEG-b-PLA-NHS. To improve immunity of subunit vaccine, SAV was designed to display repetitive antigens in nanoscale for eliciting efficient antigen delivery and immune cell activation via multivalent recognition. SAV showed enhanced cellular uptake by dendritic cells (DCs), accelerating an efficient initiation of the adaptive immune system. SAV also showed high level of induction of IgG through in vivo analysis. These results implicate that SAV facilitate both efficient B cell activation and efficient T cell mediated immune response. In animal models, we confirmed that SAV comprising of hemagglutinin (HA) efficiently protected mice from mortality following challenge with influenza A virus. These findings suggest that the self-assembled nanosystem composed of antigen-polymer conjugate can be a potent vaccine platform with effectiveness and versatility.

**BI-TuP-7 Polymersome Based Co-Delivery System of Antigen and Immunostimulant for Improvement of Humoral Immune Response, Eulhae Ga, Chonnam National University, Republic of Korea; J. Lim, Seoul National University, Republic of Korea; J. Hwang, S. Moon, Chonnam National University, Republic of Korea; M. Yeom, D. Song, Seoul National University, Republic of Korea; W. Na, Chonnam National University, Republic of Korea**

Cellular uptake of antigen (Ag) by antigen-presenting cells (APCs) is vital for effective functioning of the immune system. Intramuscular or subcutaneous administration of vaccine Ag alone is not sufficient to elicit optimal immune responses. Thus, adjuvants are required to induce strong immunogenicity. Here, we developed nanoparticulate adjuvants that assemble into a bilayer spherical polymersome (PSome) to promote the cellular uptake of Ag into APCs. PSomes were synthesized by biodegradable and biocompatible block copolymer methoxy-poly(ethylene glycol)-b-poly(D,L-lactide) to encapsulate both hydrophilic and lipophilic biomacromolecules, such as ovalbumin (OVA) as a model Ag and monophosphoryl lipid A (MPLA) as an immunostimulant. After co-encapsulation of OVA and MPLA, the PSome synthetic vehicle exhibited the sustained release of OVA in cell environments and allowed efficient delivery of cargos into APCs. The administration of PSomes loaded with OVA and MPLA induced the production of interleukin-6 and tumor necrosis factor-alpha cytokines by macrophage activation *in vitro* and elicited effective Ag-specific antibody responses *in vivo*. These findings indicate that the nano-sized PSome may serve as a potent adjuvant for vaccine delivery systems to modulate enhanced immune responses.

**BI-TuP-8 Rapid and Effective Intradermal Application of Canine Influenza Vaccine Without Removal of Hair Using Patchless Insertion-Responsive Microneedle (Irmn) and Its in Vivo Efficacy Evaluation, Suyun Moon, E. Ga, J. Hwang, Chonnam National University, Republic of Korea; A. Kang, QuadMedicine R&D Centre, QuadMedicine, Republic of Korea; S. Baek, QuadMedicine R&D Centre, QuadMedicine, Inc., Republic of Korea; H. Jun, QuadMedicine R&D Centre, QuadMedicine, Inc., Seongnam, Republic of Korea; S. Choi, QuadMedicine R&D Centre, QuadMedicine, Inc., Republic of Korea; J. Lim, M. Yeom, Seoul National University, Republic of Korea; J. Park, Gachon University, Republic of Korea; H. Kim, Kangwon National University, Republic of Korea; D. Song, Seoul National University, Republic of Korea; W. Na, Chonnam National University, Republic of Korea**

Novel tip-separable microneedle system called Insertion-responsive microneedles (IRMNs) is painless application system, compensating the existing disadvantages of conventional vaccine administration such as intramuscular vaccination. IRMNs are composed of dissolvable hyaluronic acid (HA) tips and biocompatible polycaprolactone (PCL) bases, which is immediately isolated right after needle insertion and retraction. In this study, we conducted several *in vivo* and *ex vivo* tests to prove stability, safety and efficacy of IRMNs. *Ex vivo* porcine skin injection tests confirmed IRMNs penetrates skin and successfully releases coated components with no damage on skin tissues. Immunization in Guinea pigs using IRMNs induced two times higher hemagglutination inhibition (HI) antibodies compared to intramuscular injection groups, and complete elimination of viral shedding was found at 8 days post infection challenged with influenza A/canine/Korea/O1/2007 (H3N2) wild-type virus after second vaccination. Similar result has been shown in the H3N2 vaccine inoculation into dog's ears compared with intramuscular injection group. Tips of IRMN were well separated from the base, successfully delivering vaccine materials into dog's hairy skin without pain. The veterinarian assessed behavior of dog during injection and compared entire response of IRMN group with intramuscular administration. Dogs treated with IRMs appeared to be more comfortable and painless compared to syringe injection group. IRMNs are potential candidate of rapid and convenient vaccination, which will be particularly useful and attractive in veterinary research fields using animal vaccination.

**BI-TuP-9 Probing Bacterial Membrane Composition in the Study of Antibacterial Resistance using GCIB-SIMS, John Fletcher, University of Gothenburg, Sweden**

The spread of antibiotic resistance is an increasingly difficult problem to deal with as more bacterial infections survive treatments with commercial antibiotics. One of the main routes for the spreading of resistance among bacterial population is horizontal gene transfer, mainly through conjugation where mobile genetic elements are transferred from a donor cell to a recipient cell through a conjugative pilus.

One way to deal with the increasing levels of antibiotic resistance in bacteria is to develop new antibiotics for which resistance has not yet

emerged, which can be both laborious and not always a lucrative market. An alternative is to inhibit the conjugation itself so that the rate at which new resistance genes spread between populations is reduced and the usefulness of existing and new antibiotics is extended.

A previous study, performed at the University of Gothenburg, used a high-throughput screen to identify chromosomal *Escherichia coli* genes in the donor cells that were important for conjugation of the F-plasmid and could be potential targets to reduce conjugation. Among these hits were several genes that are involved in the cell envelope through stress response pathways, biogenesis, outer membrane protein assembly and homeostasis, which formed an interest into the role and importance of the cell envelope for conjugation.

Here, recent findings on the influence of different mutations and conditions on the highlighted mutants detected by secondary ion mass spectrometry (SIMS) using a gas cluster ion beam (GCIB) are presented.

## **BI-TuP-10 Soft, Precision Engineered Porous, Hydrogel Scaffolds Mechanically Tailored towards Applications in the Central Nervous System, Ningjing Chen, B. Ratner, University of Washington**

Largely incurable diseases and traumatic injuries to the central nervous system (CNS) demand the development of new biomaterials to improve healing and treatment options. Matching material mechanical properties to the CNS tissue and optimizing material porous structures are two central goals for improving better biomaterials for the CNS. However, biomaterials with both precision-controlled porous structure and brain-matched mechanical properties are still lacking. In this study, we developed a copolymeric hydrogel of 2-hydroxyethylmethacrylate and glycerol monomethacrylate (pHEMA-co-GMA) with mechanical properties tunable into the range of CNS tissues, and a uniform 40  $\mu\text{m}$  porous structure. The two characteristics were achieved by a new fabrication process combining phase separation and sphere templating. We used scanning electron microscopy (SEM) to image their morphology and an Instron mechanical testing apparatus to examine their compressive Young's Moduli. The resulting scaffolds are non-cytotoxic and endotoxin-free according to the ISO 10993-5 standard and commercialized endotoxin testing kit. In addition, 3D culture of microglial cells within the scaffolds demonstrates cell attachment and maintenance of a rounded, quiescent morphology, potentially due to spatial confinement. These results support further *in vivo* studies and suggest broad potentials in CNS applications, such as brain-computer interfaces, neural regeneration, and basic neurobiology.

## **BI-TuP-11 Zwitterionic Copolymer for the Bio-Compatible Coating on Medical Devices to Prevent Protein Fouling and Complement System Activation, Kan Wu, B. Ratner, University of Washington**

In the past decades, the demand for biocompatibility is increasing with the rapid development of advanced medical technologies, such as biosensors, implantable chips, and hemodialysis apparatus. Among all the biocompatible materials, zwitterionic carboxybetaine (CB) distinguishes itself for its superior hydration capability and stability as well as its potential for further functionalization. However, a reliable yet easy method to introduce the CB material onto the target surfaces is still challenging.

We developed a series of PCB-DOPA conjugates with different architectures of combining polycarboxybetaine (PCB) and mussel-inspired binding groups (DOPA) groups and investigated the structural effect on their coating performance. We found that a molecule with a linear PCB chain and quadruple DOPA groups at the chain end (PCB-4DOPA) can significantly increase the coating coverage and stability. Next, we applied the PCB-4DOPA conjugate on blood-contacting devices (hemodialysis membrane, catheters, respiratory devices, etc) to test the performance of this method in the real application. We demonstrated that the PCB-DOPA conjugate can form a protective coating on the device surfaces and significantly reduce the fibrinogen attachment and complement system activation. The complement proteins C5b9 generated by PCB interface is only 1.96  $\mu\text{g}/\text{ml}$ , compared to 12.16  $\mu\text{g}/\text{ml}$  generated by poly(poly(ethylene glycol)methacrylate)(PEGMA) at the same incubation condition. In addition, we also propose a new standard method to detect the complement reaction activation level in the blood samples for most prevailing biomaterials. This new detection method optimized the current measuring techniques and achieved a more accurate measurement.

## **BI-TuP-12 Enhanced Antithrombogenicity of 3D Templated Artificial Vascular Grafts Through Heparin Complex Conjugated with PEG Spacer, ChaeHwa Kim, Advanced Textile R&D Department, Korea Institute of Industrial Technology, Republic of Korea; J. Kim, Material & Component Convergence R&D Department, Korea Institute of Industrial Technology, Republic of Korea; J. Lee, T. Kim, Advanced Textile R&D Department, Korea Institute of Industrial Technology, Republic of Korea**

Artificial vascular grafts to replace blood vessels are necessary in cases where irreparable damage to blood vessels occurs due to circumstances such as disasters or accidents. Furthermore, blood compatibility is also vital in the transplantation of artificial blood vessels. In this study, we designed the vascular graft with 3D printing techniques and developed heparin derivatives that have better adherence to the surface and avoid thrombosis. Heparin complex conjugated with PEG spacer was synthesized and coated on the artificial vascular graft fabricated by 3D templated printing technology. We fabricated the 3D-printed polyvinyl alcohol (PVA) templates according to the blood vessel size and shape, and these were dip-coated with salt-suspended thermoplastic polyurethane (TPU). The core template was removed to obtain a customized porous TPU graft. Next, the dopamine-PEG-heparin conjugate was prepared through NHS/EDC coupling reaction. PEG spacers were also introduced to increase the heparin surface arrangement and inhibit blood component adherence. PEGs having various molecular weights were conjugated to heparin to investigate antithrombotic properties according to the length of the PEG linker. Using an NHS/EDC coupling procedure, a heparin-PEG-dopamine complex was generated and subsequently coated onto TPU vascular grafts for robust immobilization. Then, heparin release studies, blood coagulation, and platelet adsorption tests were performed. The use of a PEG linker improved the coating stability, which lowered the release rate of heparin and reduced platelet adhesion. In addition, the longer the molecular weight of the PEG linker, the stronger the antithrombotic effect. The results demonstrated that heparin-PEG-dopamine can substantially enhance the coating strength and anticoagulant properties of porous TPU vascular grafts.

## **BI-TuP-13 Impact of Amino Acid Conformation on the Efficacy of Antimicrobial Cyclic Peptides Against Medically- and Industrially-Relevant Microbes, Q. Lu, D. Regan, D. Barlow, Kenan Fears, US Naval Research Laboratory**

Microbial growth on surfaces, if unmitigated, poses health concerns and can accelerate the biodegradation of engineered materials and coatings. Cyclic peptides have emerged as a promising class of agents to combat biofouling. Cyclic peptides are more resistant to enzymatic degradation than their linear counterparts and can be designed to interact with extracellular targets, intracellular targets, and/or self-assemble into transmembrane pores. Here, we compare the antimicrobial efficacy of two pore-forming cyclic peptides,  $\alpha\text{-K}_3\text{W}_3$  and  $\beta\text{-K}_3\text{W}_3$ , against bacterial and fungal liquid cultures, and fungal biofilms. These two peptides display an identical series of side-chain chemistries, but the additional methylene group in the peptide backbone of  $\beta$ -amino acids results in two structural differences: 1)  $\beta\text{-K}_3\text{W}_3$  has a larger pore diameter, and 2) all backbone carbonyl (C=O) groups point in the same direction, with all amide (N-H) pointing in the opposite direction, leading to an enhancement in the dipole moment that drives self-assembly. In liquid cultures,  $\beta\text{-K}_3\text{W}_3$  was more efficient at reducing the number of colony forming units (CFU) when exposed to a gram-positive bacterium, *S. aureus*, and two fungal strains, *N. albida* and *P. laurentii*. To evaluate efficacy against fungal biofilms, cyclic peptides were incorporated into surface coatings of Irogran<sup>®</sup>, a polyester-based thermoplastic polyurethane. We detected no survival of *N. albida* and *P. laurentii* microcolonies ( $10^5$  per inoculation) on coatings containing either peptide a 7-day exposure. To determine if antimicrobial activity persists upon repeated exposure, we reinoculated coatings with *P. laurentii* live cells every 7 days for 4 weeks and detected no CFU after 14 and 21 days. In direct comparison, the number of CFU from Irogran<sup>®</sup> coatings without cyclic peptides was  $> 5 \log$  CFU.

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