Biomaterial Interfaces Division
Room Central Hall - Session BI-TuP

Biomaterial Interfaces Poster Session with Flash presentations

BI-TuP-1 Optimizing Micropost Arrays to Break Up Biofilms, ***James Waters***, *A Balazs,* University of Pittsburgh

Surfaces covered with periodic arrays of microposts represent an appealing avenue of fouling mitigation, as they rely on a physical mechanism without unintended environmental consequences. In addition to reducing the area for contaminant cells to bind to the surface, the flow field generated by specific configurations of posts under shear may help push particles away from the surface, or break up biofilms as they form. We represent such a system computationally using a hybrid of bulk fluid simulated via the lattice Boltzmann method, and deformable vesicles, representing cells, simulated via that lattice spring method. This simulation methodology allows us to rapidly implement and test different surface structures, and explore how the parameters of post shape and arrangement can most effectively deter the accumulation of biofilms.

BI-TuP-2 Dynamic Field Testing of Fouling Release Coatings by a Rotating Disk System, ***Julian Koc***, *K Nolte,* Ruhr-University Bochum, Germany*; A Stephens,* Florida Institute of Technology*; M Schultz,* United States Naval Academy*; G Swain, K Hunsucker,* Florida Institute of Technology*; A Rosenhahn,* Ruhr-University Bochum, Germany

The development of materials with the capability to resist the accumulation of biomass on surfaces in contact with seawater (marine fouling) is both, economically and ecologically desired. To rank the performance of novel coating technologies, different lab and field screening methods have been established. While technical coatings are tested over several months, methods for short-term testing of thin film chemistries are missing. We developed a setup for dynamic, short term field testing of coatings. To obtain a constant shear stress during colonization, a rotating disc was used. The rotating disc was designed to be easily transported and installed at various marine testing sites. The shear situation above the disk was theoretically simulated and adjusted to shear ranges identified in recent laboratory experiments to be suited to distinguish the fouling-release potential of surfaces [1]. To validate the setup, self-assembled monolayers with well characterized physicochemical properties were tested under similar shear conditions, as in our recently reported laminar flow lab assay. The same discriminations with the same trends as in the lab assay were obtained for a mixed population of marine diatoms in the ocean. In the future, the setup will be used to compare the results of lab tests of new promising coating chemistries with short term dynamic field exposure.

[1] K. Nolte, J. Schwarze, A. Rosenhahn, *Biofouling* **2017**, in press

BI-TuP-3 Bioinspired Vascularized Polymers for Controlled Drug Delivery, ***Kayla Marquis***, *A Webber, C Howell,* University of Maine

Nearly all methods that deliver bioactive compounds to the surface of a substrate rely on application from above or fail over time due to depletion of compounds. Here we explore the use of natural vascular channels embedded within polymeric matrices to allow for continuous, targeted, low concentration delivery of bioactive compounds to the surface from below. To achieve this, networks of empty 3D printed vascular channels are continuously filled with bioactive compounds. The compounds flow through the vascular network and diffuse through the polymer, eventually reaching the substrate surface of the matrix. By varying the locations and depths of these vascular channels we demonstrate that the amount of material and duration over which it is delivered to the surface can be controlled. The ability to control the diffusion of compounds both spatially and temporally is key in developing assays that test the effects of chemical gradients on various systems at both the cellular and organismal levels. This approach may prove useful in applications such as toxicity and wound healing assessment and targeted antifouling surfaces.

BI-TuP-4 Measuring the Mechanical Properties of Hydrophobic Anti-Fouling Surfaces, ***Samantha Zanetti***, *S Moorzitz, G Dickinson, M Figueroa,* The College of New Jersey

Biofouling by marine organisms causes damage to ships and underwater structures. Some anti-fouling coatings reduce adhesion by small marine organisms but are not as effective in deterring adhesion from barnacles. To develop a surface capable of further reducing barnacle adhesion, it is important to understand the chemical and mechanical interactions in the formation of bonds between the glue and surface. While some experiments have studied the mechanical properties of the cyprids and barnacles, their adhesion is complex and still not fully understood. Furthermore, there are only a few studies that have measured the adhesive properties of reattached barnacles. To study the adhesive properties of the glue, adult barnacles were removed from hydrophobic surfaces and the glue residue was characterized by atomic force microscopy (AFM).

Assessments were conducted on methylated and fluorinated self-assembled monolayer substrates. Substrates were prepared on glass slides that were cleaned with Piranha solution prior to use. Barnacles were reattached to the substrates in artificial seawater for two weeks. They were then removed via shear force following ASTM D5618-94. Separately, a mechanical testing frame was used to remove another set of reattached barnacles in a controlled manner. Force required to displace the barnacle was recorded and compared to the ASTM standard.

To determine the glue's viscoelastic properties and Young's Modulus, an AFM was used to collect force curves and images of the barnacle glue residue. The mechanical properties of the glue were recorded for each type of coating following an indentation procedure using an intermittent contact mode. Adhesion data and the deflection of the tip was used to plot applied force vs. vertical displacement. A contact model was applied to the approach and retraction curves to gather the viscoelastic properties of the samples.

The poster will present summer 2017 research results. This will include the measured mechanical properties of glue from reattached barnacles, retrieved from the AFM analysis and mechanical test strain data.

BI-TuP-5 ***In Vitro*** **Degradation Performance and Increased Biological Response of a Surface Modified Mg-Al-Zn Alloy**, ***Michael Melia***, *D Florian, J Scully, J Fitz-Gerald,* University of Virginia

As a lightweight metal with mechanical properties similar to natural bone, Mg and its alloys are great prospects for biodegradable, load bearing implants. However, the United States has yet to clear Mg for any substantial role in the body due to the concerns of electrochemically derived hydrogen gas and unpredictable loss of structural integrity as a result of a dynamic corrosion resistance varying with time. This research investigates how the chemical homogenizing effects of laser processing and the application of a corrosion resistant coating impacts the corrosion resistance, cell viability, and cell adhesion of the AZ31B (3 wt. % Al, 1 wt. % Zn, 0.3 wt. % Mn, and 95.7 wt% Mg) alloy in a physiological solution.

Cell viability and adherence measurements were carried out utilizing the osteosarcoma (MG63) cell line and were plated on the AZ31B specimens in the as-received, laser processed, and coated conditions. In vitro cell viability assays show improved cytocompatibility for both the laser processed and coated specimens over the as-received AZ31B alloy. The coated specimen performed the best with a 5 fold improvement in cell viability over the as-received alloy. Cell adhesion was further investigated by fixation of the MG63 cells and imaging using scanning electron microscopy (SEM). Electron micrographs revealed significant adhesion of cells to the coated specimen with limited adhesion for specimen in the as-received and laser processed condition.

Laser processing utilized a KrF pulsed excimer laser (λ = 248 nm and FWHM = 25 ns) which has been shown to reduce the corrosion rate of Mg alloys by an order of magnitude in NaCl containing solutions. Corrosion experimentation was performed under full immersion in a minimal essential media (MEM). Time dependent corrosion rates and electrochemical kinetics were analyzed using open circuit potential, electrochemical impedance spectroscopy, and potentiodynamic polarization measurements. The corrosion product morphology was investigated using SEM, energy dispersive spectroscopy, and x-ray diffraction. The coated specimens exhibited an order of magnitude reduction in cathodic kinetics after 24 hours of immersion in MEM compared to the as-received AZ31B alloy. The laser processed condition exhibited a 5 fold reduction in cathodic kinetics to the as-received alloy as well as maintaining an open circuit potential 150 mV lower than the coated and as-received specimen. The passivate nature of all three specimen conditions was similar.

BI-TuP-6 Interactions between Single Molecules and Surfaces, *C Klinger,* TU Bergakademie Freiberg, Germany; ***Laila Moreno-Ostertag,*** MPI for Iron Research, Germany; *C Weber, P Schiller, M Valtiner,* TU Bergakademie Freiberg, Germany

Unraveling the complexity of the macroscopic world relies on understanding single molecule interactions and their scaling towards integral interactions at the meso- and macroscopic scale [1]. Here, I will discuss how one can measure the interaction free energy of single interacting functional groups at various solid/liquid interfaces. The adhesion between single molecules and surfaces in electrolytes is a central point regarding many biological systems and the delamination of coatings.

Single molecule force spectroscopy with an AFM is a suitable tool for measuring the work and force needed to unbind single molecules. The relation between the work of non-equilibrium trajectories and the free energy of interaction can be described by Jarzynskis equation [2]. So the surface-to-molecule bond rupture can in principle be characterized fully, but systematic errors arises. First, we will discuss how the effect of contour length of typically utilized molecular linkers such as PEG potentially adds a systematic bias on the free energy determined from AFM experiments. Secondly, also experiments with varying speed of the force runs were realized and the bias due to increasing rates (i.e. further shift from the equilibrium situation), which will be discussed in this contribution.

Finally, we will discuss in detail how single molecule unbinding energy landscapes can be utilized to predict scenarios where a large number of molecules simultaneously interact, giving rise to adhesive failure under corrosive and wet conditions. As such, our experimental strategy provides a unique framework for the molecular design of novel functional coatings through predicting of large-scale properties such as adhesion and molecular interactions in various systems based on experimentally determined single molecule energy landscapes.

[1] T. Utzig, S. Raman, and M. Valtiner, Langmuir 31, 2722-2729 (2015)

[2] S. Raman, T. Utzig, T. Baimpos, B. R. Shrestha, and M. Valtiner, Nat. Commun. 5, 5539 (2014)

BI-TuP-7 Proton Transfers Involved in Melanin Biosynthesis: Binding of Cysteine to Dopaquinone Investigated by Density Functional Theory based Calculation, ***Ryo Kishida,*** Osaka University, Japan

Melanin is a natural pigment present in many types of living organisms. The color of the skin, hair, and eyes is a manifestation of melanin biosynthesis (melanogenesis). Melanogenesis is initiated by oxidation of tyrosine to form reactive dopaquinones. The formed dopaquionone rapidly reacts with cellular cysteine, resulting in the generation of yellow to reddish brown pheomelanin. At lower concentration of cysteine, dopaquionone undergoes intramolecular cyclization, resulting in the generation of brown to black eumelanin. Thus, the reactions of dopaquinone (cyclization and cysteine binding) affect the pheomelanin/eumelanin ratio, determining the body color. The color of eumelanin is further controlled by its monomer ratio. Eumelanin monomers are formed from dopachrome, which is a molecule generated after the stage of dopaquinone.

We have investigated the reactions of dopaquinone and dopachrome [1-4]. In this symposium, we present our recent mechanistic study on reactions of dopaquinone with a focus on the cysteine binding. Using density functional theory based calculation, we computed the energy profiles for the approaching of cysteine to dopaquinone and obtained stable cysteine-bound structures. We found that the cysteine-bound structures can undergo intramolecular proton transfer for further stabilization with fairly small activation energy.

[1] R. Kishida et al., Pigment Cell Melanoma Res. 27 (2014) 734.

[2] R. Kishida et al., Biochim. Biophys. Acta 1850 (2015) 281.

[3] R. Kishida et al., J. Electron. Mater. (2017) doi:10.1007/s11664-017-5299-x.

[4] R. Kishida et al., Biochim. Biophys. Acta (to be submitted).

BI-TuP-10 Interferometry: A New Way to Study Corrosion at Confined Interfaces, ***Claudia Merola,*** *H Cheng,* Max Planck Institute for Iron Research, Germany; *M Valtiner,* University of Freiberg, Germany

Understanding marine corrosion and biofouling is of central importance for designing materials for marine use that last longer and protect more effectively from biofouling. Many different types of destructive attack can occur to structures, ships and other equipment used in sea water service.

Crevice corrosion (CC), which is corrosion at an interface, still remains one of the most difficult types of corrosion to detect and to prevent. Most often CC occurs in narrow fissures where oxygen access is poor and a stagnant electrolyte solution is present. Experimentally it is a challenge to obtain in-situ information of processes in confined geometries and to establish well defined confined situations in the first place.

Here, we show how white light interferometry[1] can be utilized, for the first time, to study and monitor in situ the initial stages of the crevice corrosion process of thin layers of different metals[2] (e.g. Ni, Al, Au..) in different concentrations of NaCl solutions. Using Mica as a crevice former in an electrochemical surface apparatus allowed us to provide a deeper understanding of the initiation of the corrosion process, which also occurs at the adhesive interface of bio organism such as barnacles or mussels.

Our new approach provides a real-time view of the initial corrosion of confined surfaces, and hence may contribute to a deeper general understanding, and ultimately prevention, of localized corrosion and corrosion underneath biofoulers.

[1]J. Israelachvili *et al.*, Recent advances in the surface forces apparatus (SFA) technique. *Reports on Progress in Physics* **73**, 036601 (2010).

[2] B. R. Shrestha *et al.*, Real-Time Monitoring of Aluminum Crevice Corrosion and Its Inhibition by Vanadates with Multiple Beam Interferometry in a Surface Forces Apparatus. *Journal of the Electrochemical Society* **162**, C327 (2015, 2015).

BI-TuP-11 Stimuli-responsive Thin Films made from the Mucilage of *Opuntia Ficus-indica* Cactus, ***Zeinab Veisi,*** University of South Florida; *M Cardenas, A Cardenas-Valencia,* SRI International; *R Toomey, N Alcantar,* University of South Florida

We have used the mucilage of *Opuntia ficus-indica* cactus to fabricate ultra-thin films of surface-attached networks. The gelling properties and swelling behavior of these thin films were studied as a function of various stimuli to determine the main factors affecting the responsiveness of such layers.

Opuntia ficus-indica belongs to the cactaceae family, and is grown in dry regions. Its abundance makes it a promising commercial source of industrial pectin. Mucilage extracted from *Opuntia ficus-indica* is a heteropolysaccharide composed of a backbone chain structure of α-D-galacturonic acid and β-L-rhamnose interrupted by different neutral sugars. The carboxyl groups present in a polygalacturonic acid chain can be cross-linked in the presence of divalent ions to render hydrogel networks with conformations responsive to internal and external variables. The presence of a considerable amount of water within the polysaccharide matrices renders unique hydrophilic gels suitable to be used in a wide range of applications.

Thin films of surface-attached polysaccharide networks were fabricated by spin-casting solutions of mucilage. Ca^{2+} ions were then introduced to obtain cross-linked networks with adjustable extent of crosslinking. The fabricated surface-attached thin films of cross-linked polysaccharide were then characterized by Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) Spectroscopy and ellipsometry. Swelling behavior of the confined surfaces was studied as a function of temperature in contact with aqueous solutions, and their response was perturbed by different stimuli. Moreover, surface-attached networks were exposed to buffer solutions of pH of 9 and 2 to investigate the effect of charge concentrations on the response of networks.

The average water content in the networks as a function of temperature and the extent of crosslinking was precisely measured using ellipsometry. The results revealed that the extent of equilibrium water content and release profiles of thin films strongly depend on the degree of crosslinking. Moreover, the extent of equilibrium water uptake is affected by the values of pH of the media.

Our findings provide an improved understanding of the chemical properties, functionalities and the gelling behavior of thin films of surface-attached naturally occurring polysaccharides which can be employed for establishing responsive surfaces with tunable response suitable for the pharmaceutical and biotechnology industries.

BI-TuP-13 Effect of Topography on Retinal Stem Cell Viability and Regrowth, ***Aleksandr Filippov,*** *Y Tian, Y Xie,* SUNY Polytechnic Institute

Age-related macular degeneration is a devastation eye condition that inflicts damage to the retina and leads to irreversible vision loss. The retina is made up of several layers of light-sensing cells, which are supported and nourished by the retinal pigmented epithelial (RPE) layer. The RPE cells sit atop the Bruch's Membrane and form a highly-selective blood-retinal-barrier that is critical for retinal homeostasis. In this project, we attempt to recreate the barrier in vitro using elestrospun nanofibers. Human RPE cells were cultured on nanofibers made from natural and synthetic polymers,

such as chitosan and polycaprolactone, with Synthemax and gelatin as controls. We found that human RPE cells demonstrated proper morphology and protein expression when cultured on the chitosan substrate.

BI-TuP-14 DNA Interactions with Elastin like Polypeptide Coacervates, ***Telmo Díez***, *P Nguyen, N Carroll, J Satterfield, G Lopez,* University of New Mexico

Intrinsically disordered proteins (IDPs) are dynamic biomaterials used by mammalian cells in cell signaling, transcription, and chromatin remodeling functions. In native cells, they are frequently used in packaging and un-packaging of nucleic acids (NAs), making them promising biomaterials for drug delivery and gene delivery. Elastin Like Polypeptides (ELPs) are synthetic biopolymers that have similar structural features to natural IDPs with many similar associated functions. In this research, we focus on replicating IDPs' ability to assemble into hierarchal phase-separated granular structures and interact with nucleic acids using cationic ELPs. Importantly, ELPs condense to form coacervates above a lower critical solution temperature (LCST). Below this temperature, ELPs exist as a fully soluble random coil polymer. In this study, we use an ELP comprising 8 positive charges due the presence of 8 lysines interspersed within the chain. We demonstrate that condensed ELP coacervates provide the necessary charge density to attract and encapsulate nucleic acids. Here, ELP coacervates are incubated with fluorescently labeled DNA containing a Cy3 fluorophore on its 5' end. We characterize the amount of DNA captured by fluorescence intensity measurements that are taken prior to and following formation of a phase-separated ELP coacervate in aqueous solution. Furthermore, we use microfluidics to form aqueous microdroplets comprising ELP and fluorescent DNA to visualize DNA capture within ELP coacervate spheres via fluorescence microscopy. ELP coacervates formed by heating the microdrops above the ELP transition temperature are shown to electrostatically complex with- and capture DNA. We characterize the thermodynamic binodal boundary (i.e. temperature-dependent phase boundary) of the ELP to resolve the ELP volume fraction within the coacervate to determine the optimal temperature to maximize DNA capture. These initial studies will inform our future work to engineer smart, programmable nanoparticles for the delivery of nucleic acids for gene therapy applications.

BI-TuP-15 Bovine Aortical Endothelial Cell Encapsulation with Elastin-like polypeptides (ELP) and bis(sulfosuccinimidyl)suberate (BS3)., ***Phuong Anh Nguyen***, *T Diez Perez, H Canavan,* University of New Mexico*; N Carroll,* Universiity of New Mexico

Chronic wounds do not adequately recover through the healing process and have become a major challenge to healthcare systems worldwide. In the U.S., chronic wounds affect an estimated 6 million people per year, costing more than $25 billion annually due to complications and over $18.5 billion in associated care. Current biomaterials for wound healing scaffolds including aglinate, hydrofibers, foam, hydrogels, cadaver skins, fetal cow skin, skin grafts or fish skin to wounds to encourage healing. However, common drawbacks include poor biocompatibility, risk of disease transmission and host rejection. Bioprinting of hydrogel materials has emerged as a flexible tool with potential to obviate these problems. For example, tissue engineering by extrusion bioprinting uses robotic deposition to print cells encapsulated in hydrogel scaffolds to form new organs or tissues. However, biocompatible and biofunctional materials for printable hydrogels are lacking. We propose to encapsulate cells in novel microgel materials, elastin-like-polypeptides (ELP), to create printable bioinks that are biocompatible, bioinert, and recapitulate physicochemical cues of natural extracellular matrices. In our study, ELP hydrogels are formed by crosslinking ELPs with bis(sulfosuccinimidyl)sulfate (BS3), an amine-reactive crosslinker, to encapsulate bovine aortic endothelial cells within the formed hydrogels. Initial testing via live/dead assays shows cells are able to survive in the hydrogel scaffold for many days. Hydrogel stiffness can easily be controlled via temperature, pH, and crosslinker concentrations. Future work leveraged from these assays will be encapsulation and differentiation of mesenchymal stem cells (MCMs) for programmable wound healing.

BI-TuP-16 Direct Electron Beam Imaging of Proteinaceous Fibrils, *M Thieu,* KRISS, Republic of Korea*; T Ha,* KRIBB*;* ***SangJung Ahn***, KRISS, Korea, Republic of Korea

Direct electron beam imaging method was investigated with abnormal protein assembly of amyloid fibrils. Without and with metal coating, the fast electron beam methods such like scanning electron microscope (SEM) and transmission electron microscope (TEM) were used to observe in nanoscale and compared with slow tip-probing method, atomic force microscope (AFM). As a model protein for amyloid fibril, insulin protein (15 kDa) was chosen, whose aggregation has been believed to have a relation with type II diabetes in human. The insulin amyloid fibrils have grown under several effector molecules such as treholose, ectoines, and citrulline in order to discriminate the morphology differences in various conditions. The direct imaging of proteinaceous fibrils with electron beam was possible only in narrow windows of imaging conditions due to the facilitation of electrostatic charging effect, which is dependent on the underlying substrate. The comparisons of images with electron beams and physical tip-contact were conducted and analyzed in terms of measurement speed, charging, and mechanical damages.

BI-TuP-17 Textured TNZT Foams for Bone Implant Applications, ***Elizabeth Blackert***, *S Murguia, M Kramer, M Young, S Aouadi,* University of North Texas

TNZT alloys with compositions of Ti-35Nb-7Zr-5Ta are materials that are more biocompatible than the more widely used Ti-6Al-4V alloy since each of its constituent elements is biocompatible. In addition, it has the lowest Young's modulus of all the titanium-based alloys created so far (50-60 GPa). This property allows for a greater transfer of functional loads, which ultimately leads to bone growth stimulation. TNZT alloys were produced by arc melting of pure elements and were forged into rods. Oxide nano-scaffolds were grown on TNZT samples to investgate the potential of these nanostructures surfaces to improve osseointegration. These nanoscaffolds were grown using the hydrothermal method to create an oxide film. The alloys with and without nano-scaffolds were characterized using top-view and cross-sectional scanning electron microscopy equipped with an energy dispersive x-ray spetrometer to investigate the structure, morphology and chemistry of the resulting nanostructures. Finally, the formation of hydroxyapatitite on the modified surfaces was investigated upon immersion in simulated body fluid (SBF).

BI-TuP-18 Synthesis and Immobilization of Silver Nanoparticles in Natural Hydrogels by Directed Liquid-plasma Nanosynthesis, ***Camilo Jaramillo***, *A Shetty, A Civantos, S Arias, J Devorkin,* University of Illinois at Urbana-Champaign*; S Chang,* Nanjing University of Aeronautics and Astronautics, China*; J Allain,* University of Illinois at Urbana-Champaign

Plasma technology has seen an increased demand in nanotechnology, because of the changes in chemistry and morphology it can induce. These capabilities enable novel applications in a wide range of areas from advanced optical components to biomaterials [1]. Traditional plasma-based techniques work in low-pressure controlled environments. Compared to vacuum-based systems, atmospheric-pressure plasma (APP) systems offer reduced costs (e.g. no vacuum needed), higher reaction rates due to their high neutral-particle component and low-temperature treatment of polymer-based materials [2]. In addition, for specialized applications such as biology or catalysis, APP can offer treatment under gaseous or aqueous environments. One weakness of APP is the difficulty in controlling the coupled ion-neutral species and in turn high-fidelity modification of materials. One alternative to APP is the ability to tailor surface properties by careful control of species in the liquid plasma-material interface resulting in manipulation of nanostructured surface properties. Directed liquid-plasma nano-synthesis (DLPNS) is used in this work as the basis for systematic studies on the synthesis of silver nanoparticles (Ag NPs) in aqueous solution with DLPNS compared to in-vacuum directed plasma nanosynthesis (DPNS) on natural hydrogel matrices. Silver NPs are important for antimicrobial applications due to their unique antibacterial properties [3], but they also possess cytotoxic properties, making them harmful to human tissues [4]. Chitosan (CS) is a biodegradable, biocompatible and non-toxic natural biopolymer, which has been studied due to its antimicrobial properties [5]. DLPNS was used to treat Ag and Ag/CS solutions, driving NPs synthesis and surface nanopatterning. Surface morphology and composition were studied with SEM and EDS, respectively. Ambient-pressure *in-situ* XPS was used to measure irradiation-induced chemistry changes of CS. The antimicrobial properties of synthesized Ag NPs and nanostructured CS was systematically studied with control parameters such as energy and fluence. Notable transformation of the hydrogels was achieved, with self-organized pillar structures and porous structures produced on CS.

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[2] R. Foest, E. Kindel, A. Ohl, M. Stieber, K.-D. Weltmann, Plasma Phys. Control. Fusion 47 (2005) B525–B536.

[3] B. Le Ouay, F. Stellacci, Nano Today 10 (2015) 339–354.

[4] P. Dubey, I. Matai, S.U. Kumar, A. Sachdev, B. Bhushan, P. Gopinath, Adv. Colloid Interface Sci. 221 (2015) 4–21.

[5] I. Bano, M. Arshad, T. Yasin, M.A. Ghauri, M. Younus, Int. J. Biol. Macromol. 102 (2017) 380–383.

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