Wednesday Afternoon, November 6, 2002

Biomaterials
Room: C-201 - Session BI-WeA

Polyelectrolyte Surfaces/Cell-Surface Interactions
Moderator: A. Chilkoti, Duke University

2:00pm **BI-WeA1 Polyelectrolyte Multilayers: Design of Biofunctional Surfaces**, ***G. Decher***, Université Louis Pasteur, France **INVITED**

Layer-by-layer (LbL) assembly@super 1@ is an easy to use method for the fabrication of multi-composite films and has kindled widespread interest in such nanohybrids.@super 1-@super 7@. Electrostatic interactions between anionic and cationic compounds (synthetic or natural polyelectrolytes, DNA or proteins) offer four major advantages: (1) layer-by-layer construction due to surface charge reversal in each layer (2) restriction to single layers due to repulsion between last layer and excess material (3) low steric demand for interaction between oppositely charged ions (4) deposition on almost any solvent accessible surface. As an introduction to the LbL-technique, the guiding principles of multilayer assembly will be presented and details of the film structure will be discussed. Since the technique allows to interface a wide variety of materials with predefined spatial arrangement, it has successfully been introduced to both materials science and applied bio-sciences. At this meeting we will focus on work relevant for surfaces in contact with biological materials or environments. This will include work on films composed of natural or semi-synthetic polyions such as charged polypeptides or polysaccharides some of which has been carried out in close collaboration with the groups of P. Schaaf (ICS) and J.-C. Voegel (INSERM U 424).

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[3] Decher, G., Science 1997, 277, 1232-1237.

[4] Laschewsky, A., Europ. Chem. Chronicle 1997, 2, 13-24.

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[6] Bertrand, P. et al., Macromol. Rapid. Commun. 2000, 21, 319-348.

[7] Hammond, P. T., Curr. Opinion Coll. & Interf. Sci. 2000, 4, 430-442.

2:40pm **BI-WeA3 Poly-(L-Glutamic Acid)/Poly-(L-lysine) Multilayers used as Biomaterial Coating**, ***P. Schaaf***, Institut Charles Sadron (CNRS), France, *J.C. Voegel, C. Picart, Ph. Lavalle*, Unite INSERM U424, France, *F. Boulmedais*, Institut Charles Sadron (CNRS), France

Polyelectrolyte multilayers constitute an easy tool for multi-functionalizing biomaterial surfaces. In this paper we will discuss biological and physico-chemical properties of poly-(L-glutamic acid)/poly-(L-lysine) (PGA/PLL) multilayers. We first discuss the response of cells in contact with (PGA/PLL) multilayers functionalized by embedding active peptides within the films. We then present results relative to the cellular response when the film is functionalized by embedding proteins in the architecture. In both cases we get a cellular response even when the active molecules are embedded under up the 20 (PGA/PLL) bilayers. We discuss also possible cell recognition mechanisms of the active molecules. But the system (PGA/PLL) is also interesting because it leads to films with internal secondary structures such as beta-sheets or alpha-helices. The relation between the secondary structure of the film and the secondary structure of (PLL/PGA) complexes in solution will also be presented.

3:00pm **BI-WeA4 Cellular Interactions with Multilayered Polyelectrolyte Films**, *C. Picart*, Université Louis Pasteur, France, *Ph. Lavalle*, INSERM Unite 424, France, *L. Richert, D. Vautier*, Université Louis Pasteur, France, *P. Schaaf*, CNRS, France, ***J.C. Voegel***, INSERM Unite 424, France

The short time interactions of chondrosarcomas cells with polyelectrolyte multilayered architectures built up by the alternated deposition of poly(L-lysine) (PLL) and poly(L-glutamic acid) (PGA) were estimated in the presence and the absence of serum. Film constructions with and without protein adsorption were first characterized by means of optical waveguide lightmode spectroscopy, quartz cristal microbalance and zeta potential determinations. In the presence of a serum containing medium, the detachment forces measured by the micropipette technique were about eight times smaller for PGA than for PLL ending films. For these later ones, the adhesion forces decreased also when the films increased in thickness. In a serum-free medium the differences between the negative and positive ending films become larger : adhesion forces on PLL-ending films were by 40 % to 100 % higher, whereas no cells adhered on PGA terminating films. Also, PGA ending films were found to prevent the adsorption of serum proteins whereas large protein amounts adsorbed always on PLL ending films. These data suggest that cell interactions with polyelectrolyte films can be tuned by the type of the outermost layer, by the presence of proteins, and the number of deposited layers.

3:20pm **BI-WeA5 Entrapment of Phosphate Ester Hydrolyzing Enzymes in Polyelectrolyte Multilayers Deposited on Glass Beads and Extended Retention of Their Enzymatic Activity**, ***A. Singh***, *Y. Lee, I. Stanish*, Naval Research Laboratory, *T.C. Cheng*, Edgewood Research Development & Engineering Center, APG, MD

Recent advances in multilayer technology involving layer by layer technique indicate its utility in solving complex problems of multidisciplinary nature. We have explored layer-by-layer technology for entrapping enzymes organophosphorus hydrolase and Organophosphorus acid anhydrolase in polyelectrolyte multilayers with a goal to sustain their biological activity for a long period of time under an environment, where native enzymes turned inactive. Thus, phosphate ester-hydrolyzing enzymes were immobilized in multilayers coated on glass beads (30-50 μm). Coatings on Glass beads consist of 3 alternating layers of branched poly (ethyleneimine) (BPEI) and polystyrene sulfonate (PSS) as precursor layers, followed by five alternative layers of BPEI and OPH. Immobilized enzymes were tested for their enzymatic activity and stability at different temperature and under different humidity conditions and found active. Surprisingly, in 15 percent relative humidity environment an enhancement in enzyme activity was observed. Stability of multilayers incorporating enzymes was further improved by laying additional poly (acrylic acid) (PAA) layer on top of the multilayer assemblies and endcapping the enzyme-PAA layer with monomers, such as trimethylsilyl propylethylenediamine (TMSPED), and vinyl benzyl solketol (VBS). TMSPED end-capped OPH enzyme performed better than VBS coated multilayers and was further evaluated through salt stress test (involving 1 M aq. NaCl). An improved performance of end-capped OPH glass beads was demonstrated than their uncapped counterpart. Efforts on the synthesis of novel support beads will also be presented.

3:40pm **BI-WeA6 In-situ Measurements of Polyelectrolyte Multilayer Build-up using Ellipsometry and QCM-D : 9**, ***T.J. Thurell***, *U. Elofsson*, YKI, Sweden

Polyelectrolyte multilayers are easily constructed by alternately exposing a charged surface to positively and negatively charged polymers. The aim of this study was to create a biocompatible surface for use in implant technology. The charged poly-amino-a cids PGA (Poly (L)Glutamic Acid) and PLL (Poly (L)Lysine) were coated on an initial layer of PEI (Polyethylimin) on both silica and titanium surfaces. Multilayer build-up was monitored in-situ using both ellipsometry and QCM-D. The polyelectrolytes adsor b ed firmly, with insignificant desorption upon rinsing, on both substrates used. In the ellipsometer up to 12 layer pairs were easily built while monitored in-situ. Comparing adsorbed amounts obtained from the ellipsometer, with those calculated from QC M-D measurements, one can see that these polymer-multilayer-films are highly hydrated (app. 70% water content). In the QCM-D measurements, an almost linear mass increase/layer are obtained from the second layer pair and up, whereas linear mass increase/l a y er is not achieved until the eighth layer pair in the ellipsometer. The observations indicate that the polymer film become denser with each added layer. This is also reflected in the increasing refractive index, which eventually level out at 1.457 af t er abo ut 10 layer pairs. The large fluctuations in refractive index and thickness found initially suggests that the polyelectrolytes in the two first layer pairs are unevenly adsorbed with gaps and holes where water may get trapped. One possible explanat io n for the variations in film density would be that the gaps and holes will eventually fill up resulting in constant film density and linear mass growth. This work is a part of the SIMI project (Surface Improvement of Metal Implants GRD1-2000-26823) funded by the European Commission.

4:00pm **BI-WeA7 Nanoscale Surface Properties of Microbial Cells**, ***Y.F. Dufrene***, Universita Catholique de Louvain, Belgium **INVITED**

Biological events such as microbial adhesion, microbial aggregation and molecular recognition play a pivotal role in the natural environment, in medicine and in biotechnological processes. Understanding the molecular bases of these phenomena requires knowledge of the structural and physical properties of microbial cell surfaces. With atomic force microscopy (AFM), it is now possible to explore the surface of single cells with nanometer lateral resolution and under physiological conditions. AFM can be used for visualizing surface ultrastructure (crystalline arrays, appendages), for following physiological changes (germination, growth) and for monitoring the effect of external agents (antibiotics, metals). These studies open the door to new applications in biotechnology and biomedicine, such as the

rapid detection of microorganisms and the rationale design of drugs. AFM is actually much more than a microscope in that it also enables physical properties to be probed quantitatively: (i) surface hydrophobicity and electrical properties can be mapped using probes functionalized with defined functional groups (CH3, OH, COOH); (ii) surface softness can be measured by pressing the probe onto the cell surface; (iii) the elasticity of surface macromolecules, such as polysaccharides, can be addressed by means of force spectroscopy. These measurements have a great potential for elucidating the structure-function relationships of microbial surfaces (molecular recognition, conformational changes, surface interactions). In this contribution, I will discuss recent data obtained on fungal spores, yeasts and bacteria to highlight these unique capabilities.

5:00pm **BI-WeA10 Development of a Fluorescent Based Assay for Quantifying Ligand Surface Density on IPN-Modified PS for High Throughput Applications**, ***G.M. Harbers***, Northwestern University, *T.A. Barber*, University of California, Berkeley and University of California, San Francisco, *K.E. Healy*, University of California, Berkeley, *S.L. Golledge, D.G. Castner*, University of Washington

Biomimetic surface engineering exploits the power of specific ligand-receptor engagement to control cell-biomaterial interactions independent of bulk material characteristics. Accurate characterization of ligand surface density (Γ) is crucial for interpreting cellular response to these engineered surfaces. Currently, low throughput techniques including ellipsometry, SPR, and radiolabeling are employed to make these measurements. Lack of high throughput alternatives provided the motivation for the development of a fluorescence microplate reader based assay to measure Γ on a modular biomimetic surface developed to rapidly screen the adhesive potential of bioactive peptides. Poly(acrylamide-co-ethylene glycol/acrylic acid) interpenetrating polymer networks [p(AAm-co-EG-/AAc) IPNs] were grafted on to 96-well polystyrene (PS) plates. Fluorescently labeled peptides were subsequently coupled to the IPN using different input concentrations (0.01-100 μM) to modulate Γ. Surface characterization (contact angle goniometry and XPS) and cell-surface interactions were consistent with the results on previously developed IPN modified metal oxide surfaces. Reproducible control of Γ was observed over four orders of magnitude (~ 0.1-100 pmol/cm^2). Furthermore, competitive binding experiments using labeled and unlabeled peptides facilitated the determination of the equilibrium dissociation constants (K_d) of the various peptides. Although this technique may not be as sensitive as the others mentioned above, it allows for the characterization and rapid development of well defined biomimetic surfaces for high throughput applications.

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