

SURFACE ENGINEERING STRATEGIES FOR CONTROL OF PROTEIN AND CELL INTERACTIONS

H.Ma, J.Hyun N.Nath & A.Chilkoti

Department of Biomedical Engineering, Duke University, Durham, NC 27708.

INTRODUCTION: The ability to control the adsorption of proteins and the interaction of cells on a substrate are important for the development of cellular biosensors, biomaterials, and high-throughput drug screening assays. The critical problem in spatially directing cellular interactions at a surface is the rapid adsorption of a complex layer of proteins within minutes of contact with serum in cell culture or upon implantation in vivo.

We describe two different strategies for the synthesis of biologically-nonfouling polymer coatings that are applicable to diverse substrates. The first strategy involves physically coating a surface with an amphiphilic comb polymer that presents short oligoethylene glycol side chains [1]. Reorientation of the oligoethylene glycol side chains at the solid-water interface presents an oligoethylene glycol brush at the interface, thereby rendering the surface protein and cell resistant. A number of different fabrication methods to synthesize these nonfouling coatings will be described including spin-coating, dip-coating and surface-initiated polymerization using an atom transfer radical polymerization (ATRP) initiator immobilized on gold through the formation of an alkanethiol self-assembled monolayer (SAM). The second, "active" strategy involves the grafting of a stimuli-responsive biopolymer, derived from an oligomeric sequence found in mammalian elastin. This polypeptide, which we term an elastin-like polypeptide (ELP), is a biopolymer with the repeat unit Val-Pro-Gly-Xaa-Gly, and exhibits a lower critical solution temperature (LCST) transition in aqueous solution [2]. Surfaces grafted with these polypeptides exhibit a hydrophilic-hydrophobic transition in response to increased temperature or salt concentration. The change in the interfacial properties can be exploited to create "active" non-fouling polymer grafts that enable reversible, triggered binding of proteins onto surfaces in response to an external stimulus [3].

METHODS:

Synthesis of Comb Polymer. The comb polymer was synthesized by free radical polymerization of methyl methacrylate (MMA), poly(ethylene glycol) methacrylate (referred to herein as hydroxy-poly(oxyethylene) methacrylate (HPOEM), $M_n \sim 526$ g/mol, corresponding to $m \sim 10$) and poly(ethylene glycol) methyl ether methacrylate, (referred to herein as poly(oxyethylene) methacrylate (POEM), $M_n \sim 475$ g/mol, corresponding to $n \sim 8.5$) [1]. Composition: 61 wt% MMA, 21 wt% HPOEM, 18 wt% POEM. Molecular weight: $\sim 24,000$ Da; $M_w/M_n \sim 1.7$.

Expression of ELP and ELP Fusion Protein. An ELP with a molecular weight (MW) of 71 kDa and a thioredoxin-ELP (Trx-ELP) fusion protein where the same ELP was fused to the C-terminus of Trx were synthesized by overexpression of a plasmid-borne synthetic gene in *Escherichia Coli*, as reported elsewhere [2].

RESULTS:

Physical Deposition of Nonfouling Comb Polymer Films and Microstructures. The amphiphilic comb polymer was spin-cast onto different substrate from a water/methanol mixture to create a homogeneous coating. Alternatively, we found that microstructures of the comb polymer can be created on most substrate by spin-coating the comb polymer onto a micropatterned polydimethylsiloxane (PDMS) stamp from solution, and then selectively transferring regions of the comb polymer film to the substrate by bringing the stamp into physical contact with the surface. Both homogeneous coatings and microstructures of the comb polymer are stable in water and are protein and cell resistant, as long as the polymer is allowed to hydrate prior to incubation with proteins or cells. These nonfouling microstructures provide an experimentally robust method to pattern proteins and cells, as shown by the fact that cellular patterns are retained in the presence of exogenous serum for up to a month [1]. An important feature of this patterning methodology is that nonfouling topographical structures of the comb polymer can be created, whose height can be varied from ~ 50 nm up to several microns, and whose lateral dimensions can be controlled from several hundred nanometers to several hundred microns by nano and micro-contact printing. This system allows seamless integration of topographical and biochemical cues to direct cell behavior such that the presentation of both set of cues to cells can be independently controlled. This system has application in fundamental studies of cell-substrate interactions, cell-cell signaling, as well as in biotechnology and pharmacological pharmaceutical screening assays that require isolation of individual cells.

Surface Initiated Polymerization of Comb Polymer. More recently, we have synthesized an alkanethiol functionalized with a terminal ATRP initiator, and have used a SAM of this initiator-functionalized thiol to carry out surface initiated polymerization (SIP) of the amphiphilic comb polymer on gold. A SAM of the ATRP initiator thiol, w-mercaptopundecyl bromoisobutyrate was prepared by immersing a gold-coated silicon wafer

into a 1 mM solution of the thiol overnight. The SAM was rinsed with methanol and dried under a stream of nitrogen. SIP was carried out in an oxygen-free environment in a water/methanol mixture with POEM macromonomer. Results will be presented that demonstrate that oligoethyleneglycol-functionalized polymer brushes of tunable thickness in the 5-50 nm range, a thickness inaccessible to SAMs or polymer grafts, can be easily synthesized by SIP, that these polymer brushes exhibit no detectable adsorption of proteins and are cell-resistant for up to a month under typical cell culture conditions, and that the synthesis method is compatible with a range of patterning techniques from the nano- to the micro-scale, which enables the patterning of cells in a biologically relevant milieu over extended periods of time.

“Active” Control of Protein Adsorption. “Active” approaches to dynamically modulate the binding and release of proteins will also be demonstrated. In one implementation of this concept, which we have named TRAP –Thermodynamically Reversible Addressing of Proteins– we have shown that proteins can be reversibly bound to a surface in a functionally active orientation directly from cell lysate by exploiting a thermodynamically reversible hydrophilic-hydrophobic LCST transition exhibited by a genetically engineered, stimuli responsive ELP [2]. In TRAP, an ELP is covalently micropatterned on a glass surface against an inert BSA background. The ELP patterned surface is incubated with the soluble fraction of E. coli lysate containing an expressed Trx-ELP fusion protein, which is appended with the same ELP as on the surface. The LCST transition of the grafted ELP and the Trx-ELP fusion protein is simultaneously triggered by an external stimulus. The LCST transition results in capture of the ELP fusion protein from solution onto the immobilized ELP by hydrophobic interactions between the grafted ELP and the ELP fusion protein. The captured ELP fusion protein is oriented such that the fusion partner is accessible to binding of its

target from solution. We also demonstrate that TRAP is reversible; the bound protein-ligand complex is released from the surface by reversing the LCST transition [3]. The triggered control of interfacial properties provided by an immobilized stimuli-responsive polypeptide at the solid-water interface is an enabling technology that allows reversible and functional presentation of ELP fusion proteins on a surface directly from cell lysate without the necessity of intermediate purification steps and subsequent recovery of the protein-ligand complex for downstream analysis by other analytical techniques. TRAP has application in lab-on-a-chip bioanalytical devices as well as in the fabrication of peptide and protein arrays.

CONCLUSIONS: An amphiphilic comb polymer film can be stably coated onto diverse substrate by a simple spin coating process from an environmentally benign solvent (water/alcohol) such that the comb polymer coating is stable in water and confers nonfouling properties to the substrate. The comb polymer can also be grafted from a gold surface using surface-initiated ATRP, leading to the formation of a polymer brush. These passive nonfouling coatings have diverse applications in the fabrication of biomaterials and biosensors. An active nonfouling coating strategy has also been developed whereby the interaction of a recombinant protein can be reversibly controlled at a surface that is grafted with a genetically engineered stimuli-responsive polypeptide. Such active coatings can be used to selectively and reversibly capture a protein of interest from solution, and are likely to prove useful in microfluidic BioMEMS devices and peptide and protein arrays.

REFERENCES: ¹J. Hyun, Z. Zhang, T.P. Beebe, Jr., and A. Chilkoti (2003) *Adv. Mat.* **15**: 573-576. ²D.E. Meyer and A. Chilkoti (1999) *Nature Biotechnology* **17**: 1112-1115. ³N. Nath and A. Chilkoti (2003) *Anal. Chem.*, **75**: 709-715.