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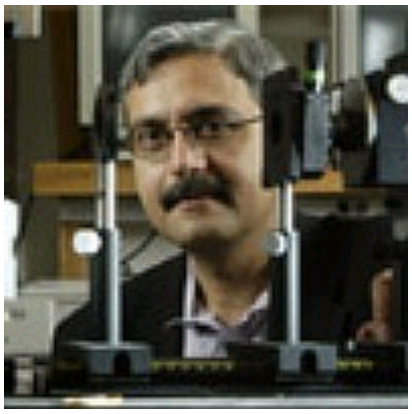
ASHUTOSH CHILKOTI, THEO PILKINGTON PROFESSOR OF BIOMEDICAL ENGINEERING



Ashutosh Chilkoti is a professor with Duke's Department of Biomedical Engineering, and Associate Director of the Center for Biologically Inspired Materials and Materials Systems (CBIMMS).

My research in biomolecular materials and surface science emphasizes the development of applications that span the range from bioseparations, biosensors, patterned biomaterials, and targeted drug delivery. The first area of research in my laboratory is the genetically encoded synthesis, characterization, and application of artificial elastin-like polypeptides (ELPs). ELPs are biopolymers composed of a VPGXG peptide repeat, which undergo a thermally reversible phase transition. Below a characteristic inverse transition temperature (Tt), ELPs are soluble in aqueous solution, but when the temperature is raised above Tt, they desolvate and form visible aggregates. We have synthesized a number of ELPs with different composition's and molecular weights encoded by synthetic genes in E. coli, and exploit the reversible thermal behavior of these polypeptides in different molecular applications, which include:

Thermal purification of recombinant proteins by fusion with an ELP expression tag. We have observed that fusion proteins with N- or C-terminal ELP tags also undergo a transition similar to that of the free polypeptide. We have developed a ne w method, based on this observation, which we term "inverse transition cycling" (ITC), for purification of soluble fusion proteins incorporating an ELP tag. The fundamental principle of ITC is remarkably simple: it involves rendering the ELP fusion protei n insoluble in aqueous solution by triggering the inverse transition, by increasing the temperature above the Tt, or alternatively by depressing the Tt below solution temperature in the presence of contaminating host proteins present in the cell lysate. T his results in highly selective aggregation of the ELP fusion protein only, and the aggregated protein is collected by centrifugation. The aggregated protein is redissolved in buffer at a temperature below the Tt, which reverses the inverse transition, yi elding soluble, functionally active and purified protein. We believe that ITC has major advantages over other protein purification methods currently used: technical simplicity, low cost, ease of scale up and multiplexing.

Thermally targeted delivery of anticancer therapeutics to solid tumors by thermally responsive ELP carriers. We hypothesized that ELPs conjugated to drugs would enable thermally targeted drug delivery to solid tumors if their Tt were between bod y temperature (Tb) and the temperature in a locally heated region (Th). We synthesized an ELP with a Tt of 41 °C as well as a thermally insensitive control ELP, by recombinant DNA techniques in E. coli, to test this hypothesis. In vivo studies of ELP delivery to implanted tumors in nude mice demonstrated that thermal targeting provides a 2-fold increase in tumor localization versus compared to the thermally insensitive control polypeptide. We observed aggregates of the thermally responsive ELP by fluo rescence videomicroscopy within the heated tumor microvasculature but not in control experiments, which demonstrates that the phase transition of the thermally responsive ELP carrier can be induced in vivo at a specified temperature. By exploiting the pha se transition-induced aggregation of these polypeptides, this method provides a new method to thermally target polymer-drug conjugates to solid tumors.

Synthesis of bulk, crosslinked ELP hydrogels for application as Injectable tissue engineering scaffolds. The overall goal of this collaborative project with the research group of Prof. Lori Setton (Biomedical Engineering, Duke University) is to investigate the potential for crosslinked ELPs to promote tissue regeneration while restoring the native mechanical function of cartilaginous tissues in situ. The first aim of this project is to synthesize and cross-link several formulations of geneticall y engineered ELPs. The crosslinked ELP gels will be extensively characterized for their mechanical properties, dimensional swelling changes, toxicity and degradation properties. This specific aim will provide important structure-function relationships for genetically engineered ELPs which will serve as the Materials Engineering foundation for synthesis of a hydrogel of any targeted specification. The second aim is to evaluate cartilage regeneration in ELP scaffolds in vitro. Cells from articular cartilage and the intervertebral disc will be cultured in three-dimensional scaffolds, crosslinked ELP gels. Cellular viability and proliferation and measures of gene expression and immunolabeling for

phenotypic matrix proteins will be quantified in these cell-ELP constructs. In addition, biodegradation and mechanical properties of the cell-ELP constructs will be evaluated. Together, these data for cell-ELP constructs in vitro will be essential for assessing the potential for these scaffolds to provide for functio nal tissue repair in vivo. With the knowledge gained in this initial in vitro study, we will select cell-specific formulations appropriate for tissue repair to be tested in our in vivo animal models of osteoarthritis and intervertebral disc degeneration.< /p>

In a second area of research in biomolecular surface science, we are investigating new methods to micro- and nano-pattern proteins and other biological ligands onto self-assembled monolayers (SAMs) and polymers for application in multianalyte biose nsors, patterned molecular recognition biomaterials, and protein chips. These methods are:

- Light-activated micropatterning (LAMP), which exploits spatially-precise light-activated deprotection of affinity ligands on functionalized SAMs to achieve step-and-repeat patterning of multiple biomolecules. LAMP is a multi-step patterning process with considerable flexibility in its implementation. First, a COOH terminated SAM is formed on gold. Next, the carboxylic acid end groups in the SAM are coupled to methyl ?-nitropiperonyloxycarbonyl biotin succinimidyl ester ("caged'' biotin este r) through a diamine linker. The caged biotin is then deprotected in regions irradiated by masked UV light, and subsequent incubation with streptavidin results in selective binding of streptavidin to the irradiated regions. Micropatterning of various prot eins has been demonstrated with a spatial resolution of ~6 µm by confocal microscopic imaging of fluorophore-labeled proteins, and a contrast ratio of ~4:1 was determined by direct ellipsometric imaging of streptavidin. Immobilization of biotinylate d antibodies on the streptavidin pattern indicates that LAMP can enable spatially resolved micropatterning of different biomolecules by repeated cycles of spatially-defined photodeprotection of biotin, streptavidin incubation, followed by immobilization o f the biotinylated moiety of interest.

- Microstamping onto activated polymer surfaces (MAPS), which involves surface-selective functionalization of polymers, followed by microcontact printing (µCP) of reactive biological ligands. We have demonstrated that polyethylene (PE), p olystyrene (PS), polymethylmethacrylate (PMMA) and polyethylene terephthalate (PET) films can be successfully modified to introduce COOH groups on their surface, which can be subsequently patterned by reactive µCP of amine-terminated biotin. We have used the streptavidin-biotin interaction to subsequently pattern a cell adhesive biotinylated RGD peptide onto these surfaces with good S/N and micrometer spatial resolution, and have demonstrated cellular patterning on these peptide micropatterns. This methodology provides a generic route to pattern any biotinylated biomolecule of interest on a wide variety of polymeric biomaterials.

Thermodynamically addressable reversible patterning (TRAP): Given a thermodynamic "address" - a surface template which exhibits patterned domains with different surface energies - TRAP functions by the selective adsorption of a n elastin fusion protein above its phase transition temperature specifically on the patterned hydrophobic regions, but not on the protein-resistant hydrophilic background. Unlike other methods for protein patterning, TRAP is reversible, and modulating the solution environment (e.g., T, ionic strength), can erase protein patterns.

Ultraflat nanosphere lithography (UNSL). UNSL combines conventional nanosphere lithography and ultraflat template stripping to create an ultraflat pattern of a material M1 embedded in a matrix M2. We have created ultraflat, ~100 nm structure s of Au and Ag (M1) embedded in chemically orthogonal materials (M2) such as Al and SiO2, and have demonstrated that the surface roughness of these nanocomposites is < 1 nm (rms) over areas of several hundred µm2. These topographically flat, chem ically distinct nanostructures are convenient templates for bottom up nanofabrication using orthogonal chemical self-assembly and for nanopatterning of biomolecules.

Contact Info:

Office Location: 3381 CIEMAS
Office Phone: (919) 660-5373
Email Address: [redacted]
Web Page: http://www.chilkotilab.pratt.duke.edu

Teaching (Spring 2010):

- BME 310.01, *BME GRADUATE SEMINARS*

Education:

PhD, University of Washington, 1991
B. Tech., Indian Institute of Technology, 1985

Specialties:

Polymer and Protein Engineering
Drug Delivery
Biological Materials
Sensing and Sensor Systems
Nanomaterial manufacturing and characterization

Research Interests:

Chilkoti's research focuses on biomolecular materials and surface science and emphasizes the development of applications that span the range from bioseparations, biosensors, patterned biomaterials, and targeted drug delivery.

Areas of Interest:

Biomolecular materials
Biointerface Science

Awards, Honors, and Distinctions

3M Nontenured Faculty Award, 2002
Distinguished Research Award, Duke University, Pratt School of Engineering, 2003
Stansell Family Distinguished Research Award, Duke University, Pratt School of Engineering, 2005

Recent Publications (More Publications)

1. D. L. Nettles and A. Chilkoti and L. A. Setton, *Early Metabolite Levels Predict Long-Term Matrix Accumulation for Chondrocytes in Elastin-like Polypeptide Biopolymer Scaffolds*, Tissue Engineering Part A, vol. 15 no. 8 (August, 2009), pp. 2113 -- 2121 [abs].
2. T. Christensen and M. Amiram and S. Dagher and K. Trabbic-carlson and M. F. Shamji and L. A. Setton and A. Chilkoti, *Fusion order controls expression level and activity of elastin-like polypeptide fusion proteins*, Protein Science, vol. 18 no. 7 (July, 2009), pp. 1377 -- 1387 [abs].
3. A. Hucknall and A. J. Simnick and R. T. Hill and A. Chilkoti and A. Garcia and M. S. Johannes and R. L. Clark and S. Zauscher and B. D. Ratner, *Versatile synthesis and micropatterning of nonfouling polymer brushes on the wafer scale*, Biointerphases, vol. 4 no. 2 (June, 2009), pp. FA50 -- FA57 [abs].
4. A. Hucknall and S. Rangarajan and A. Chilkoti, *In Pursuit of Zero: Polymer Brushes that Resist the Adsorption of Proteins*, Advanced Materials, vol. 21 no. 23 (June, 2009), pp. 2441 -- 2446 [abs].
5. S. Zauscher and A. Chilkoti, *Biological Applications of Polymer Brushes*, Biointerphases, vol. 4 no. 2 (June, 2009), pp. FA1 -- FA2 .

Biomedical Engineering Department
Pratt School of Engineering | Duke University
Room 136 Hudson Hall • Box 90281 • Durham, NC 27708-0281
Phone: (919) 660-5131 • Fax: (919) 684-4488