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Polymeric Nanostructures for Delivering Drugs and Imaging Agents

Adsorption of Immunoglobulin on Cellulose and Chitin Films Using Surface Plasmon Resonance

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Chitin and cellulose are useful as biomaterials because of their mechanical properties, abundance in nature, and biocompatibility with humans. Potential applications of chitin and cellulose include wound healing agents and drug delivery. In order to achieve and enhance these applications, it is important to understand how proteins will interact with chitin and cellulose. The goal of this study was to compare the degree to which the antibody Immunoglobulin G (IgG) adsorbs onto cellulose and chitin using surface plasmon resonance (SPR). In order to accomplish this objective, thin polysaccharide films were generated by spincoating solutions of trimethylsilyl (TMS) derivatives of the polymers. The TMS groups were then cleaved by placing the sensors over a 10% HCl solution. Changes in the resonant angle due to IgG adsorption onto these films as a function of time was tracked by SPR. Typical SPR spectra consisted of a baseline established using a phosphate buffer solution (PBS) (pH=7.4), an increase in the resonant angle arising from IgG adsorption, and finally another baseline established using PBS to remove all protein that had reversibly adsorbed. The change in the resonant angle due to adsorption was then converted to surface concentration (Γ) as a function of IgG solution concentration. Freundlich adsorption isotherms were then used to model the data for these surfaces. These isotherms suggest greater IgG adsorption onto chitin than cellulose which could be due to stronger van der Waals interactions between IgG and the acetyl groups of chitin.

Design and Fabrication of Chitosan Nanoparticles for the Delivery of Rosmarinic Acid

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Drugs face many complex environmental challenges that lower their bioavailability and effectiveness. Nanoparticles have been able to effectively encapsulate, deliver, and release drugs, improving the ability to deliver active pharmaceutical ingredients (APIs) with poor solubility and stability. Rosmarinic acid is a naturally occurring API that could be an important tool in developing therapies for both cancer prevention as well as Alzheimer's disease. Unfortunately, rosmarinic acid has poor solubility and dispersion stability. The ionic gelation method was used to encapsulate rosmarinic acid in chitosan nanoparticles. Chitosan was chosen due to its biodegradability and biocompatibility. Dynamic light scattering showed that the loaded particles were 27.4 ± 6.9 nm. Particles were purified using two different methods: dialysis followed by freeze drying and flocculation using ammonium hydroxide. Particles that were purified using ammonium hydroxide had a yield of 70% based on mass of material used and an encapsulation efficiency of 5.6%. Particles that were purified using dialysis had a yield of 92% and an encapsulation efficiency of 54.9%. These results are very promising and show that chitosan nanoparticles could be an effective tool in encapsulating and delivering rosmarinic acid.

Design and Isolation of Cargo-Carrying Bacteria for Cancer Therapy

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Facultative anaerobic bacteria have been shown to preferentially colonize within tumor sites and successfully treat metastatic cancer in immunecompromised mice. However, to date, a bacterium has not shown success in inhibition of tumor growth in fully immunocompetent hosts. We strive to uniquely combine the advantages of bacteria as delivery vectors with the benefits of chemotherapeutics. We have previously developed Nanoscale Bacteria-Enabled Autonomous

Delivery Systems (nanoBEADS) in which 10 or more nanoparticles are assembled onto a bacterium using a controlled self-assembly approach. In this work, we first construct NanoBEADS using the attenuated *Salmonella enterica S*erovar Typhimurium VNP20009 whose safety has been established by an earlier phase I trial. For *in vivo* drug delivery, the drug must be precisely contained as progression occurs throughout the vasculature of the body, i.e., the nanotherapeutic must be securely attached to the bacterium and not remain free in solution. A density gradient centrifugation strategy was developed to separate the low density nanoparticles from higher density bacteria. Finally, constructing a viable drug delivery system requires adequate *in vivo* modeling prior to subject testing. Thus, the deeply penetrating characteristics of the infrared spectrum were deemed a desirable feature for fluorescence of the bacterium carrier. Construction of an infrared fluorescent protein (IFP1.4) encoding bacterial plasmid was shown to be successful using the conventional BioBrick system. Furthermore, chemical transformations and utilization of a restriction-deficient and modification-proficient bacterial strain allowed for the successful incorporation of IFP1.4 into *S.* Typhimurium VNP20009. The purified NanoBEADS suspension with constitutive IFP expression will be used to assess the efficacy of such bacteria-based chemotherapeutic delivery vectors in cancer therapy.

Synthesis of Heteroditopic AB Monomer for Polypseudorotaxane Host-Guest Supramolecular Polymer System

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Supramolecular polymers are composed of monomeric units held together by noncovalent, reversible interactions including hydrogen bonding, van der Waals forces, and π-π stacking. Host-guest systems consist of a hosts and guest molecule that form a complex through non-covalent interactions. Host-guest systems can be used to effect self-assembly of supramolecular polymers. A monomer may be end functionalized with two host molecules or two guest molecules (AA-BB system). A monomer may also be end functionalized with both the host and the guest molecules (AB, or heteroditopic system). To create a self-assembling system capable of yielding high degrees of polymerization (DP), a high association constant (K_a) value between the host-guest must exist. Pyridyl cryptands (a macrocyclic, pre-organized crown ether) are good hosts for paraquat derivative guests as they exhibit high K_a values. The complex is called a pseudorotaxane. Main chain polypseudorotaxanes are supramolecular polymers that rely on host-guest interactions (pseudorotaxane formation) to form the polymer chain. The focus of this investigation is to synthesize a heteroditopic AB monomer to yield a self-assembling supramolecular polymer; a polypseudorotaxane. Polypseudorotaxanes exhibit smart properties, such as responding to external stimuli, such as pH, temperature, and solvent as well as self-heal. We expect the resulting heteroditopic AB monomer to produce a system with smart properties and possibly self-heal. In addition to synthesizing the heteroditopic AB monomer in high yields, the Gibson group will attempt to characterize the mechanical properties of the supramolecular polymer, contributing to an area of study that has not received much attention yet.

Time-Resolving Unimer Exchange In Block Copolymer Micelles

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Full comprehension of micelle dynamics is required if polymeric micelles are to be used as targeted drug delivery vehicles. Contrast-matching Time-Resolved Small Angle Neutron Scattering (TR-SANS) has proven useful in understanding unimer (polymer chain) exchange rates, however, it is a time and resource exhaustive technique. In this work, we explore the utility of NMR spectroscopy techniques, such as spin-lattice relaxation time and pulsed-field-gradient diffusometry, as an alternative to TR-SANS. We use ^{1}H- and ^{2}H-based poly(ethylene oxide)-

polycaprolactone diblock copolymers that self-assemble into spherical micelles at 1% w/v. Analogous to the contrast-matching TR-SANS experiment, mixing of ^{1}H- and ^{2}H-based micelles shows an increase in spin-lattice relaxation time with time due to mixing of the unimers. We further evaluate the mechanism of unimer exchange through NMR diffusometry to show two-component diffusion coefficients, suggestive of both free unimer and micelles existing in solution. The combined data is suggestive of free unimer exchange as the dominate exchanging mechanism. As an alternative to traditional characterization techniques, NMR is a much more easily accessible and cost effective method of analysis. We believe that NMR spectroscopy and diffusometry will prove to be promising techniques for more general characterization of micelle dynamics.

Negative Isolation of Monocytes from Mouse Whole Blood via Polydimethylsiloxane Microfluidic Device

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Monocytes are a type of white blood cell that play a key role in the innate immune system by defending our bodies from infections and diseases. Isolating monocytes from mouse whole blood would allow chromatin immunoprecipitation (ChIP) and gene sequencing tests to be performed. This paper proposes the design and fabrication of a microfluidic separation channel made from a flexible and biocompatible polymer – polydimethylsiloxane (PDMS). Using softlithography, a fabrication procedure was established for creating a master silicon wafer, to be used indefinitely to produce PDMS devices. This paper clearly defines the parameters used to create a successful silicon wafer through all steps of manufacturing: spin coat, soft bake, exposure, post-exposure bake, and development. With a working PDMS device, a packed-bed of streptavidin-coated magnetic microspheres (Spherotech) was created in the separation channel to allow the flow of red blood cells, plasma, and platelets to the outlet, while retaining white blood cells. Tests were performed to show the capability of a PDMS device to support the creation of a packed-bed of microspheres. Once a packed-bed was created, THP-1 human monocytic cells in PBS were flowed to prove the retention of the cells within the packing. Future work for this project includes creating an antibody cocktail to allow the negative selection of monocytes by the microspheres, and to perform ChIP and gene sequencing tests to reveal how monocytes function.

Breast Cancer Single-Cell Motility and Contractile Force Measurements on STEP Suspended Fibers

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Metastasis, which is responsible for 90% of cancer deaths, is influenced by both chemical and mechanical stimuli. Studies of breast cancer in particular highlight the importance of cellular interaction with aligned collagen fibers of the native extracellular matrix (ECM). Both fiber alignment and matrix stiffness have been shown to strongly influence the migration and invasive behavior of cancerous cells in mammary tissue. This work utilizes highly-aligned, suspended polymeric (polystyrene) nanofiber networks fabricated using the non-electrospinning Spinneret-based Tunable Engineered Parameters (STEP) technique to analyze single-cell motility and contractile forces. Cancerous MDA-MB-231 and non-cancerous MCF-10A cell lines were compared. Motility was quantified for two distinct cell morphologies by migration speed, persistence and directionality in relation to structural stiffness (k, N/m), which depends on Young's modulus and fiber geometry. Contractile forces were measured via beam mechanics in the elastic limit from observable fiber deflections caused by cells. The two cell types were observed to respond differently to variable structural stiffness of suspended fibers. MCF-10A migrated ~8 μm/h slower in the higher k range (3+ mN/m) than in the lower range (0-3 mN/m) whereas MDA-MB-231 migrated ~5 μm/h faster when k>3 mN/m. In agreement with prior literature, healthy cells exerted higher contractile forces (61±16 nN) than their cancerous counterparts (50±3 nN). Results

obtained from the study highlight the importance of varying responses of normal and cancerous cells to changing structural stiffness. In future, the platform could be coupled with microfluidic devices to introduce chemical gradients which would allow us to investigate the interplay of mechanical and chemical stimuli during cell migration.

Nucleobase Functionalized Polymers for Enhanced Interlayer Adhesion in 3D Printing

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3D printing is a rapidly emerging advanced manufacturing technique, which constructs a 3D object layer-by-layer. One of the limitations of this method is the anisotropic mechanical properties due to low interlayer adhesion. In this study, novel adenine and thymine poly(ethylene glycol) acrylates were synthesized through a Michael addition reaction to analyze their effects on this interlayer adhesion in the 3D printing method of mask projection microstereolithography (MPμSL). MPμSL uses UV exposure to cure polymeric precursors on a micron-scale which would enable medical applications such as tissue scaffolding or drug delivery. Alternating layers of acrylates functionalized with complementary nucleobases will provide hydrogen bonding between adjacent layers that will influence the interlayer adhesion. Poly(ethylene glycol) diacrylate (PEGDA) was crosslinked into hydrogels in a biocompatible water-based system as a control to test the interlayer adhesion through tensile analysis using three distinct geometries of samples developed for this purpose. PEGDA exhibited lower stress and strain at break for samples mimicking the layer-by-layer process of 3D printing such as the end-to-end jointed sample in contrast to the single film control samples. The end-to-end sample had 0.706 ± 0.138 MPa stress and 0.107 ± 0.027 mm/mm strain at break in contrast to the control which had 1.29 ± 0.23 MPa stress and 0.238 ± 0.033 mm/mm strain at break. The PEGDA gels also show similar thermal and thermal mechanical properties upon drying despite different curing times or water content. With the incorporation of nucleobases in the nucleobase-functionalized gels, DSC and DMA show a lowering of the T_g while TGA shows an increase in weight loss at lower temperatures. These studies set the basis for future work investigating the amplification of 3D printing through increased interlayer adhesion of novel polymers.