REU Program, Summer 2014
Polymeric Nanostructures for Delivering Drugs and Imaging Agents

Synthesis of PEG-*b*-PCL Block Copolymers for a Theranostic System

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A theranostic system brings advancement in personalized medicine through targeting a site, delivering a drug, time-controlled release of a loaded drug, and imaging the treatment. Polymer coated metal organic frameworks (MOF) can allow for both imaging and drug delivery. Biocompatible polycaprolactone (PCL)/poly(ethylene glycol) (PEG) block copolymers were investigated as MOF coatings. To prepare an amine-terminated block copolymer, caprolactone was first polymerized to yield a 1,500 g/mol oligomer with one carboxylic acid end and one hydroxyl end. Subsequently, a 1,000 g/mol PEG was coupled to the carboxylic acid terminal of the PCL, then the terminal alcohols on the PEG and PCL blocks were reacted with FMOC-Glycine. Finally, the FMOC protecting groups were removed to yield the primary amine functionality on each end. ^{1}H NMR confirmed the structures and molecular weights of the intermediate and final products. The PEG-*b*-PCL copolymer was coordinated to a manganese center of the MOF, and the complex was confirmed using TGA (the degradation temperature of the polymer was 206 °C; 10% of the weight remained at 600°C). Additionally, we proposed a new system to direct the PCL toward the center of the nanoparticle using carboxylate functionality. To synthesize NH_2-PEG-*b*-PCL-COOH, tosylation and HCTU coupling were both investigated as routes to synthesize bifunctional NH_2-PEG-OH. We first tosylated the PEG oligomer with a 1:1 stoichiometric ratio of toluenesulfonic acid, then obtained the mono-toslyated chains using column chromatography. The mono-tosylated PEG was functionalized with an azide then reduced to the corresponding amine, yielding an amine functional group on one end of the PEG oligomer and the original hydroxyl group on the other. This method led to low yield and a new synthetic route was performed where FMOC-glycine was coupled to the PEG in a 1:1 ratio and purified using column chromatography. The purified bifunctional PEG was then used to initiate ring-opening polymerization of caprolactone. MOF theranostic systems using PEG-*b*-PCL polymers is a promising field for nanomedicine.

Novel Photopolymers for use in Mask Projection Microstereolithography

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Mask projection microstereolithography (MSTL) is a form of additive manufacturing that involves the use of a photocurable resin to build a 3D structure in a layer-by-layer process. Up to this point, resin printability has been explored through a trial and error process using test prints. This paper proposes the use of photorheology to quantify resin properties affecting cure time to facilitate discovery of resins for use in mask projection MSTL. In this study, photorheology monitored evolution of the elastic and storage moduli of a resin as it was cured and thus was used to polymerize poly(ethylene glycol dimethacrylate) (PEGDMA), poly(propylene glycol diacrylate) (PPGDA), and Pluronic® L31. The cure time was estimated using the modulus crossover, shown to be within the vicinity of the gel point. For the initial study, 0.5 mL of sample was exposed to a UV light intensity of 5 mW/cm^2. It was found that PPGDA cured in 7.9 seconds, PEGDMA in 18.8 seconds, and Pluronic® L31 in 54.2 seconds. The differences in cure time are attributed to molecular weight, methyl substituents, and incomplete functionality of Pluronic® L31. Additionally, PEGDMA was copolymerized with the ionic liquid 4-vinylbenzyl trioctyl phosphonium bis(trifluoromethylsulfonyl) imide (TOPTf2N) and PPGDA was copolymerized with Tinuvin® 400. TOPTf2N with a functionality of one, hindered crosslinking

therefore increasing the cure time from 18.8 seconds to 41.9 seconds at 10 mol% TOPTf2N. Tinuvin® 400, a UV absorber, delayed PPGDA gelation from 7.9 seconds to 66.2 seconds at 0.15 wt% Tinuvin®. Increasing light intensity from 5 mW/cm^2 to 20 mW/cm^2 decreased cure time from 18.8 seconds to just 5.9 seconds for PEGDMA. Print resolutions were investigated using microCT and SEM imaging. Improved print resolution was observed with longer cure times for the PEGDMA and PPGDA systems. Photorheology is a powerful tool for exploring structure-processing relationships with the ultimate goal of creating viable resins with improved printability.

Hydroboration of Cellulose Esters with 9-BBN

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Hydroxyl functionalized cellulose derivatives have been shown to provide novel properties such as hydrophilicity and water dispersity. These properties make them potentially good candidates to be used in the formulation of amorphous solid dispersions to increase drug aqueous solubility and bioavailability. Synthesis of hydroxyl functionalized cellulose ester derivatives via hydroboration/oxidation has been shown to be successful for the first time. Cellulose ester derivatives with terminal olefin side chains of five and eleven carbons (cellulose omega (ω)-unsaturated esters) were first synthesized by esterification of commercially available cellulose esters (i.e. cellulose acetate, cellulose acetate propionate, and cellulose acetate butyrate) with either 10-undecenoyl chloride or 4-pentenoyl chloride. Subsequent one-pot, two-step hydroboration/oxidation reactions of these cellulose ester derivatives were performed, using 9-BBN as the hydroboration agent, and then oxidizing the intermediate to a hydroxyl group with H_2O_2. Both NaOH and NaOAc were studied as bases in the oxidation step. The much weaker NaOAc minimized the hydrolysis of ester linkages and chain-scission, yielding product. In the ^{13}C NMR, complete disappearance of the terminal olefin peaks at 114 and 139 ppm, and the appearance of a peak at 61 ppm for the methylene alpha to the hydroxyl group confirmed the hydroxylated product. Other characterization methods, including FTIR, ^{1}H NMR, and HSQC 2D NMR, also confirmed the synthesis of a family of novel hydroxyl functionalized cellulose esters unattained by previous methods. Additionally, SEC was performed to examine polymer degradation. Cellulose acetate butyrate undecenoate with M_n = 87.6, showed a decrease in M_n to 31.4 following hydroboration, indicating that a shorter reaction time might be preferable.

Adsorption of Human Hemoglobin onto Chitin and Cellulose Surfaces

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The adsorption of hemoglobin onto chitin and cellulose has yet to be modeled. In this work, hemoglobin solutions were prepared in 50 mM, pH = 7.4 phosphate buffer at concentrations of 0.0125 – 0.5 mg·mL^{-1} and adsorption onto gold, chitin, and cellulose surfaces was studied with surface plasmon resonance (SPR). First, chitin and cellulose were modified with trimethylsilyl groups to improve solubility in common organic solvents. Solutions with concentrations of 0.05 mg/mL were prepared in chloroform for chitin and toluene for cellulose for the purpose of preparing spin-coated films. Polymer films were spin-coated onto gold SPR surfaces, and the hydroxyl groups were regenerated using 10% aq. hydrochloric acid vapor. Hemoglobin solutions were flowed across gold, chitin, and cellulose surfaces and resonant angle changes from SPR were converted into surface concentrations (Γ_{SPR}). In all systems, hemoglobin failed to cover the entire surface, therefore the equilibrium Γ_{SPR} were less than a monolayer. Hemoglobin adsorbed onto gold surfaces with $\Gamma_{SPR} = 1.2$ mg·m^{-2} and reached equilibrium within 70 minutes, independent of hemoglobin concentration. Hemoglobin

adsorbed faster onto gold than chitin, however Γ_{SPR} for adsorption onto chitin exceeded gold at high solution concentrations. For cellulose surfaces, hemoglobin adsorption led to smaller Γ_{SPR} at all concentrations tested relative to chitin. Adsorption rates for hemoglobin onto cellulose were substantially smaller than those observed with gold and chitin. These studies revealed stronger interactions between hemoglobin and chitin than hemoglobin and cellulose and these findings are consistent with strong interactions observed between hemoglobin and chitosan derivatives that are used in drug delivery formulations.

nanoBEAD: A Revolutionary Biohybrid Vehicle

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Due to the active tumor targeting property of bacteria, biohybrid vehicles, bacteria interfaced with nanoparticles, are predicted to improve drug delivery by reducing the required drug dosage and improving interstitial transport. A revolutionary biohybrid vehicle, termed a nanoBEAD, is an optimal design because it exhibits a higher loading capacity and it is small, thus allowing for efficient penetration of tumors and drug delivery. Recent studies showed that nanoBEADs can successfully penetrate tumor spheroids, but the mechanism of transport is unknown. It was hypothesized that proliferation may be the source of the bacterium's intratumoral penetration instead of chemotaxis. The goal of my research was to compare the transport of non-proliferating bacteria and proliferating bacteria. To inhibit proliferation x-ray irradiation was used. Through experiments it was shown that the motility medium impacts the viability and motility of bacteria, and that researchers should investigate alternative media. The size of the tube also impacted the motility and viability of the bacteria. It was proven that the largest Eppendorf provided the best environment for bacteria to grow. Future research will investigate the optimal dosage of x-ray irradiation that will inhibit the proliferation of bacteria. Increasing our knowledge about the mechanism of intratumoral transport of bacteria will allow researchers to improve biohybrid vehicles and ultimately improve drug delivery.

Synthesis and Solution Rheology of Imidazolium Containing Polyesters for Electrospinning

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Synthesis of novel imidazolium containing monomers was investigated due to the potential to create electrospun scaffolds for biomedical applications. Butyl derivatives of imidazolium polyesters have the potential to lyse endosomal membranes and provide a route for endosomal escape and DNA transfection. Both methyl and butyl derivatives of imidazolium containing diol monomers were synthesized via a 3-step organic synthesis. Molecular structures of products were confirmed using ^{1}H NMR and mass spectrometry. Poly(3-(3-hydroxy-2-(hydroxymethyl)-2-methylpropyl)-2-methyl-1H-imidazol adipate) (PHI) was synthesized from the methyl diol via step growth polymerization at 130° – 220°C. Gel binding studies indicated that the PHI bound DNA, which offers promise in DNA transfection and drug delivery. The butyl derivative was unable to be synthesized due to the diol's low thermal stability. Thermogravimetric analysis (TGA) in N_2 indicated a T_d of 137 °C while isothermal TGA above 130 °C showed degradation within 30 minutes. Poly(neopentyl adipate) (PNPA), an uncharged analog of PHI, was synthesized and electrospinability was compared to the charged, imidazolium containing polyester. Electrospinning of PNPA did not result in fiber formation due to its low molecular weight. Solution rheology confirmed the lack of an entanglement concentration (Ce). Initial electrospinning of methyl imidazolium PHI resulted in droplets forming on the collector plate instead of fibers. In order to improve electrospinability, PHI was blended at various ratios with poly(ethylene oxide) (PEO, 200,000 g/mol). An optimal blend ratio of 80:20

PHI and PEO (respectively) resulted in uniform fibers with consistent morphology and diameters of 1-2 microns.

Fabrication and Characterization of Quantum Dot-Incorporated Carboxymethyl Cellulose Acetate Butyrate Nanoparticles

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The distribution and transport of nanoparticle drug delivery systems are not fully understood. There are many barriers that limit the effectiveness of orally administered nanoparticles. Thus, fully understanding the subcellular biochemical processes involved in the transport of these nanoparticle carriers would augment our knowledge of drug activity and disease. Quantum dots (QDs) present new opportunities to advance intra- and extracellular imaging due to unique optical properties not otherwise seen with traditional chemical fluorophores. The novel cellulose ester, carboxymethyl cellulose acetate butyrate (CMCAB), was selected to provide the polymer matrix that encapsulated the quantum dots. CMCAB shows promise for use in drug delivery systems because of its low toxicity, high stability, pH responsivity, and high glass transition temperature. Flash nano-precipitation, a technique that utilizes high supersaturation to control particle size, was used to fabricate the nanoparticles. Dynamic light scattering of CMCAB-only nanoparticles showed sizes of 233±37 nm with a PDI by dynamic light scattering of 0.20±0.04 after redispersion in deionized water. Dynamic light scattering of CMCAB nanoparticles with hydrophobic CdSe/ZnS quantum dots incorporated at a 3 wt% loading yielded particle sizes of 217±4 nm with a PDI of 0.17±0.02 after redispersion in deionized water. Examination of the fluorescent characteristics of this amalgam, obtained via inverted fluorescence microscopy, were promising and revealed visible fluorescence of the quantum dot-incorporated CMCAB particles.

Microfabricated Magnetic Patterns for Improving Microfluidic Immunomagnetic Separation

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In order to analyze cell samples for rare circulating tumor cells (CTCs), developing microfluidic devices for immunomagnetic cell separation is of high clinical interest. Microfluidic devices are networks of channels created in polymers such as polydimethylsiloxane (PDMS) in which fluid samples can be flowed through. Building on the strength of combining microfluidics and immunomagnetic separation, this paper proposes the fabrication of a magnetic pattern on the surface of a microfluidic channel to improve cell capture efficiency in the presence of an external magnetic field. Cells (RAW 264.7 macrophages) were mixed with antibody-coated magnetic nanoparticles (Dynabeads) before passaging. Cell capture was improved significantly at several flow rates in the presence of a magnetic pattern in addition to the external magnet, compared to capture in a channel with no magnetic pattern and only an external magnet. The capture rates with the pattern at 0.92, 1.84, and 2.76 μL/min flow rate, respectively, were 48.8±18.1%, 33.3±3.9%, and 31.5±9.1%. With no pattern, the capture rates at 0.92, 1.84, and 2.76 μL/min flow rate, respectively, were 20.8±6.4%, 7.9±4.1%, and 7.1±4.9%. The statistical significance of capture improvement was confirmed by t-test. A correlation between increased flow rate and increased proportion of captured cells bound directly to the pattern supports the role of the magnetic strip as the primary cause of additional capture; an average of 40.6% of captured cells adhered directly to the pattern at 0.92 μL/min, increasing to an average of 76.2% at 2.76 μL/min. Future techniques for directing cell capture may be developed based on the improved efficiency afforded by this technique.

Single Cell Directionality on Nanofibers

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In developmental biology, the extracellular matrix (ECM) provides biomechanical cues for stem cell specification. On flat substrates (i.e. polyacrylamide gels) with varied stiffness, cells exhibit directional commitment and "durotaxis": a preference for stiffer regions. Cell-ECM interactions on a more realistic model of a suspended fibrous scaffold is a groundbreaking field. Scaffolds were prepared using STEP (Spinneret based Tunable Engineered Parameters), a non-electrospinning, pseudo-dry spinning, nanofiber fabrication technique allowing for the continuous deposition of fiber arrays. The first layer of 600-nm diameter nanofibers were spun 500 μm apart from a 2M, 12% by weight polystyrene-xylene solution. A second layer of 400-nm fibers were spun orthogonally with a separation of 8 μm by using a 2M, 7% by weight polystyrene-xylene solution. The structural stiffness, k (N/m), of the aligned nanofibers follows a parabolic distribution along the suspended fiber length (minimum k~0.004 N/m at the center). This relates size, organization, and shape of the material to its ability to resist deformation. Mesenchymal stem cells (MSCs) were seeded onto the scaffold. This work characterizes the cell commitment process on a fibrous scaffold with varied structural stiffness, which can have regenerative applications when considering driving stem cell differentiation. MSCs on fibers were found to have a less rigid commitment to directionality than on flat substrates, switching directions often between 5-minute time lapse images. The protrusion counts observed at the leading and trailing edges of the cells, ranging from 2 to 5, can influence which direction the cell will travel. Both edges cooperated in directionality when the leading edge had 4 more protrusions than the trailing edge. By fixing and staining MSCs for paxillin, a key focal protein, we measured focal adhesions at the protrusions. Paxillin stain lengths were longer at the center of a suspended fiber, where the structural stiffness was lowest, and the leading edge migrated 1 μm/min faster through these low structural stiffness regions.

Hydrogen Sulfide Delivery

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In this novel study, reversible addition-fragmentation chain transfer (RAFT) polymerization and ring-opening metathesis polymerization (ROMP) were employed sequentially to prepare brush-first star polymers with H_2S-releasing capability. A novel, water soluble poly(acryloyl morpholine) macromonomer was prepared via RAFT polymerization using a norbornene-funtionalized chain transfer agent. A bis-norbornene thiooxime-containing crosslinker was utilized to impart thiol-triggered H_2S-release. This study focused on constructing large star polymers containing high payloads of hydrogen sulfide, which would readily degrade in the presence of cysteine and other free thiols into hydrogen sulfide and biologically friendly by-products. Size exclusion chromatography was used to determine molecular weight and polydispersity of all polymers. The macromonomer prepared by RAFT was 3,100 g/mol, matching the targeted molecular weight of 3000 g/mol, with good monodispersity at a PDI of 1.02. ROMP of this macromonomer yielded a bottle brush of 28,200 g/mol consistent with the desired degree of polymerization of 10. In the final step of the star synthesis, the concentration in THF was varied. At 50 mg/mL, dimers formed and at 75 mg/mL octimers formed. One hundred mg/mL was the optimal concentration to achieve high molecular weight without gel formation. The crosslinking converted the bottle brush to an 840,000 g/mol polymer containing approximately 32 arms and a PDI of 1.04. The results of this synthesis lend high potential for application work in the direct future for cell and hydrogen sulfide release studies.