FUNCTIONALIZATION OF CELLULOSE NANOCRYSTALS WITH FITC-LABELED GRGDS PEPTIDE FOR CANCER TARGETING

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Cancer cells overexpress $\alpha_v\beta_3$ integrin receptors, which are barely detectable on healthy human cells.[1] It has been shown that RGD peptide sequences bind strongly to these integrin receptors, and thus it was expected that conjugates of such peptide segments with cellulose nanocrystals would result in targeting of the nanocrystals to cancer cells. Our objective was to prepare and characterize a cellulose nanocrystal-GRGDS-FITC conjugate and compare it's structure to a cellulose nanocrystal-FITC control. Fluorescein-5'-isothiocyanate (FITC) was conjugated to a GRGDS peptide to form the fluorescent FITC-GRGDS. Cellulose nanocrystals were oxidized in the presence of TEMPO to form carboxylic acids on their surfaces, then the FITC-GRGDS was grafter onto the cellulose crystal surface by forming an amide bond. A control was also prepared for comparison where FITC was treated with ammonia, then was bonded to the cellulose nanocrystals (no peptide). Comparison of the UV-Vis spectra of the FITC-GRGDS-CNC with a strong absorbance at ~450 nm and the control FITC-CNC product confirmed that the FITC-GRGDS had been successfully attached to the CNC.

1. Park J. H., et al. *Journal of Controlled Release.* **2004***, 95,* 579-588.

Evaluation of the Bioactivity and Biocompatibility of Cellulose Nanocrystals for Bone Tissue Engineering

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This research sought to provide a basis for the use of cellulose nanocrystals (CNCs) and surface oxidized-nanocrystals (SO-CNCs) in bone tissue-engineering applications. The CNCs were prepared through sulfuric acid hydrolysis of wood pulp powder, then the crystals were oxidized to form carboxylic acids on their surfaces in a TEMPO-mediated oxidation. Atomic force microscopy (AFM), conductometric/potentiometric titrations and X-ray diffraction were used to characterize the the materials. The unmodified and modified cellulose crystals were evaluated to determine whether they could support calcium phosphate crystallization. The mineralization study was conducted by incubating CNCs and SO-CNCs at 37°C in Dulbecco's Phosphate Buffered Saline (PBS) containing Mg^{2+} and Ca^{2+} and in Simulated Body Fluid (SBF) 1x for 60 hours. Aliquots were removed and purified at 10-hour intervals. The height increase in AFM caused by deposition of calcium phosphates was considered a marker of mineralization, and XRD identified the minerals as calcium phosphates. The carboxylate-functional CNCs exhibited an earlier onset of calcium phosphate deposition as compared to the unmodified crystals. The relative fluorescence of the CellTiter-Blue® reagent secreted from pre-osteoblast MC3T3 cells confirmed that neither the SO-CNCs or the CNCs were toxic to the cells.

Study of Polymer Beacons for DNA Delivery by MRI

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Polymer beacons are being developed that allow simultaneous delivery of DNA therapeutics and tracking of the delivery vehicle through Magnetic Resonance Imaging (MRI). Results suggest that these polymer beacons can potentially track nucleic acid transfer *in vitro,* providing researchers with a better understanding of DNA delivery in order to develop more efficient theranostic agents. To obtain reliable and reproducible results for initial *in vitro* studies, experiments were conducted using an artificial matrix to mimic the properties of natural tissue. Our group optimized a MRI method to measure the diffusion properties of contrast agents through these artificial matrices. The rate of penetration of a clinical contrast agent, Magnevist©, through a *Gelatin A* tissue-mimic was measured by placing a solution of the agent on top of the hydrogel matrix and monitoring its transport downward through the gel. The signal intensity at each voxel was extracted from Paravision© and converted using a Bruker© converter program to a format that could be analyzed via Matlab©. We wrote a program that allowed us to visualize the MR images and average the signal intensity at vertical and horizontal positions throughout the image. The intensity at certain positions was plotted vs. time to estimate the distance the Magnevist© traveled through the tissue-mimic. The estimated value paralleled the calculated mean-square displacement value, and this led us to propose that self-diffusion was the main driving force governing contrast agent migration.

Synthesis of Anionic Copolymers with Anti-HIV Microbicide Activity

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Anionic polymers have been found to have a high amount of anti-HIV microbial activity through inhibition of the gp120 protein complex on the surface of the HIV virion. A sulfonate-protected neopentyl styrene sulfonate monomer was synthesized and copolymerized with maleic anhydride using reversible addition-fragmentation chain-transfer (RAFT) and conventional free-radical polymerizations, then the protecting groups were removed to form polyelectrolytes. Size exclusion chromatography of the protected copolymers showed that the number average molecular weights of two of these oligomers were 2100 and 4000 g/mol. In addition, a 2500 g/mol poly(ethylene oxide) macroRAFT chain-transfer agent was synthesized using DCC coupling and Grignard reactions. This macroRAFT agent was used to synthesize a block copolymer of 2.5kPEG-*b*-(styrene-*alt*-maleic anhydride), which was then post-reacted to yield the polycarboxylate

Polymers for Anti-HIV Microbicides

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In 2011, 2.5 million people became newly infected with HIV, and there were 1.7 million deaths due to AIDS. This epidemic rises at an alarming rate, showing the need for an effective topical microbicide that women can use to provide efficient protection against sexually transmitted diseases. Over the past two decades, researchers have investigated polymers as potential topical microbicides that can be applied vaginally to protect women from HIV and unintended pregnancies. Research has found that polyanions inhibit HIV-1, but in human clinical trials, the polyanions were ineffective in preventing the spread HIV-1. This research has investigated the synthesis and characterization of oligomeric polyzwitterions as potential microbicides. N,N-dimethylamino styrene was copolymerized with *tert*-butyl 4-maleimidobenzoate by conventional free radical polymerization. The *tert*-butyl esters were subsequently removed with trifluoroacetic acid to form anionic carboxylates. Size exclusion chromatography showed that the protected t-butyl ester form of the polymer had a M_n of 2163 g/mol and polydispersity of 1.6.

Adsorption of Mixed Linkage Glucans onto Regenerated Cellulose Surfaces

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Mixed linkage glucan, a hemicellulose component of the cell wall, is confined to the order Poales along with a few others. Among the vast order of Poales is the economically important family of grasses. Quartz crystal microbalance with dissipation monitoring (QCM-D) was utilized to measure the adsorption of mixed linkage glucans (MLGs) onto cellulose surfaces regenerated from spincoated trimethylsilyl cellulose films (RG). The RG surfaces had a thickness of 10 nm and a root-mean-square surface roughness of 1 nm. The adsorption of two MLG samples from different sources, lichenan and barley beta-glucan, was studied as a function of temperature and concentration. Temperatures ranged from 15 to 50 °C while concentrations ranged from 0.01 to 0.1 percent by mass. A Voigt-based viscoelastic model was used to fit the time dependent adsorption profiles for the soft MLG layers. Barley, which had a molecular weight four times larger than lichenan, formed a more rigid layer i.e., a higher value of elasticity. An increase in temperature led to a decrease in viscosity, density, and thickness and an increase in elasticity for lichenan. This observed temperature dependence is evidence that a more rigid, thinner, and less dense layer formed at higher temperatures. It also suggests that at lower temperatures the layers have more bound water. No clear conclusions could be drawn about the effect that differences in structure between lichenan and barley MLG had on the adsorption process. Differences in elasticity between lichenan and barley MLG surfaces were most likely due to differences in molecular weight between the two polysaccharides.

Manganese-Organic Frameworks Embedded in Hydrogels and Microspheres

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Photodynamic metal-organic frameworks (MOFs) were synthesized by microwave assisted solvothermal synthesis of $MnCl_2$ and azobenzene-4,4'-dicarboxylic acid (ABDA). These Mn/ABDA MOFs were characterized by dynamic light scattering and scanning electron microscopy, showing particle sizes of around 100 nm. Mn/ABDA MOFs were loaded into agarose hydrogels and gelatin microspheres and their dispersion from the hydrogels and release from the microspheres were investigated. The gelatin microspheres coordinated to the Mn/ABDA MOFs through lysine residues and their presence was confirmed by electron microscopy. Diffusion of the MOFs was measured through UV-Vis spectroscopy by monitoring the absorbance of trans-ABDA. The release profiles indicated that MOF diffusion rates depended on the crosslink density of the agarose matrices.

Synthesis of Melt Processable Poly(Acrylonitrile-*co*- Methyl Acrylate) Copolymers

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Polyacrylonitrile (PAN)-based copolymers are currently solution-processed to produce acrylic fibers, and the use of solvents detracts from the economics of the process. Melt extruded PAN copolymer fibers and films could have many useful properties, but there is no way to melt process these materials at this time. Our research focuses on the synthesis of PAN copolymers via suspension free radical polymerization. Ideally, we want to create a copolymer with sufficiently high acrylonitrile content to retain the desirable properties acrylonitrile has such as, thermal stability, chemical resistance, and low permeability, as well as synthesizing a high strength and melt processable copolymer. The results showed the amount of dodecyl mercaptan (chain transfer agent) incorporation in the product calculated via NMR analysis provided a good indication of molecular weight assuming the polymer yield is high. Also, ammonium persulfate (APS) produced higher molecular weight AN/MA copolymers due to its high solubility in the immiscible water phase of the suspension polymerization. Thermal gravimetric analysis (TGA) showed that using AIBN as an initiator produced copolymers that degraded at the same temperature (~300°C) as ammonium persulfate initiated polymers. This indicated that AIBN produced polymers with high enough molecular weight to maintain chemical stability as effectively as very high molecular weight APS initiated copolymers.

Synthesis and Characterization of Diblock Polymer Zwitterions Containing Phosphonate Anions

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Poly(ethylene oxide) and poly(ethyloxazoline) are water soluble polymers with potential to be utilized in drug delivery and bioimaging due to their biocompatibility. Poly(ethylene oxide-*b*-ethyloxazoline) diblock copolymers were synthesized in a ring-opening cationic polymerization of ethyloxazoline using a monomethoxy poly(ethylene oxide) macroinitiator that was tosylated at one end, then the endgroups were terminated with piperidine. After the diblock copolymers were formed, controlled concentrations of the pendent amides on the poly(ethyloxazoline) block were removed under acidic conditions to yield poly(ethylene oxide-*b*-ethyleneimine) diblocks. The degree of hydrolysis of amide to amine was controlled at both 20% and 55% hydrolysis. The secondary amine groups on the polymer backbone were subsequently added across the vinyl bond of diethylvinyl phosphonate in a facile Michael reaction conducted in water, then the phosphonate ethyl groups were removed in a two-step deprotection reaction first using trimethylsilyl iodide, then methanol. Greater than 95% addition of the phosphonate groups was achieved. It was found that the resultant polyammonium phosphonate zwitterions could bind high concentrations of Mn^{++} ions that are under investigation as potential bright field contrast agents for magnetic resonance imaging.

Polyurethane Electrolytes Based on Phosphonium Cations and their Polyplexes with Nucleic Acids

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Nucleic acid delivery is a novel therapy developed with the potential to treat cancer, genetic disorders, and infectious diseases. Cationic polymers, usually containing ammonium ions, have been investigated to form multivalent complexes with anionic DNA (i.e., polyplexes) through electrostatic interactions. Recent research in our laboratories utilized phosphonium ions as the positively charged source, which resulted in increased thermal stability, DNA binding, and minimized cytotoxicity. In this study, a series of thermally stable phosphonium-based polyurethanes were synthesized with ethyl- and butyl- alkyl groups with weight average molecular weights greater than 20 kg/mol. The polymers with higher charge content bound DNA at a lower polymer to DNA ratio, while lower charge content in the polymer resulted in binding at higher polymer to DNA ratios, or no binding at all. Polyplex sizes and colloidal stability studies revealed hydrodynamic diameters from 125-75 nm in diameter with good colloidal stability in water. Polyplexes in salt solution ranged from 225-350 nm in diameter and were not colloidally stable. Future studies will investigate serum stability, zeta potentials to assess charge characteristics, cytotoxicity, transfection efficiency and cellular uptake.

Amphiphilic Pullulan Ethers as Biocompatible Micelles

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Pullulan is a maltotriose polysaccharide synthesized from starch by the fungus *A. pullulans.* It is used commercially as an edible film and a thickening agent. Pullulan and its derivatives are being studied for use in drug delivery applications. The synthesis of 6-carboxypullulan ethers could provide amphiphilic pullulan derivatives for enhanced drug delivery. In this research, pullulan was first oxidized by sodium hypochlorite and sodium bromide using TEMPO (2,2,6,6-tetramethylpiperidin-1-yl)oxyl) as a catalyst. Complete oxidation was confirmed by NMR and etherification reactions were performed with various bromoalkyl and iodoalkyl reagents. The degree of alkyl substitution obtained was determined by proton NMR data. Shorter chain alkyl halides produced high degrees of substitution, while long chains only yielded low degrees of substitution. Hydrolysis experiments were carried out to confirm that ethers had been formed at the carbon 2 and 3 hydroxyls as opposed to esters on the carbon-6 carboxylates. NMR data showed no significant change in the degree of substitution following hydrolysis, supporting the formation of ethers. Etherification was also supported by the lack of ester peaks in the IR spectra. These amphiphilic polymers formed micelles at low concentrations, offering another possibility for their use in drug delivery. Future research will include evaluating the polymer's affinity for specific drugs and the synthesis of carboxypullulan esters.

Synthesis of Acetylated Alginates

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Alginates are unbranched polysaccharide copolymers comprised of β-D-mannuronic acid (M) and its C-5 epimer, α-L-guluronic acid (G). As natural polysaccharides, alginates play an important role in medicine. They are used as dressings to treat wounds, and alginate hydrogels can also be used in treating type II diabetes. Several strategies to synthesize alginate derivatives have been practiced, but the ability to conduct the reactions under moderate temperatures with organic solvents would open up new possibilities towards drug delivery. This paper focuses on the acetylation of alginate. The acetylation reaction was optimized by varying concentrations of pyridine and acetic anhydride. ^{1}H NMR and titration were used to analyze the degree of substitution (DS) of the alginate product. DS was controlled by varying the molar equivalents of the reagents. In addition, freeze drying large volumes of extremely viscous material is time consuming, so strategies were developed to purify alginates by precipitation in organic solvent mixtures.

Synthesis of Chemical Linkers for Bionanoconstructs

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Poly(ethylene glycol) (PEG) lipids play important roles in modern drug delivery methods. Recent research has led to the use of PEG lipids for drug encapsulation by micelles. Other studies have focused on the utilization of PEG chains on the exterior of liposomes for drug delivery. PEGylation increases the therapeutic effectiveness of the liposomes. A novel class of drug delivery vehicles, polymer nanoparticles, hold potential for superior performance relative to conventional liposomes due to enhanced stabilities. The design of bilayer-coated nanoparticles and other bionanoconstructs requires the synthesis of “linker” molecules that comprise a head-spacer-lipid architecture. Our research has focused on the synthesis of linker molecules to form tricarboxyl-PEG-cholestanol. Synthesis of PEG-cholestanols was accomplished by initiating the living polymerization of ethylene oxide with cholestanol alkoxide and acidification of the endgroup with HCl. PEG lipids were synthesized with a systematic series of oligomeric molecular weights (500 – 5000 g/mol). Each crude polymerization product was fractionated via column chromatography to reduce the poydispersity index (PDI) below 1.05. Fractionated products were then reacted with Weisocyanate™, and subsequently fractionated via column chromatography. Deprotection of the triester was accomplished by reaction with trifluoroacetic acid and purification by carbon treatment.

Synthesis and Characterization of Novel Cellulose ω-Carboxyalkanoates for the Oral Delivery of Crystalline Drugs

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The formation and properties of amorphous solid dispersions of cellulose ω-carboxyalkanoates with hydrophobic drugs have been investigated. The objective is to trap the drug in an amorphous state in a blend with the polymeric matrix to inhibit nucleation and crystal growth. These cellulose ω-carboxyalkanoates were synthesized by reacting acyl chlorides with cellulose hydroxyl groups using a protection/deprotection strategy to avoid crosslinking. Long chain dicarboxylic acids were partially esterified with benzyl groups and purified by pH separations. The monobenzyl half esters were converted to acid chlorides and reacted with cellulose acetate and cellulose acetate propionate. The benzyl protecting groups were removed through hydrogenolysis with a $Pd(OH)_2/C$ catalyst. The chemical structures of the cellulose derivatives were analyzed by ^1H NMR. These novel polymers were used to produce solid dispersions with the hydrophobic HIV drug Ritonavir. X-ray diffraction and differential scanning calorimetry showed that the Ritonavir was trapped in the amorphous state with the polymer. A proof-of-concept drug release test and analysis by HPLC confirmed that these cellulose ω-carboxyalkanoates enhanced drug solubility and also afforded enhanced solubility over time.

Properties of Novel Amphiphilic Copolymers in Water

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The human immunodeficiency virus (HIV) causes an incapacitating disease of the immune system that has been famously difficult to treat. The increasing population of HIV patients in concert with the challenges facing available pharmaceutics motivates research aimed at enhancing modern treatment capabilities. This research has focused on characterizing a novel series of poly(ethylene glycol-*b*-butylene oxide-*co*-ethoxy vinyl glycidyl ether) (PEG-*b*-P(BO-EVGE)-*b*-PBO) tri-block copolymers used to form micelles for delivering HIV therapeutics. The polymers consisted of a unique tapered block with a functionalizable vinyl group positioned between a hydrophobic and hydrophilic block. The vinyl ether functional groups were post-reacted with functional thiols to place carboxylic acids and alternatively amines between the hydrophilic and hydrophobic blocks so that the series of materials containing 1) vinyl ethers, 2) anionic carboxylates and 3) cationic ammonium ions. The structures of the polymers were investigated in water as functions of pH. Micelles formed by the parent polymer with the vinyl ethers in the backbone had the lowest critical micelle concentration (CMC) of 0.01 mg/mL, and the largest hydrodynamic diameter, 36 nm (neutral pH). The large diameter suggested a cylindrical micelle shape. Incorporation of charged moieties in the middle block decreased the bulkiness of the hydrophobic tail. The functionalized polyelectrolytes exhibited both higher CMCs and smaller diameters at neutral pH, suggesting that spherical micelles had formed. Varying the pH revealed that the assemblies of the polyelectrolytes became similar to that of the "parent polymer" as the charge was suppressed. The low CMC, small micelle size, and capacity for crosslinking in the core suggest that this polymer has great potential for drug delivery with increased bioavailability and control of drug release rates.

Analysis of Block Copolymers by SEC with Multiple Detectors

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In Size Exclusion Chromatography (SEC), there are two methods for determining absolute molecular weights of polymers. One method utilizes a *Universal Calibration* that relies on the combination of a concentration (i.e., refractive index, RI) detector and the viscosity detector. The other method utilizes a static light scattering detector (i.e., multi-angle laser light scattering, MALLS) in combination with an RI detector to measure the concentration dependence of the refractive index signal (dn/dc). Both of these methods assume that dn/dc remains constant over the entire distribution of molecular weights. Analysis of block copolymers is often complicated by variances in dn/dc across the distribution that result from aspects of the synthetic method. This research analyzed poly(ethylene oxide-*b*-ethyloxazoline) diblock copolymers by SEC that were prepared by utilizing a poly(ethylene oxide) macroinitiator having a tosylate ester to initiate and polymerize ethyloxazoline in a living cationic polymerization. The materials were analyzed in N-methylpyrrolidone utilizing three detectors (viscosity, static light scattering and refractive index). The refractive index increment of the poly(ethylene oxide) in this system was extremely low relative to the polyethyloxazoline. The viscosity detector signal at the elution volume of the poly(ethylene oxide) macroinitiator showed a strong signal while there was no significant refractive index concentration signal at that elution volume. Unfortunately, this showed that there was significant macroinitiator remaining in the diblock copolymer materials. This meant that the initiation rate of the ethyloxazoline was very slow relative to propagation and signified that different initiators would be needed. This would not have been evident without the viscosity detector since the dn/dc of the macroinitiator was low and its oligomeric molecular weight was too low for the light scattering signal to be significant.