Optimization of polymeric nanoparticle synthesis for drug delivery to lung cancer

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Motivation/Background: Nanomedicines have shown promise in the treatment of many diseases, including cancer. However, the formulation of nanomedicines requires consistent biological behavior and pharmacological profile. Unfortunately, nanoparticles are typically produced in small, non-homogenous batches which leads to difficulty in reproducibility and scale up for commercial production.

Objective Statement: Evaluate and optimize the factors that most affect particle size, polydispersity, and zeta potential. Test predictive equation to determine proper processing parameters for desired particle size.

Method/Approach: To study processing parameters, nanoparticles are produced with flash precipitation method using polystyrene as the model polymer. Optimization factors investigated thus far include solvent to anti-solvent ratio, stir rate, and injection flow rate.

Results: Size was not statistically different between predicted and measured values for both medium ($p = 0.187$) and large nanoparticles ($p = 0.159$). Batch to batch size and polydispersity were consistent for all sizes using controlled nanoprecipitation method.

Conclusions: We have developed a predictive model based on operating parameters for nanoparticle synthesis by controlled precipitation.

Protecting Biological Implants from Host Immune Response

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Motivation/Background: Foreign materials introduced into biological systems initiate an innate host immune response called the foreign body response which leads to clinical device failure. Interventions to minimize this response include altering the surface to minimize protein absorption and release of anti-inflammatory drugs. A method to inhibit the foreign body response is needed.

Objective Statement: Develop an *in vitro* model that mimics the innate immune response and inflammation.

Method/Approach: The THP-1 cell line is a human leukemia monocytic line which will be the basis of our immune response model. These cells can differentiate into M1 and M2 macrophages and into dendritic cells (DC). Marker genes combined with qPCR provide quantifiable data of differentiation. Surface markers and cytokine levels are obtained through immunofluorescence and benchmarked against inflammatory agonists to quantify the level of inflammation response.

Results: Initial experiments will focus on characterizing the phenotypical changes between the monocyte and differentiated cell types (M1, M2, DC), followed by characterizing changes when stimulated with lipopolysaccharide.

Conclusions: Ultimately this model will be used to screen interventions designed to protect biomaterials from the foreign body response.

Synthesis and degradation of Biodegradable polymers

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Motivation/Background: Biodegradable polymers (Polyanhydrides, Polyesters and Polyethers) having different properties which can be tailored into copolymers for use in biomedical applications.

Objective Statement: Optimization of reaction parameters (Temperature, Pressure, Reaction Sequence and Reaction Time) for the synthesis of homopolymers of lactic acid (L.A) and sebacic acid (S.A). Development and validation of degradation (COMSOL) model which accounts for polymer connection bonds and monomers used.

Method/Approach: In this study PLA and PSA were synthesized using direct polycondensation polymerization without catalysts, harsh solvents and initiators, thereby avoiding additional purification steps and possible toxicity from those components. In this study the following parameters varied are: temperature (140- 250°C), pressure (1 atmosphere to vacuum), time (5 hours to 12 hours), and pre-acetylation of monomers.

Results

Synthesized homopolymers were characterized by proton (H)-NMR to determine polymer composition and determine average molecular weight. The synthesized homopolymers of sebacic acid and lactic acid are show in figures.

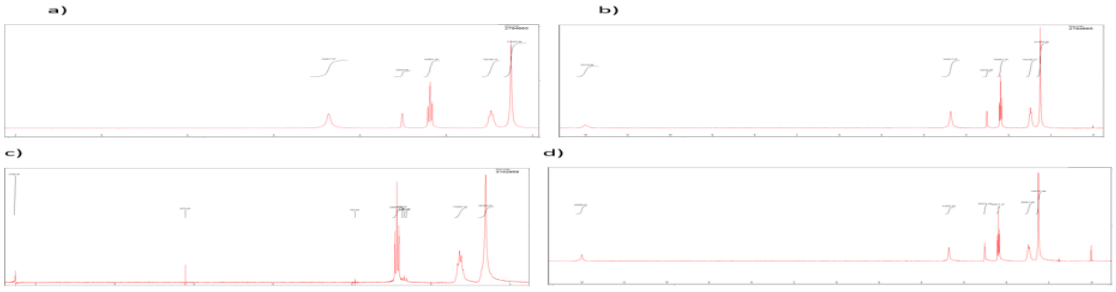

Figure 4: H-NMR spectra of monomers and polymer. a) Sebacic acid b) Lactic acid. c) Poly Sebacic anhydride d) Poly Lactic acid.

Conclusion

We have successfully synthesized and characterized homopolymers from sebacic acid and lactic acid by melt polycondensation. These operating conditions will inform further experiments into the synthesis of random, diblock and triblock copolymers.

The Spontaneous Behavior of Adhesion Molecules of Vascular Smooth Muscle Cells in Predicting Atherosclerosis Onset.

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During Atherosclerosis vascular smooth muscle cells (VSMC) alter in structure and function. N-cadherin mediated attached VSMCs detach from neighboring cells and migrate towards the intima. This event highlights one of the key and crucial steps in atherosclerosis progression. Several disciplines have come together to study and observe live cellular behavior. The atomic force microscope is a strong tool used to investigate real-time dynamics of VSMCs. Our aim was to merge AFM data with a unique analytical method to extract and monitor spontaneous oscillations released by VSMCs. Atherosclerotic B6.129P2-Apoe^{tm1Unc}/J and wild type (WT) mouse VSMCs were used to study their t time series N-cadherin and α5β1 Integrin mediated adhesion and monitor their component-wise relationship with oscillatory cell adhesion behavior. Results showed that the total N-cadherin and α5β1 Integrin mediated adhesion forces for WT and ApoE VSMCS had different profiles. This distinctive tool might serve as a means to analyze diseased and healthy VSMCs physiological function and reveal the component-wise relationship between oscillatory behaviors of cell-cell adhesion.

Transparent Titanium Dioxide Nanotubes: Processing, Characterization, and Application in Establishing Cellular Response Mechanisms

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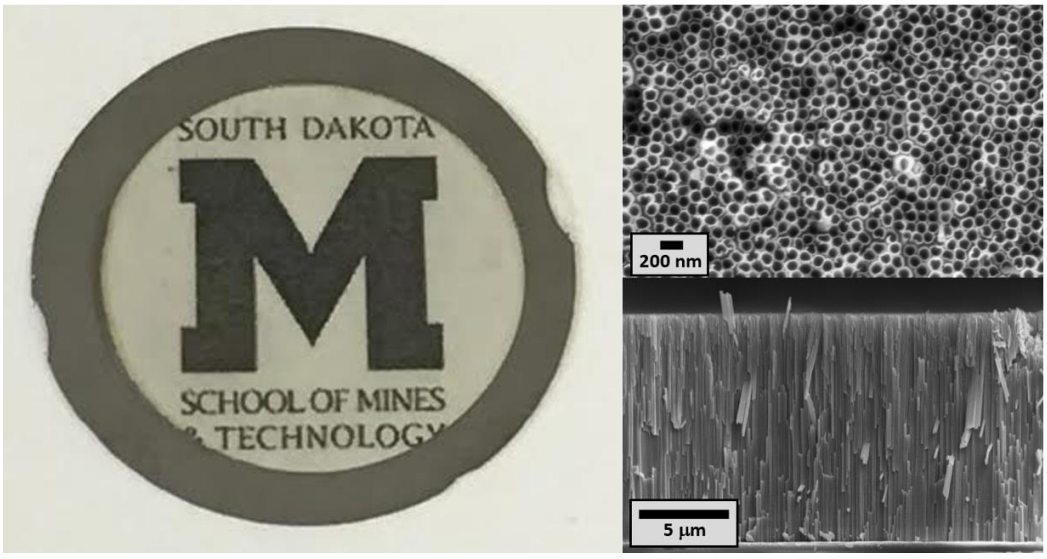
Motivation/Background: Titanium dioxide (TiO_2) nanotubes have been shown to strongly enhance bone cell behavior and, consequently, have gained attention as potential osteogenetic surface treatments for titanium-bone implants. The exact mechanism by which TiO_2 nanotubes influence cellular function remains controversial, partly due to limitations in existing cellular imaging methods for opaque substrates (such as titanium).

Objective Statement: By fabricating transparent TiO_2 nanotube imaging platforms, this work aims to establish the mechanistic relationship between nanostructure and bone cell response to ultimately inform the development of advanced osteogenetic surface treatments for titanium-bone implants.

Method/Approach: This work began by identifying and establishing the relationship of TiO_2 nanotube fabrication conditions to improve the production of transparent TiO_2 nanotube coatings with controlled diameters. Pre-osteoblast mouse cells (MC3T3-E1) containing fluorescently-tagged focal adhesion protein vinculin and cytoskeletal filament actin were implemented to record real-time, cell-substrate interaction mechanisms via conventional confocal fluorescent microscopy and fluorescent lifetime imaging (FLIM).

Results: Transparent TiO_2 nanotube platforms demonstrated high optical clarity when compared to glass substrates, and were used to clearly capture cellular adhesion, outgrowth, and migration responses to TiO_2 nanotubes.

Conclusions: It is the hope of this work to further develop advanced imaging techniques including FLIM, FRET, and lattice-light sheet microscopy to begin defining important surface material properties influencing initial and prolonged cell growth.



Title: Polyethylene Oxide as a Next Generation Platform for Drug-Coated Balloon [Single and Multiple Release Applications]

Authors: Jordan Anderson[1], Sujan Lamichhane[1], Thomas Vierhout[1], Tyler Remund[2], Katie Pohlson[2], Amber Wolf[2], Angela Vandenhull[2], Brandon Whipple[2], Melanee Clark[2], Daniel Engebretson[1], Patrick Kelly[2]

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Drug-coated balloons (DCBs) are currently used to treat various cardiovascular diseases by opening up the narrowed arteries and locally deliver antiproliferative drugs such as paclitaxel (PAT) to prevent restenosis (artery re-narrowing). Recently, we have developed a DCB that contains a polymeric coating (polyethylene oxide – PEO) to precisely control the delivery of drug (PAT) from the balloon. The PEO based DCB has shown to be safe and effective through minimal particulates shed and therapeutic drug retained in the tissues at day-30, respectively. However, multiple stenosis or blockages are often present in peripheral artery disease patients, particularly those with diabetes. Current DCB technology limits them to treating a single stenosis or blockage site; therefore, multiple DCBs are needed to treat the typical patient. Hence, there is a need in the marketplace for a single DCB able to treat multiple sites, which would be of great benefit, both procedurally and financially, to the patients. In this study, we have developed multiple-release bilayer and multiple-release multilayer PEO drug-coated balloons for the treatment of two inflation sites. The characterization, efficacy, and safety of the PEO balloons were determined through an in vitro model. The coating on the balloons was smooth and mostly homogenous before each treatment inflation. Both the multiple-release bilayer and multiple-release multilayer balloons provided an excellent control in delivering similar drug concentrations in two separate arteries in an in vitro model. Also, the particulates produced from the PEO balloons were negligible compared to the commercially available DCB. Thus, this study demonstrated the use of PEO as a next generation platform to safely and effectively control the delivery of drug from balloons in two separate treatment sites for the treatment of multiple blockages.

A 3-Dimensional Tubular Scaffold for Treating Esophageal Atresia

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Motivation/Background: Esophageal Atresia (EA), is a disease that is defined as a malformation of the esophagus that is found in newly born children. This disease is fatal if not properly treated. The current treatment of the disease involves immediate surgery to correct for the anomaly.

Objective Statement: In this study the focus was using the biocompatible and biodegradable poly (glycerol sebacate) (PGS) as our polymer material to serve as the tubular structure for our scaffold. This structure would serve as a bridge to allow for the growth and development of a new esophagus.

Method/Approach: PGS polymer is formed in two stages. The first being the development of the pre-polymer, followed by the 3D tubular scaffold being developed by finishing the last of the cross-linking steps in a mold.

Results: Tested the mechanical properties both longitudinally and around its circumference to get an idea of its tensile properties. Check with SEM and MTT assay that cells were able to grow on the material.

Conclusion: Obtained a 3D tubular polymer that matches the mechanical properties of the target organ. Cells were proven in both quality and quantity grow on the scaffold.

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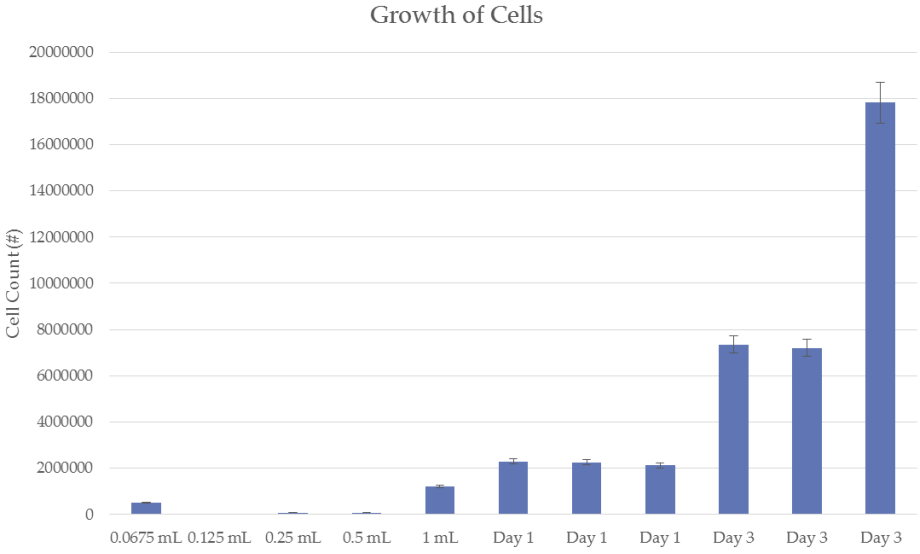

The effects of membrane cholesterol and substrate stiffness on the mechanics of vascular smooth muscle cell

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Although there have been many different medical advances atherosclerosis still remains the major cause of cardiovascular disease (CVD). Cholesterol and arterial wall stiffening play crucial roles in contributing to the cause of atherosclerosis. Vascular smooth muscle cells (VSMCs), which help make up the arterial wall, are dynamic systems constantly interacting in a bidirectional manor with neighboring cells and the external matrix that surrounds the cells. In the atherosclerotic environment VSMCs experience major changes that induce a phenotypic switch to occur. Recent studies have shown that approximately 40% of foam cells, the major contributors to plaque growth, are smooth muscle cell derived. Cellular adhesion to the extracellular matrix (ECM) is critical for cellular migration to occur, thus it is important to study how a major contributor to atherosclerosis, such as cholesterol and varying substrate stiffness, affects this process. Cholesterol is a potential important regulator of VSMC mechanical functions. In this study we tested the hypothesis that cholesterol manipulation in VSMCs may have a regulatory role in integrin-mediated adhesion, and alter the VSMCs sensory abilities to ECM mechanical properties. Our data shows that cholesterol manipulation and extracellular mechanics may synergistically regulate biomechanical functions of VSMCs in an atherosclerotic environment.

NanoStringDisc: a NanoString data analysis workflow for the discovery of significant differentially expressed genes

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Motivation/Background: The NanoString nCounter© system is a recent technology that can be used to overcome the key clinical challenge of reproducible gene expression analysis. This technology is uniquely able to address this challenge due to the sensitivity of the technique, the capability to directly read mRNA or miRNA counts without amplification, and the superior performance with regard to formalin fixed paraffin embedded (FFPE) samples. Because of the newness of this technology, there are limited options for analysis workflows.

Objective Statement: This study describes a generalized workflow for the differential expression analysis of NanoString data that is open source and modular and has the potential for additional downstream analysis.

Method/Approach: The data from raw RCC files is imported, extracted, normalized, and assessed using a quality control package. NanoStringDiff and DESeq2 will be utilized for differential expression analysis and then the results will be visualized using cross-method heatmaps and cross-experimental condition heatmaps. Output data includes the genes within the heatmap clusters, which can be utilized for further downstream analysis. The utility of the workflow will be verified by analysis of a custom miGRE assay.

Conclusions: Results from the differential expression workflow applied to the NanoString data suggest that PTK7 may be a gene of interest in ATRT.

Food Grade Protein Biopolymer Based Nanoparticles for Oral Drug Delivery Applications

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Motivation: The goal of this study is to develop and test the feasibility of using food grade protein biopolymers for oral drug delivery applications.
Objective statement: Core-shell nanoparticles were developed using Zein (Z), a water insoluble corn protein as the core and β-lactoglobulin (LG) and whey protein isolate (WP) as the shell.
Method: Nanoparticles were characterized by measuring particle size and zeta potential. Nanoparticles were evaluated *in vitro* in respect of encapsulation, release, enzymatic stability, cellular uptake study, bioadhesion and *in vivo* in rat after oral delivery.
Results: The average particle size was 200nm diameter and zeta potential varied from -17.7 to -36mV depending on the shell composition. The release of NR was sustained both in SGF and SIF for 24 hours. There was time dependent increase in cellular uptake of nile red (NR) loaded nanoparticles in Caco-2 cells. Cy5.5 loaded nanoparticles were found to be retained in the gastrointestinal tract in rats up to 24 hours. Taken together, the enhanced bioadhesion and cell uptake can lead to prolonged and higher drug absorption from the nanoparticles.
Conclusions: Findings from this study can be used to develop a safe and effective, food compatible oral pediatric drug delivery system.

Deconstructing Fabrication of the Squid Pen

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Motivation/Background: Chitin based bandages are a newer alternative to cellulose (gauze) dressings and provide many benefits for promoting wound healing. Most efforts have focused on the chitin and not the proteins that are always found with chitin in nature. We are characterizing fabrication of the chitinous squid pen to understand nature's natural 3D printer used for layering chitin and protein into a supple yet strong biomaterial. More complex biodegradable structures may be fabricated for tissue engineering by combining chitin and native proteins.

Objective Statement: Identify the molecular composition of the squid pen and characterize the chemical environment in the shell sac in which it is constructed.

Methods and Results: Squid proteins used for fabrication of the pen are being identified using tandem mass spectrometry in combination with transcriptomes from the surrounding shell sac and whole organism. The ionic environment enclosing the shell sac has also been identified.

Conclusions: A number of structural and enzymatic proteins have been identified with chitin binding domains and appear to function in a normal extracellular fluid-like environment. A significant number of proteins with unknown functions require further efforts for identification.

3D printing acrylated poly(glycerol sebacate) using DLP printing

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Motivation/Background: Manufacturing complex tissue scaffolds with porosity is difficult and depending on the type of scaffold, may be impossible.

Objective Statement: Design a 3D printer capable printing a biodegradable and biocompatible acrylated polyglycerol sebacate to fabricate scaffolds with elastic properties is being developed.

Method/Approach: Acrylated polyglycerol sebacate (PGSA) was selected because of its rheological and crosslinking behavior (mechanical properties) that can be easily controlled by changes in curing time, temperature and pressure. The polyglycerol sebacate (PGS) was acrylated in order to become a photopolymer resin that could be used with digital light processing (DLP) 3D printing. This material was also proven to be cytocompatible and can replicate tissue shapes, according to detailed computer-aided designs. DLP printing consists of a vat of photocurable resin that is suspended above a DLP projector. The projector shines the desired slices of the 3D model from underneath the vat.

Results: The DLP printer has been developed and used to create samples using both a flexible photocurable resin and PGSa prepolymer. Initial results of the PGSa prepolymer cured through DLP 3d printing show similar results mechanical properties (modulus of elasticity and ultimate tensile strength) to PGS manufactured using standard techniques.

Conclusions: DLP 3d printing is a viable option for 3d printing tissue scaffolds with porosity. More testing is needed to develop repeatable scaffold manufacturing processes. Testing the scaffolds using live cells is also still needed.

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Engineered EP4A for Bone Regeneration by Modulating both Inflammation and Osteogenesis

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Introduction: Most recent studies suggest prostaglandin E_2 (PGE_2), known as a marker and promoter of inflammation, can potentially promote bone formation, it also presents severe adverse side effects (e.g. diarrhea) when either delivered systemically or in high dosage form. In our lab, we will determine the function of PGE2 EP4 receptor agonist (EP4A) in bone healing mechanism and hypothesized EP4A can concomitantly exhibit pro-osteogenic and anti-inflammatory capability to promote endogenous tissue regeneration while reducing adverse effect.

Materials and Methods: The macrophages used are from immortalized cell lines from murine and mouse myoblasts were used for osteogenic differentiation. Both immortal cells were stimulated and treated with EP4A and enzyme-linked immunosorbent assays were performed to analyze protein expression. Gelatin 3D nanofibrous scaffolds were fabricated through thermally induced phase separation technique in combination with porogen leaching methods (TIPS&P).

Results: Tumor necrosis factor α (TNFα) is a primary inflammatory cytokine produced by macrophages and has detrimental effects on bone formation. Our result indicates that EP4A can significantly inhibit lipopolysaccharide (LPS)-induced TNFα expression in J774A.1 cells (Fig. 1). Decreasing the expression of TNFα can lower the risk of developing chronic inflammatory disease and progress the recovery forward into reparative phase.

Conclusion: As a physiological mediator, EP4A can be a promising target for bone repair due to its unique role in both inflammation and osteogenesis.