Development of a Surface Adhesion Screening Assay and Construction Designs of an Activatable Recognition Protein (Actibody) Library using the FimH Scaffold

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Many non-immunoglobulin binding proteins mediate specific high-affinity interactions and find applications in medicine and biotechnology. These antibody-like binding proteins represent alternative scaffolds for design of surface display libraries of novel binding molecules but with advantages over antibodies. Here, we propose to develop an activatable recognition protein (actibody) library using the affinity regulated bacterial adhesive FimH scaffold. FimH binding has been engineered to respond to different regulatory stimuli without altering its specificity. Therefore, FimH represents an attractive starting point for the development of display library of activatable antibodies. The diversity library will be constructed by varying the amino acid composition of three complementarity determining regions (CDRs) that make up the ligand-binding pocket of FimH. A set of skewed degenerate oligonucleotides will be designed to introduce variations in the CDRs while maintaining a wild-type bias in conserved, structurally-important or solvent-inaccessible residues. Further optimizations will condition for adequate FimH expression, proper folding and maintenance of the scaffold conformation prior to screening. Avidity enhanced, competitive surface adhesion assay was developed to specifically screen the FimH actibody library. Kinetic and equilibrium screening parameters were derived from competitive surface elution model of bacteria FimH libraries detaching from glycosylated surface in the presence of soluble glycan. In artificial test experiments, the selection method allowed for identification, isolation and amplification of one high performing variant among one million non-binding variants. The bacterial fimbriae display of pre-conditioned FimH libraries combined with the competitive adhesion screening and additional fluorescence-activated cell sorting (FACS) presents remarkable potential for isolating variants that bind to novel targets in a regulated manner. The mechanical properties of the FimH scaffold and its ability to form catch bonds could be utilized in biological systems for a number of useful recognition purposes such as molecular force biosensors and force-regulated selective and microfluidic diagnostic tools to detect pathological agents.

Predicting Mutations that Alter the Force Responsiveness of Fluorescence-Activating Proteins

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Mechanical forces play a significant role in protein activity. Although force spectroscopy enables researchers to artificially apply forces to and observe proteins in vitro, there is no direct method for detecting and measuring intrinsic forces. The overall goal of this project is to design a genetically expressible biosensor that can measure forces via fluorescence. This force biosensor adapts a fluorescence-activating protein (FAPs), a two-domain peptide which dramatically enhances the fluorescence of a single fluorogen molecule by trapping it within an interdomain cleft. Interdomain interactions at the interface can be ruptured by applying tensile force across the peptide. The specific aim of this project is to predict mutations that will modify the force-responsiveness of this protein. Domain separation is modeled by molecular dynamics (MD) simulations and analyzed to determine force-bearing residues and associated rupture forces. MD analysis informs mutations that can alter the force-responsiveness of the FAP. These mutations will be expressed and validated using MD simulation and fluorescence microscopy. With these mutants, we can design an array of biosensors that are sensitive to different levels of forces.
Designing a Skeletal Muscle Implant to Improve Trauma Healing Outcomes
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Skeletal muscle healing following severe trauma is often limited by the fact that electro-mechanical conduction between myocytes is damaged. This compromised interface means that stimulatory signals generated in the brain and peripheral nerves might not reach the muscle tissue distal to the traumatic site, leading to denervation atrophy, which can increase the risk of limb amputation. Currently, muscle flaps can be transferred to the wound to replace major muscle loss; however, success in increasing the level of neuromuscular signal transduction across the trauma site is rare because these additions generally do not become innervated. We present a seeded skeletal muscle implant to act as a unifying bridge between proximal and distal sides of the wound. The work was approached in a series of three phases. In the first, cell lines from rat muscle-derived stem cells were generated from three muscles in the leg. These cell lines maintain the potential to differentiate into the desired skeletal muscle cells, and thus were valuable for the seeding of the implant. Following this work, a series of collagen implant designs were created and evaluated for their mechanical properties. This design step ensured the highest possible level of uniformity between the implant and the surrounding tissue, and also encouraged proper stem cell differentiation. The final project phase was a biomechanical study of the seeded implant, focusing on the ability of muscle-derived stem cells to align and eventually produce contractile force when subjected to electrical stimulation.

Targeted Delivery of CWP1-Conjugated Photodynamic Therapy in *Giardia lamblia*
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*Giardia lamblia* is a waterborne pathogen that annually infects nearly 2 million Americans and 200 million people worldwide. The *Giardia* lifecycle has a non-infectious trophozoite and an infectious cyst. The *Giardia* cyst has evolved to withstand months in freshwater and digestion by the host through the development of a hardy cyst wall. The cyst wall is composed of three cyst wall proteins and a unique homopolymer composed of B1,3-linked GalNAc, found only in the *Giardia* cyst wall. Conventional treatment of *Giardia* infection involves the antibiotic metronidazole which has been identified as a potential carcinogen that only affects the trophozoite. The potential exists for alternative Giardiasis therapies that utilize the properties of the cyst wall against itself. We present a novel method of specifically targeting and delivering photodynamic therapy to the *Giardia* cyst wall. The photodynamic therapy would disrupt normal *Giardia* cell processes without the damaging side effects of conventional antibiotics. Application of the developed delivery method could be applied to other pathogens that have an identifying sugar or protein and has the advantage of being more stable than antibodies used in conventional targeting modalities.

Methacrylated Hyaluronan and Poly(vinyl alcohol) Scaffolds for Periodontal Tissue Regeneration
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Periodontal disease (PD) is a major global oral health problem lacking ideal treatment due to inconsistent outcomes and insufficient alternatives to radical surgery. Thirty one percent of the United States adult population display mild forms of PD, 13% exhibit moderate severity, and 4% suffer from advanced PD symptoms. PD is characterized by the inflammatory response and tissue damage due to the infection of periodontal tissue. Chronic inflammation results in the destruction of the periodontal ligament (PDL) and alveolar bone resorption therefore leading to tooth loss. A major goal of periodontal therapy is to restore the structural integrity and physiological function of the damaged tissue. For the purpose of periodontal tissue regeneration, porous tissue engineering scaffolds of methacrylated hyaluronan/poly(vinyl alcohol) (HA-MA/PVA) were developed and fabricated using an efficient freeze-drying method. HA acts as the bioactive component of the scaffold as it is an essential component of PDL extracellular matrix and has been shown to promote bone differentiation. An interconnected porous network was obtained by repeated freeze-thaw sequences and subsequent lyophilization of polymer blend solutions, confirmed via scanning electron microscopy. Further covalent crosslinking of the HA-MA by photopolymerization was carried out. A preliminary three day study with primary human mesenchymal stem cells (hMSCs) demonstrated non-cytotoxicity of scaffold materials and cellular adhesion to exposed surfaces. Unconfined dynamic mechanical compression tests and a 28-day hMSC study are currently underway to examine material properties and cellular proliferation and differentiation. Future work with a bilayered scaffold will investigate the use of different materials to direct cellular differentiation and regeneration of adjacent periodontal tissue.
Enhancing Immune Defenses: Controlling Transfection Efficiency of Dendritic Cells for Vaccine Application
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Dendritic cells (DCs) are host immune sentinel cells. Not only do DCs sense and monitor the presence of invading pathogens, they specialize in internalizing invaders and presenting them to B and T lymphocytes in the host immune system. As professional antigen presenting cells (APCs), DCs represent the primary population responsible for generating adaptive immunity necessary for vaccinations. DCs internalize antigens and migrate to secondary lymph organs to present these antigens to lymphocytes modulating host adaptive immunity. Hypothesis: the environment of DCs can be engineered to enhance the efficiency of the host immune system. This study focuses on how a DC cell line, JAWS II, responds to specific chemokines by examining the expression of DC markers, selective migration, and T cell stimulation. Utilizing chemokines, bioactive molecules, and/or biomaterials we will develop a microenvironment that enhances DC internalization and presentation of antigens. Then, using a gene therapy approach, DCs will be transfected with vaccine non-viral vectors to develop either an anti-infective or anti-cancer host immune response.

Calibration of a Protein-Based Force Sensor
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Mechanical force regulates essential cellular processes in humans including blood clotting and bone restructuring. To study this mechanical regulation on the subcellular level, a protein-based force sensor is being developed to measure forces within cells. The force sensor consists of two fluorescence activating protein (FAP) subunits connected by an amino acid linker. The sensor loses its ability to induce a dye known as a fluorogen to fluoresce if the two subunits are separated from one another. In order to make quantitative measurements with the sensor, the force at which the two subunits separate must be known. To determine the force of separation, force must be applied to the construct in a controlled manner. Experimental results have shown that most of the interactions between the tags on the protein and the antibodies specific to these tags are not stable enough under tension to allow the separation measurement to be made. The exception to this finding is the interaction between streptavidin and biotin. To create a stable mechanical anchor for the protein, a SNAP-tag is being expressed with the protein. The SNAP-tag enzymatically attaches itself to benzyl guanine-biotin, which can be bound by a streptavidin. Currently, experiments are being performed to determine the functionality of the SNAP-tag. If the tag is functional, force can be applied to the resulting protein through these mechanical anchors, which may ultimately enable quantitative measurements of force with the sensor. These force measurements may add to the understanding of mechanically regulated cellular processes.