

Evelyn Bracho-Sanchez
Graduate student
J. Crayton Pruitt Family Department of Biomedical Engineering
University of Florida

Targeted Extracellular Indoleamine 2,3-Dioxygenase Suppresses Immune Responses In Vitro and In Vivo

Evelyn Bracho-Sanchez, Azadeh Hassanzadeh, Kevin Y. Koenders, Antonietta Restuccia, Margaret M. Fettis, Mark A. Wallet, Gregory A. Hudalla, Benjamin G. Keselowsky.

Indoleamine 2,3-dioxygenase (IDO), an enzyme that catalyzes the rate limiting step of tryptophan catabolism, has been shown to play a critical role in the promotion of immune tolerance in pregnancy, cancer and transplant models¹. Two mechanisms have been proposed for this effect: 1) depletion of this essential amino acid increases susceptibility of T cells to death by starvation, and 2) downstream metabolites, collectively known as kynurenines, directly interact with immune cells to induce anergy, apoptosis or halt proliferation of effector T cells by inducing regulatory T cells. Therefore, much interest has developed for the use of IDO to direct immune metabolism and induce a tolerogenic environment. IDO is preferentially expressed in the cytosol of antigen presenting cells and most efforts have focused on inducing expression of the enzyme by dendritic cells (DCs) and macrophages (MØs)². In this study we aim to establish IDO as a potent extracellular immunomodulator and develop a strategy for its targeted delivery in vivo by fusion with Galectin 3 (Gal3). Gal3 is a member of the carbohydrate binding lectin family, with strong affinity for N-Acetyl-D-lactosamine present in proteins of the extracellular matrix and surface receptors on various immune cells³. C80/86/MHC II evaluation and cytokine release profile revealed an immature DC phenotype was maintained when treated with extracellular IDO even in the presence of LPS. Suppression of antigen specific proliferation by IDO-treated DCs was also observed. This effect was reversed in the presence of MT suggesting suppression is mediated by the active enzyme. NanoLuc-Gal3 demonstrated retention at the injection site for up to 7 days post treatment with minimal localization to other organs and tissues. Following assessment of Gal3 as a retention strategy, evaluation of inflammatory cytokine gene expression in response to IDO-Gal3 revealed significantly reduced levels out to 5 days post-treatment. In this study we have established IDO as a potent extracellular immunomodulator capable of maintaining immature DCs and suppressing antigen specific proliferation in vitro. We have also designed an in vivo targeted delivery mechanism which allows for the localization of active enzymes up to 7 days at the injection site by fusion with Gal3. Treatment with fusion construct, IDO-Gal3, is able to modulate localized metabolism and significantly decrease inflammatory cytokine gene expression upon challenge with LPS.

Christopher S. O'Bryan
Graduate student
Department of Mechanical & Aerospace Engineering
University of Florida

Epithelial Cells at the Air-Gel Interface

Christopher S. O'Bryan; Tristan Hormel; Tapomoy Bhattacharjee; Thomas E. Angelini

Telomerase-immortalized human corneal epithelial cells (hTCEpi) grown at the air interface share many of the characteristics of human corneal epithelium in vivo, including stratification and apoptotic cell death of surface cells. Traditional methods of culturing epithelial cells at the air interface, including air-lifted cultures and hanging drops, limit the ability to image and physically interact with cells at the air-interface, preventing systematic in vitro studies. Recent work has shown that jammed granular microgels, with tunable mechanical properties, are capable of supporting cell growth. In this study, we investigate the growth of hTCEpi cells at the air-gel interface and explore their long term viability.

Tapomoy Bhattacharjee
Graduate student
Department of Mechanical & Aerospace Engineering
University of Florida

3D Cell Motion in Jammed Granular Microgels

Tapomoy Bhattacharjee, W. Gregory Sawyer, and Thomas E. Angelini

Soft granular polyelectrolyte microgels swell in liquid cell growth media to form a continuous elastic solid that can easily transition between solid to fluid state under a low shear stress. Such Liquid-like solids (LLS) have recently been used to create 3D cellular constructs as well as to support, culture and harvest cells in 3D. Current understanding of cell migration mechanics in 3D was established from experiments performed in natural and synthetic polymer networks. Spatial variation in network structure and the transience of degradable gels limit their usefulness in quantitative cell mechanics studies. By contrast, LLS growth media approximates a homogeneous continuum, enabling tractable cell mechanics measurements to be performed in 3D. Here, we introduce a process to understand and classify cytotoxic T cell motion in 3D by studying cellular motility in LLS media. General classification of T cell motion can be achieved with a very traditional statistical approach: the cell's mean squared displacement (MSD) as a function of delay time. We will also use Langevin approaches combined with the constitutive equations of the LLS medium to predict the statistics of T cell motion.

Aniruddha Kulkarni
Graduate student
Department of Chemical Engineering
University of Florida

Sensitive Colorimetric Detection of Nitrite Ions Based on the Aggregation of Gold Nanoparticles

A. Kulkarni, V. Bird and K. J. Ziegler

Determination of nitrite both environmentally and in life processes has been of importance as its presence above a threshold causes detrimental effects. Hence, there is a need to develop new, simple and highly sensitive analytical assays. Nitrite detection has been achieved through various methods, including chemiluminescence, electrochemistry, and surface-enhanced Raman scattering which involve use of expensive equipments, tedious procedures and time consumption. However, colorimetric assays can provide visual, on-site analysis, providing a simple and instantaneous detection method. In this work, a facile colorimetric assay has been proposed based on anti-aggregation of gold nanoparticles. Gold nanoparticles coated with aromatic amines react with nitrite ions that alter the aggregation state of the nanoparticles. The change to the aggregation state causes a shift in the local surface plasmon resonance bands, triggering a colorimetric response.

An increase in the concentration of nitrite ions changes the color of the solution, which can be detected by the naked eye. While many approaches have used thiol-based linkers, our use of disulphide cross-linkers, which bind more strongly to gold has increased the sensitivity of detection by an order of magnitude. Furthermore, the effect of this assay on synthetic urine has been explored to understand the effect of the presence of foreign ions on the detection of nitrite.

Cameron Morley
Graduate student
Department of Mechanical & Aerospace Engineering
University of Florida

Contractile motion of cells in jammed microgels

Cameron Morley, S. Tori Ellison, Tapomoy Bhattacharjee, W.G. Sawyer and T.E. Angelini

Cells are often dispersed in extracellular matrix (ECM) gels like collagen and Matrigel as minimal tissue models. Generally, large-scale contraction of these constructs is observed, in which the degree of contraction and compaction of the entire system correlates with cell density and ECM concentration. The freedom to perform diverse mechanical experiments on these contracting constructs is limited by the challenges of handling and supporting these delicate samples. Here, we present a method to create simple cell-ECM constructs that can be manipulated with significantly reduced experimental limitations. We 3D print mixtures of MCF10A cells and ECM (collagen-I and Matrigel) into a 3D growth medium made from jammed microgels. With this approach, we are able to apply shear stresses to the cell constructs at arbitrary times after printing and observe the collective response. Our preliminary results reveal that, following shear deformations that exceed 300% and dramatically smear cells and matrix in space, the cells actively re-contract and re-compact the construct toward the original, un-sheared construct. These results suggest that new principles of collective recovery can be employed for tissue engineering applications using jammed microgels as a re-configurable support medium.

S. Tori Ellison
Graduate student
Department of Mechanical & Aerospace Engineering
University of Florida

Collective cell behavior on basement membranes floating in space

S. Tori Ellison, Cameron Morley, Tapomoy Bhattacharjee, and T.E. Angelini

The basement membrane is an essential part of the polarity of endothelial and epithelial tissues. In tissue culture and organ-on-chip devices, monolayer polarity can be established by coating flat surfaces with extracellular matrix proteins and tuning the trans-substrate permeability. In epithelial 3D culture, spheroids spontaneously establish inside-out polarity, morphing into hollow shell-like structures called acini, generating their own basement membrane on the inner radius of the shell. However, 3D culture approaches generally lack the high degree of control provided by the 2D culture plate or organ-on-chip devices, making it difficult to create more faithful in vitro tissue models with complex surface curvature and morphology. Here we present a method for 3D printing complex basement membranes covered in cells. We 3D print collagen-I and Matrigel into a 3D growth medium made from jammed microgels. This soft, yielding material allows extracellular matrix to be formed as complex surfaces and shapes, floating in space. We then distribute MCF10A epithelial cells across the polymerized surface. We envision employing this strategy to study 3D collective cell behavior in numerous model tissue layers, beyond this simple epithelial model.