Wake Forest®
School of Medicine

Institute for Regenerative Medicine

2019

Summer Scholar Program Research Day & Poster Symposium

A NSF and NIH Undergraduate Research Site

AUGUST 8, 2019

Piedmont Triad Community Research Center
Winston-Salem, NC

WFIRM 2019 Summer Scholars Program
Research Day & Poster Symposium

Celebrating Multidisciplinary Research Experiences for Undergraduate Scholars in Challenging Areas of Regenerative Medicine

Thursday, August 8, 2019

The Wake Forest Institute for Regenerative Medicine (WFIRM) is an international leader in translating scientific discovery into clinical therapies. Our mission is to improve patients' lives through regenerative medicine.

A significant challenge in this promising field is developing the next generation of engineers, scientists, clinicians and entrepreneurs cognizant of the challenges and approaches needed to solve regenerative medicine problems and design functional replacement tissues and organs. Here at WFIRM, inherently tied to our mission is the training of next generation experts to whom we will look toward to continue to advance and deliver upon the promise of this field and make a lasting impact on health conditions ranging from heart disease, diabetes, injury and aging.

Congratulations to our WFIRM Summer Scholars 2019.

Today we invite you to join us in celebrating the graduation of 24 summer undergraduate scholars, who participated in our annual 10-week summer research program. June 3rd, 2019 marked the beginning of WFIRM's Annual, 10-week, Summer Scholar Program. Today's final Research Day with oral presentations and scientific poster session provides the opportunity for undergraduate researchers who join us from across the United States and globe, to present their summer research. We offer congratulations to our talented scholars, their faculty mentors and near-peer mentors comprised of teams of graduate, postdoctoral fellows and lab technicians. All providing important contributions and support. Much success is extended to our 2019 cohort of undergraduate scholars in their future educational and career pursuits. WFIRM is privileged to be a part of their intellectual and professional growth. We thank you all for joining us today and the support you have extended to these fine young adults.

Anthony Atala, MD
Director, Wake Forest Institute for Regenerative Medicine

Joan F. Schanck, MPA
Summer Scholars Program Director

Introducing the 2019 WFIRM Summer Scholars

Summer Scholar	Primary Faculty Mentor(s)
Honour Adewumi Jarvis Christian College Chemistry and Biology, Junior	**Giuseppe Orlando, MD, PhD** Assistant Professor
James Bennett Bucknell University Biomedical Engineering, Junior	**Anthony Atala, MD** Professor and Director of WFIRM
Olivia Cain Spelman College Biology, Freshman	**Steve J. Walker, PhD** Professor
James Day North Carolina State University Chemical Engineering, Sophomore	**Thomas Shupe, PhD** Assistant Professor
Joselyn De Jesus Gonzalez University of Puerto Rico – Rio Piedras Cell and Molecular Biology, Junior	**Graca Almeida-Porada, MD, PhD** Professor and **Christopher Porada, PhD** Professor
Anna Deal Georgia Institute of Technology Biochemistry, Junior	**Anthony Atala, MD** Professor and Director of WFIRM
Lauren Drake University of Pennsylvania Bioengineering, Junior	**Emmanuel Opara, PhD** Professor
Anushka Gerald University of Maryland Bioengineering, Junior	**Sang Jin Lee, PhD** Associate Professor
Caterina Grasso Rice University Bioengineering, Sophomore	**Anthony Atala, MD** Professor and Director of WFIRM
Albert Han Rice University Bioengineering, Junior	**James Yoo, MD, PhD** Professor
Jada Jackson Tuskegee University Chemical Engineering, Sophomore	**Giuseppe Orlando, MD, PhD** Assistant Professor
Brandon Kassouf Georgia Institute of Technology Biomedical Engineering, Freshman	**Yuanyuan Zhang, MD, PhD** Associate Professor
Emma Koukos Saint Michael's College Biology, Junior	**Steve J. Walker, PhD** Professor
Daniel Lee Winston-Salem State University Exercise Science, Senior	**Sang Jin Lee, PhD** Associate Professor
David McGuirt Elon University Biology, Junior	**Khalil Bitar, PhD** Professor
Samuel Moss University of Wisconsin – Madison Biomedical Engineering, Junior	**Aleks Skardal, PhD** Assistant Professor

Introducing the 2019 WFIRM Summer Scholars

Summer Scholar	Primary Faculty Mentor(s)
Alexandra Saldana LaTourneau University Biomedical Engineering, Junior	**Anthony Atala, MD** Professor and Director of WFIRM
Macaiah Sheffield Georgia Military College Sports Medicine, Sophomore	**Tracy Criswell, PhD** Assistant Professor
Emma Carin Statt University of Dayton Pre-Med, Junior	**Emmanuel Opara, PhD** Professor and **Yuanyuan Zhang, MD, PhD** Associate Professor
Mallory Thomas North Carolina State University Biological Sciences, Freshman	**Graca Almeida-Porada, MD, PhD** Professor and **Christopher Porada, PhD** Professor
Jacob Thompson University of Iowa Biomedical Engineering, Junior	**Shay Soker, PhD** Professor
Nikhil Vettikattu University of Southern California Human Biology, Junior	**Vijay Gorantla, MD, PhD** Associate Professor
Olivia Zyniewicz University of Notre Dame Neuroscience and Behavior, Junior	**Young Min Ju, PhD** Assistant Professor

WFIRM 2019 Summer Scholars Program
Research Day & Poster Symposium

Thursday, August 8, 2019

Oral Presentation Venue: Piedmont Triad Community Research Center (PTCRC)
115 S. Chestnut St., Winston-Salem, NC 27101

Poster Session and Luncheon Venue: Wake Forest Institute for Regenerative Medicine
391 Technology Way, Winston-Salem, NC 27101

Schedule

7:45am-8:00am	Summer Scholars arrival at PTCRC: photo session
8:00am-8:30am	Guest arrival at PTCRC: registration with refreshments
8:30am-8:45am	Welcome and Overview Anthony Atala, MD, Director, WFIRM and Joan Schanck, MPA, Summer Scholars Program Director
8:45am-10:00am	Summer Scholars' Presentations - Part I (Note: Group Q&A follows each session)

Session 1

1 Nikhil Vettikattu University of Southern California	*NONINVASIVE NEAR INFRARED FLUORESCENCE IMAGING OR MACROPHAGE MEDIATED INFLAMMATION*
2 Joselyn De Jesus Gonzalez University of Puerto Rico – Rio Piedras	*DEVELOPMENT OF IN VITRO ASSAYS TO VALIDATE IMMUNOLOGICAL OUTCOMES IN SHEEP UNDERGOING CELL AND GENE THERAPY FOR HEMOPHILIA A*
3 Macaiah Sheffield Georgia Military College	*THE EFFECT OF MUSCADINE GRAPE EXTRACT (MGE) TREATMENT ON MACROPHAGE ACTIVITY IN SKELETAL MUSCLE AFTER INJURY*
4 Olivia Zyniewicz Notre Dame University	*TARGETED ANGIOGENESIS*

Session 2

5 Emma Koukos Saint Michael's College	*INTEGRATIVE MICRORNA-MRNA CO-EXPRESSION ANALYSIS OF RIGHT-SIDED COLONIC HYPOMOTILITY IN CHILDREN WITH AUTISM*
6 Olivia Cain Spelman College	*COMPARING MIRNA AND GENE EXPRESSION PROFILES OF BLADDER MUCOSA IN IC/BPS PATIENTS*
7 Mallory Thomas North Carolina State University	*THE EFFECTS OF SPACE RADIATION ON GASTROINTESTINAL TISSUES*
8 Anna Deal Georgia Institute of Technology	*HUMAN PLACENTAL-DERIVED STEM CELLS ATTENUATE EXPERIMENTAL NECROTIZING ENTEROCOLITIS*

Session 3

9 Honour Adewumi Jarvis Christian College	*REMODELING THE EXTRACELLULAR MATRIX OF THE LUNGS CREATES A PRE-METASTATIC NICHE*
10 Jacob Thompson University of Iowa	*EFFECTS OF MICROBIOTA METABOLITES ON COLON CANCER AND ITS IMMUNE MICROENVIRONMENT IN AN ORGANOID MODEL*
11 James Bennett Bucknell University	*DIGITAL ANALYSIS OF FULL-THICKNESS WOUNDS CONFIRMS ENHANCED EPIDERMAL REMODELING THROUGH THE APPLICATION OF BIOPRINTED SKIN*

12 Samuel Moss University of Wisconsin - Madison	*BIOPRINTING OF MULTIPLE MYELOMA MICROENVIRONMENT TO EVALUATE PATIENT-DERIVED ORGANOIDS*
13 Davis McGuirt Elon University	*FABRICATION OF ELECTROSPUN PCL SCAFFOLDS FOR REPLICATION OF SMALL INTESTINE SMOOTH MUSCLE TISSUE*

10:00am-10:15am Coffee Break

10:15am-11:20am Summer Scholars Presentations - Part II
(Note: Group Q&A follows each session)

Session 4

14 Albert Han Rice University	*EFFECTS OF PRINTING CONDITIONS ON PRINTABILITY FOR EXTRUSION-BASED BIOPRINTING*
15 Caterina Grasso Rice University	*A NOVEL ORGANOID THAT IS STRUCTURALLY AND FUNCTIONALLY REPRESENTATIVE OF HUMAN SKIN*
16 James Day North Carolina State University	*CHARACTERIZATION OF HUMAN PRIMARY CELLS GROWN IN REMDO'S CHEMICALLY-DEFINED AND XENO-FREE MEDIUM*
17 Daniel Lee Winston-Salem State University	*UTILIZATION OF DECELLULARIZED EXTRACELLULAR MATRIX FOR THE DEVELOPMENT OF BONE TISSUE-SPECIFIC BIOINK FOR CELL-BASED 3D BIOPRINTING*
18 Alexandra Saldana LaTourneau University	*MATHEMATICAL AND PHYSICAL MODELING OF GELMA BIOPRINTING FOR OPTIMIZED URETHRA BIOPRINTING*
19 Anushka Gerald University of Maryland	*IN VITRO LIVER MODELS USING PEG BASED HYDROGELS*

Session 5

20 Lauren Drake University of Pennsylvania	*ENGINEERING AN ALGINATE MATRIX TO MIMIC THE PANCREATIC MICROENVIRONMENT TO ENHANCE ENCAPSULATED ISLET CELL FUNCTION*
21 Emma Carin Statt University of Dayton	*CONTROLLED DELIVER OF IGF1 USING ALGINATE MICROBEADS*
22 Brandon Kassouf Georgia Institute of Technology	*THE REGENERATIVE POTENTIAL OF EXOSOMES FROM URINE-DERIVED STEM CELLS*
23 Jada Jackson Tuskegee University	*EXTRACELLULAR MATRIX AS A CELL CULTURE MEDIA SUPPLEMENT FOR LONG-TERM ISLET CULTURE*
24 Anna Jones University of North Carolina	*THE BIOLOGICAL EFFECTS OF PANCREAS EXTRACELLULAR MATRIX HYDROGEL IN RECAPITULATING HUMAN ISLETS' BIOLOGICAL NICHE*

11:20am-11:35am Wrap-Up/Certifications of Completion

Note: Walk to WFIRM for Poster Session & Luncheon

11:45am-12:30pm Poster Session at WFIRM (2nd Floor Collaboration Area)

12:30pm-1:50pm Lunch at WFIRM with Scholars (2nd Floor Collaboration Area)

1:50pm-2:30pm Lab Tours at WFIRM

Note: Guests sign-up during registration. Scholars assist with demos, overview of their work in lab at stations)

1

Noninvasive Near Infrared Fluorescence Imaging Surveillance of Macrophage Mediated Inflammation

N. T. Vettikattu*, F. Selek, H. Kapucu, H. Karagoz, Lu Liu, F. Zor, J.M. Janjic, V. S. Gorantla
*Summer Scholar, Wake Forest Institute of Regenerative Medicine

Aims and Objectives: Macrophages (MΦ) play key roles in innate and adaptive immunity during sterile and non-sterile inflammation. Recent studies have highlighted their role in acute rejection (AR), graft vasculopathy, nerve regeneration and autoimmune disease. The central role of macrophages (MΦ) in the immune response makes them an attractive target for noninvasive monitoring of inflammation. Recent development of MΦ-targeted, perfluorocarbon (PFC) nanoemusions (NE) with near infra-red fluorescence (NIRF) probes enable tracking of peripheral macrophages to sites of inflammation. Other NIRF probes like, MMPSense™ 680 are selectively cleaved by proteases from activated MΦ in inflamed tissues. The aim of this study was to develop an in-vivo, non-invasive strategy for real-time, sequential, monitoring of MΦ migration, trafficking and activation that could help guide and determine responsiveness to treatment in acute or chronic inflammatory states.

Methods: Complete Freund's Adjuvant (CFA) (InvivoGen, San Diego, CA) was used to cause a lasting immune response and induce localized sterile inflammation in the rat hind limb. 100 µl of CFA was injected in the right leg of each rat and 100 µl of PBS was injected in the left leg (control). 50 µl of MMPSense™ 680 (Perkin Elmer, Akron, OH) was injected alone or co-injected with CFA in the right leg (or with PBS in opposite control legs) according to study group. PFC-NE (500 µl) was systemically administered on day -1 via penile vein. NIRF imaging was performed on a LI-COR ® Pearl (LICOR Biosciences, Lincoln, NE) in both legs at 700 nm (MMPSense™ 680) and 800 nm (PFC-NE) on Day 0 [0,1,3 6 hours] and POD 1, 2, 3, and every third day till end point of day 30. NIRF signal intensity was quantified and correlated with histology of skin samples to determine tissue inflammatory cell infiltration.

Results: The time course of signal changes on imaging showed that MMPSense ™ signal started as early 1 day and peaked between 1 and 3 days. The PFC-NE signal began to increase between 1 and 2 days and peaked between 3 and 6 days after CFA. Signal intensity was significantly different at these time points between experimental versus control limbs. Both signals co-localized to the site of CFA injection, indicating both the migration and subsequent activation of systemic MΦ at the site of inflammation . The delayed time to peaking of NIRF signal on PFC-NE indicates the time for peripheral macrophages to traffic to the inflamed limb tissues (24-48 hours post- injury), whereas the earlier peaking of signal on MMPSense™ 680 indicates the activation of *in situ* macrophages in the immediate proximity of CFA injection. The co- localization of the MMP and NE signal between days 1 and 6 demonstrate that MΦ from the periphery are homing to the site of inflammation and becoming activated, working in conjunction with the resident MΦ of the tissue.

Conclusion: Our non-invasive real-time, surveillance strategy for imaging peripheral infiltrating and locally activated MΦ could help preventive or personalized treatment of acute or chronic inflammatory conditions and improve the safety and efficacy of therapies. Since inflammation is an early precursor of the immune response, monitoring inflammation can provide valuable information for the regulation of immunosuppressants in transplant patients, ultimately reducing the substantial toxicity burden of these drugs. Correlation with histology can increase the positive predictive value of these strategies.

Acknowledgement: This summer research position was made possible by NSF REU grant #1659663 entitled, "Engineering New REU Approaches to Challenges in MultiTERM" (Atala, Schanck).

2

DEVELOPMENT OF IN VITRO ASSAYS TO VALIDATE IMMUNOLOGICAL OUTCOMES IN SHEEP UNDERGOING CELL AND GENE THERAPY FOR HEMOPHILIA A

*J. De Jesús González, M. Rodríguez**, A. Atala, C.Porada, G. Almeida-Porada
* Summer Scholar, Wake Forest Institute for Regenerative Medicine

Hemophilia A (HA) is an X-linked disorder caused by the lack, or the reduced activity, of functional clotting factor VIII (FVIII) and is one of the most common severe bleeding disorders. It may be inherited or arise from a spontaneous mutation, affecting 1 in 5000 males (1). The most common treatments for HA patients are based in replacement or on-demand therapy with plasma derived- or recombinant- FVIII intravenous infusions. Although HA patients have several therapeutic options available, treatment is limited by high cost, and lifelong treatment. In addition, HA patients develop immunological responses against the infused FVIII protein. Cell and/or gene therapy is expected to become a therapeutic alternative to FVIII factor concentrates (2).

The overall goal of this research is to provide a lifelong cure to HA using a prenatal cell and gene therapy delivery approach, to allow long-term engraftment of FVIII secreting cells and induction of central immune tolerance to both, FVIII and the gene modified donor cells.

In order to test prenatal stem cell and gene delivery (PNTx) platforms to treat HA, we use sheep, as a large animal model. Thus, in order to validate these therapies and evaluate immunological outcomes, reliable and rigorous tests have to be developed for this animal model.

We hypothesize that prenatal exposure to FVIII–transduced donor cells in sheep fetuses induces central immune tolerance postnatally and deletion of T cell FVIII specific clones, thus here we aim to determine the normal levels of T cells in our animals, and develop and validate methods to determine antigen-specific T cells.

Lymphocytes and monocyte-derived dendritic cells (MDDC) were isolated from peripheral blood mononuclear cells (PBMC) of sheep. For the characterization of the T cell subsets, the cells were labeled with antibodies that bind specific cell markers for the identification of CD4, γδ, and FoxP3 T cells via flow cytometry. Next, a protocol was designed, and optimized to detect antigen-specific T cells against the FVIII transgene product. In parallel, we also developed a universal positive read out for the experiment, since it would be possible that FVIII-specific T cells were deleted after PNTx due to induction of central tolerance. Thus, we examined whether we were able to detect the presence of keyhole limpet hemocyanin (KLH)-specific T cells in sheep. To this end, MDDC (3) were cultured from PBMC in AIM V, cytokines and growth factors. CD4 T and γδ T-cells were isolated through negative selection. T cells and MDDC were then co-cultured with keyhole limpet hemocyanin (KLH) for a duration of 14 days on a growth media of 10% fetal bovine serum with cytokines to stimulate T cell differentiation and proliferation (4). The CD4 T cells that became activated in response to KLH were identified by activation markers expressed on the cell surface via flow cytometry.

Data analysis shows that the baseline of T cells in sheep is predominantly composed of CD4 T cells (17.82%) and γδ T cells (23.71%). In addition, our results demonstrated the detection of KLH-specific CD4+ T cells via presence of key activation markers. Based on the current data in our animal model, we conclude that 1) frequencies of T cell subsets can be identified and 2) antigen-specific T cells can be detected, both serving as fundamental findings for subsequent identification of FVIII-specific T cells in PNTx recipients.

Acknowledgement: This summer research position was made possible by NSF REU grant #1659663 entitled, “Engineering New REU Approaches to Challenges in MultiTERM” (Atala, Schanck).

References:

1. Graw, J.et al. Haemophilia A: from mutation analysis to new therapies. Nat. Rev. Genet. 6, 488-501 (2005)
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3. da Silva Simoneti G, Saad ST, and Gilli SC (2014) An efficient protocol for the generation of monocyte derived dendritic cells using serum-free media for clinical applications in post remission AML patients. Ann Clin Lab Sci 44:180–188.
4. Jacquemin, M. (2002). CD4+ T-cell clones specific for wild-type factor VIII: a molecular mechanism responsible for a higher incidence of inhibitor formation in mild/moderate hemophilia A. Blood, 101(4), 1351–1358.

3

THE EFFECT OF MUSCADINE GRAPE EXTRACT (MGE) TREATMENT ON MACROPHAGE ACTIVITY IN SKELETAL MUSCLE AFTER INJURY

*M. Sheffield, J. Poteracki, Y. Zhou, T. Criswell
*Summer Scholar, Wake Forest Institute for Regenerative Medicine

Background:

Muscadine Grape Extract (MGE) is a nutraceutical derived from grapes that contains polyphenols and other compounds. Macrophages are vital for inflammation. They activate inflammatory responses, fight pathogens, and initiate tissue repair at the site of injury. We tested the effect of MGE to differentiate monocytes (M0) to M1 pro-inflammatory and/or M2 anti-inflammatory macrophages, which are involved in the healing process.

Objectives:

Our goal for the project was to see if MGE enhanced macrophage conversion from M1 to M2 *in vitro* and in skeletal muscle *in vivo* after injury.

Methods:

In vitro, we treated a monocyte cell line (M0) with LPS to for induction of M1 macrophages and IL4 for induction of M2 macrophages. We cultured the different macrophage populations with MGE 24hr prior to differentiation. qPCR was used to determine the expression of markers specific for either M1 or M2 macrophage16 hours after treatment. In vivo experiments consisted of providing MGE in the drinking water to rats 2 weeks prior to injury. Muscles were harvested from the rats 3, 7 and 14 days post-injury and stained for the pan-macrophage marker CD68.

Results:

Using qPCR, we determined that MGE treatment resulted in a significant decrease in the M1 marker *Il6*, but not the M1 marker *Il1b*. Furthermore, MGE had no effect on any of the M2 markers examined. *In vivo*, MGE decreased macrophage count early after injury (day 3 – acute inflammatory phase) and increased macrophage count at day 7 after injury (remodeling inflammatory phase).

Conclusions:

This data suggests that MGE may prove to be an effective anti-inflammatory treatment for injured skeletal muscle.

Acknowledgement:

This summer research position was made possible by NSF REU grant #1659663 entitled, “Engineering New REU Approaches to Challenges in MultiTERM” (Atala, Schanck).

TARGETED ANGIOGENESIS

*O.M. Zyniewicz, J. H. Park, Y. M. Ju
*Summer Scholar, Wake Forest Institute for Regenerative Medicine

Regenerative medicine has made many advancements in the production of regenerative tissue; however, these approaches are limited in size and physiological relevance due to the tissues' need to maintain a constant supply of nutrients and oxygen (1, 2, 3). The delivery of these resources occurs via angiogenesis, or the growth of blood vessels (1, 2, 3). As the natural process of angiogenesis is relatively time-consuming (5 μm/hour), methods have been developed to enhance and control the angiogenic process (2). These methods include the introduction of natural and artificial pro-angiogenic growth factors, implantation of prevascularized constructs, induced vascularization of natural and synthetic scaffolds, and production of synthetic vascular architecture. However, these methods are limited with regard to robustness, viable longevity, and replication of physiological sizing (1, 2).

The complexity of the human body adds a level of difficulty to angiogenic process; natural vasculature develops with specific hierarchy, geometry and exerts paracrine activity to the parenchyma (5). The proximity of nonvascular and vascular tissue necessitates meticulous control over the location of angiogenesis, thus angiogenesis of regenerating tissue must be controlled (4, 5).

In this study, we hypothesized that simultaneous delivery of pro- and anti-angiogenic factors via a hydrogel scaffold will allow for control of angiogenesis and promotion of vasculature in the targeted area. Vascular endothelial growth factor, VEGF, was the pro-angiogenic factor investigated in this experiment. Bevacizumab is an FDA approved anti-cancer drug that was used as the anti-angiogenic factor in this experiment.

An *in vitro* study was performed to determine the efficacy of the pro- and anti-angiogenic factors by measuring the effects of varying concentrations of VEGF and bevacizumab on cell cytotoxicity, proliferation and migration. All concentrations of bevacizumab and VEGF showed no cytotoxic effects on cells. Cell proliferation testing under fetal bovine serum supplementation conditions showed a clear trend where increased concentrations of bevacizumab correlated with low cell proliferation.

To evaluate angiogenic properties *in ovo*, a chorioallantoic membrane assay was performed. Egg shell windows were made in fertilized eggs after three days of incubation. VEGF and bevacizumab were transferred onto the egg membrane via a silicon rubber plate. Blood vessel growth measured on Day 10 showed decreased blood vessel growth after treatment with bevacizumab compared to treatment with VEGF. For future studies, a drug-eluting scaffold was designed on an IOP2 printer with 80 kpa at a feed rate of 200mm/min, using F-127 DA biomaterial. The proposed model will contain two channels to elute VEGF and bevacizumab respectively in order to direct angiogenesis to the targeted area.

Acknowledgement: This summer research position was made possible by NSF REU grant #1659663 entitled, "Engineering New REU Approaches to Challenges in MultiTERM" (Atala, Schanck).

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2. Laschke, M.W. Vascularization in Tissue Engineering: Angiogenesis vs Inosculation. Eur Surg Res 2012; 48: 85-92.
3. Dew, L.; MacNeil, S.; Chong, C.H. Vascularization strategies for tissue engineers. Regen Med 2015; 10: 211-224.
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5

INTEGRATIVE miRNA-mRNA CO-EXPRESSION ANALYSIS OF RIGHT-SIDED COLONIC HYPOMOTILITY IN CHILDREN WITH AUTISM

*E. Koukos, A. Krigsman, T. Simon, S. J. Walker
*Summer Scholar, Wake Forest Institute for Regenerative Medicine

Gastrointestinal (GI) symptoms are more common and contribute to defects of cognition and behavior in children with autism spectrum disorder (ASD) compared to typically developing (TD) children.[1-2] Furthermore, of the GI symptoms most commonly observed in children with ASD chronic constipation is reported by parents as being especially problematic.[2] We have observed children with ASD who have sought medical assistance for chronic constipation on a background of colonic inflammation. Two clinical trends (phenotypes) were observed based on the children's response to anti-inflammatory therapy: (1) patients who experience remission from constipation while undergoing anti-inflammatory therapy (*fast responders*), and (2) patients who experience recurrent right-side fecal loading while undergoing anti-inflammatory therapy (*slow responders*).[3] Total gene expression derived from right colon biopsies of 35 patients (*15 fast responders, 20 slow responders*), distinguished ASD fast responders from ASD slow responders.[3] The objective of this study is to further compare the two ASD subgroups through microRNA (miRNA) and total gene (mRNA) co-expression analysis to identify potential molecular regulators of the atypical constipation.

Expression profiles for miRNA were assessed for 12 of the 35 original right colon biopsies (*6 fast responders, 6 slow responders*). The samples were chosen based on hierarchal clustering of similarly expressed transcripts in each subgroup, respectively. Total RNA was quantified using NanoDrop and miRNA expression was assessed based on ~800 known miRNAs using NanoString nCounter SPRINT protocol. Differential expression of miRNA in fast vs. slow responders was determined using nSolver software and miRNA-mRNA co-expression was evaluated using Ingenuity Pathway Analysis (IPA).

We identified 12 significantly differentially expressed miRNAs ($p=0.05$) between fast and slow responders (nSolver) and 8 of those had been experimentally observed to target 400 unique mRNAs (IPA). IPA co-expression analysis showed that of the 400 mRNA targets ($p=0.05$), there were 24 mRNAs that overlap with the 1500 total differentially expressed transcripts (DETs) ($p=0.01$) between slow vs fast responders. Our results demonstrate an upregulation of transcripts involved in oxidative phosphorylation, reactive oxygen species and oxidative stress, and a downregulation of oxidative-stress response elements in slow responders that suggest an overall decreased mitochondrial efficiency in cells.

The central finding of the co-expression analysis was an increased expression of genes involved in mitochondrial dysregulation pathways in slow responders compared to fast responders that result from a decreased expression of their respective inhibitory miRNAs. As mitochondrial function contributes to intestinal epithelial cell homeostasis[4], its dysregulation in the right colon indicates mitochondrial differences which impact the atypical hypomotility that is observed in slow responders through co-expression analysis.

Acknowledgement: This summer research position was made possible by the NSF REU grant #1659663 entitled, "Engineering New REU Approaches to Challenges in MultiTERM" (Atala, Schank).

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(1) Hollingue, C., Newill, C., Lee, L.-C., Pasricha, P. J. & Fallin, M. D. Gastrointestinal ascertainment in autism spectrum disorder: a review of the literature on ascertainment and prevalence. *Autism Research* 11, 24–36 (2018).

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6

CORRELATION OF MICRORNA AND GENE EXPRESSION PROFILES IN BLADDER MUCOSA FROM IC/BPS PATIENTS

*O. Cain; R. J. Evans, MD; G. Badlani, MD; C. Matthews, MD; T. Simon, BS; S. J. Walker, PhD
*Summer Scholar, Wake Forest Institute for Regenerative Medicine

A diagnosis of interstitial cystitis/bladder pain syndrome (IC/BPS) is given when a patient presents with bladder pain, urinary urgency, and urinary frequency, and all other known or suspected causes have been ruled out. This results in a patient population that is clinically heterogeneous and challenging to treat. Of the 3-8 million women and 1-4 million men diagnosed with IC/BPS, approximately 10% have Hunner lesions (HL) which provides a definitive IC/BPS diagnosis. We previously used gene expression profiling in bladder biopsies from IC/BPS patients to identify subgroups based on bladder capacity (BC) and the presence or absence of Hunner lesions. Here we expand these studies by evaluating microRNA (miRNA) expression in the same samples and comparing it to the gene expression profiles.

Total RNA was isolated from mucosal bladder tissue from patients representing three IC/BPS subgroups. **Group 1** patients (N=4) had **low BC** (≤400cc) and were **HL+**. **Group 2** patients (N=4) had **low BC** and were **HL-**. **Group 3** patients (N=4) had **non-low BC** (>400cc) and were **HL-**. Using an expression array consisting of ~800 miRNAs we performed a comparison of differential miRNA expression between Groups 1 & 2 (to identify molecular mechanism related to HL) and between Groups 1 & 3 (to identify mechanisms related to low BC). Next, previously generated gene expression data from the same 12 samples was analyzed together with miRNA expression data for each of the 2 comparisons.

In the Group 1 v 2 comparison there were 917 differentially expressed genes ($p \leq 0.01$) as well as 16 differentially expressed miRNAs ($p \leq 0.05$). Using the miRNA Target Filter feature of Ingenuity Pathway Analysis (IPA) software, we mapped the differential expression of 4 miRNAs to 13 unique genes. Using the same strategy for the Group 1 v 3 comparison, we identified 744 differentially expressed genes ($p \leq 0.01$) and 54 miRNAs ($p \leq 0.05$). The miRNA Target Filter Feature of IPA identified 11 miRNAs that mapped to 40 different genes.

Within the group of differentially expressed genes in the HL+ compared to HL- patients there are several candidates, including SHROOM2 (which is regulated by hsa-miR-29c-3p), that may be involved in the development of the HL+ phenotype. The SHROOM2 gene codes for a sodium channel activity gene that is expressed in endothelial cells and facilitates the formation of a contractile network within these cells. Hsa-miR-29c-3p was down regulated and SHROOM2 was up regulated. For the comparison of patients with a low versus non-low BC, we identified one particularly interesting mRNA, BLCAP, that is regulated by hsa-miR-613. Hsa-miR-613 and BLCAP are both up regulated. Upregulation of BLCAP can result in an increase of apoptosis in cells, a finding which has been linked to chronic pelvic pain caused by a urological related condition versus a gynecological related issue. This gene could be a biomarker in a subgroup of IC/BPS patients.

In conclusion, we found significant differences in miRNA expression across the different subgroups of IC/BPS, allowing for a more detailed understanding of the molecular mechanisms that may account for the phenotypically unique subgroups within the disease.

Acknowledgement: This summer research position was made possible by the NIH NIDDK R25 Grant (#R25DK121572) entitled, Tissue Engineering and Regenerative Medicine Undergraduate Research Addressing Challenges in Diabetes, Kidney, and Urologic and Hematologic Diseases (E. Opara, PI).

THE EFFECTS OF SPACE RADIATION ON GASTROINTESTINAL TISSUES

*M. Thomas, B. Kuhlman, G. Almeida-Porada, C. Porada
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NASA plans to launch manned missions to the Moon by 2024 and to Mars by the mid 2030s. These missions will expose astronauts to radiation not present on Earth. Unlike all missions previously undertaken, these trips will involve humans traveling for prolonged periods of time beyond low-earth orbit and the protective magnetosphere. In addition to the types of radiation we experience on Earth, space radiation is comprised of energetic protons from solar particle events (SPE) and galactic cosmic radiation (GCR) which consists of high energy and charge ions stripped of their electrons. These particles are capable of penetrating through biological tissue and damaging DNA (1). It is essential that we understand the effects of space radiation on the gastrointestinal system before humans endeavor upon these missions to the Moon and Mars, as these tissues are highly radiosensitive due to their high rate of cellular proliferation.

This research aims to provide a better understanding of the damage caused by heavy ion radiation during long term missions beyond low-earth orbit. Using a wild-type (C57BL/6) murine model, we assessed the gross morphological changes in intestinal tissue after whole body exposure to Mars mission relevant doses of space radiation, as well as differences in the presence of tight junction proteins within the epithelium of gastrointestinal villi. The mice were exposed to one of the following mission-relevant doses of space radiation: Sham (control), Iron (^{56}Fe, 50 cGy), Oxygen (^{16}O, 75 cGy), or 5-ion GCR Simulation at the Brookhaven National Laboratory. They were then euthanized at the following times post-exposure: 15 minutes, 90 minutes, 4 hours, and 24 hours. Duodenal samples of intestine were flushed, formalin-fixed, paraffin embedded, sectioned, and mounted on microscope slides. Tissues were stained with hematoxylin and eosin; gross morphological alterations such as the presence of edema, enlarged lymph nodes, and blunted villi tips were then observed via brightfield microscopy. Immunohistochemistry was utilized to label tight junction proteins between the epithelial cells to assess epithelial membrane integrity. We specifically visualized expression of Claudin-3 tight junction protein because of its prevalence in the duodenum.

If damaging effects to the duodenum are found alterations may suggest a decrease in function following space radiation exposure. Variations in tight junctions may cause the barrier created by the epithelial cells to be compromised, leading to unwanted molecules passing in and out of the villi (2).

Additionally, novel radioprotective and radiomitigative pharmaceutical countermeasures are currently being developed to counteract deleterious effects, if found within these tissues. The hematopoietic system, also highly radiosensitive, is being studied for evidence of DNA damage and leukemogenesis using a humanized mouse model.

This preliminary research is a crucial step in understanding the risk of carcinogenesis in astronauts. We seek to provide knowledge that will enable their protection during future missions involving long-term space travel.

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HUMAN PLACENTAL-DERIVED STEM CELLS ATTENUATE EXPERIMENTAL NECROTIZING ENTEROCOLITIS

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Necrotizing Enterocolitis (NEC) is a life-threatening intestinal disease that occurs in approximately 10% of premature infants[1]. Despite medical advancements in NICU care, NEC mortality rates have remained consistent at 20-40%[2]. There are three main insults that contribute to the onset of NEC including intestinal immaturity, bacterial imbalance, and prominent immune response. While research has begun to elucidate the pathogenic interplay between these insults, to date, none have produced a successful clinical treatment. however, the exact mechanism is not completely understood. NEC has an infant mortality rate of approximately 20-40% with no specific medical therapies to treat infants with NEC.[2] Recent studies from our group have shown perinatal stem cells are a promising therapeutic that repairs the damage induced in NEC. Thus, we hypothesized that human placenta-derived stem cells (hPSC) therapeutically attenuate NEC damage in the intestine.

The cellular-level impact of hPSC treatment was evaluated using an established newborn rat pup model of NEC. Newborn pups were separated into two groups: Breastfed control pups (BF) and NEC pups. BF pups remained with the mother with no external stressors. To induce NEC in the NEC pup group, pups were isolated from the mother and received a regimen of 3 NEC stressors: hypoxic stress, bacterial toxin (lipopolysaccharide, LPS), and formula feeds for 4 days. Half of the NEC pups received intraperitoneal injections of hPSC at 30 hours into the NEC induction. After 4 days (96 hours), the cellular composition and damage in the intestine was assessed. As the epithelial cells establish the protective barrier in the intestine, we first evaluated the differentiated epithelial cell types and the intestinal stem cell population. Additionally, we quantified the epithelial cell proliferation, migration, and apoptosis. We utilized multi-labelling immunohistochemistry techniques and a customized object detection code to assess the cellular changes along the crypt to villus axis in the distal small intestine. Specifically, EdU, Ki67, Ezrin, OLFM4, ChgA, and TFF3 were used to identify the different cell populations. EdU, injected into the rats 24 hours before sacrifice, marked proliferating intestinal epithelial stem cells and tracked their migration. Ki67 identified proliferating epithelial stem cells at the Day 4 endpoint. Further, OLFM4 labeled the active epithelial stem cell population. To delineate differentiated epithelial cell types, Ezrin, ChgA, and TFF3 marked the absorptive enterocytes, sensory enteroendrocrine cells, and mucin-producing goblet cells, respectively. The total number and localization along the crypt to villus axis of each cell type was quantified to thoroughly evaluate cellular changes.

NEC pups treated with hPSC had a statistically significant increase in stem cell proliferation (via EdU) compare to untreated NEC pups. There was also a significant increase in the number of active intestinal stem cells in rats with NEC treated with hPSC compared to untreated NEC pups. Further, there was a significant increase in the differentiated cell types of enterocytes and goblet cells, but a decrease in enteroendocrine cells present in hPSC-treated NEC pups compared to untreated NEC pups. These cellular changes demonstrate the therapeutic effect of of hPSC as they begin to restore the intestinal cellular composition found in healthy BF control pups. Together, these results show hPSC therapy supports epithelial restitution and improves the overall intestinal cell function. Overall, these studies provide compelling data for the use of hPSC therapy in NEC.

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Remodeling the Extracellular Matrix of the Lungs Creates a Pre-Metastatic Niche

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Fibrosis is typified by a change in the architecture of a tissue's extracellular matrix (ECM). Generally, this phenomenon is associated with wound healing and is driven by the body's immune system. However, in the context of cancer, fibrosis is often a site of increased cancer growth and engagement. It has been previously shown that paclitaxel (PTX), a common chemotherapeutic, can induce fibrosis in the lung leading to metastasis of distal cancer (most notably from the breast) to the lung tissue. Previously, we have developed an organoid model of the tumor microenvironment and demonstrated that changes to ECM can drive altered cancer response. In an effort to simulate PTX-mediated fibrosis of the lung, and understand its role in metastasis and cancer progression, we have developed an organoid model of the lung complete with an immune component (naive macrophages), fibroblast population (human lung fibroblasts), and breast cancer compartment (human cancer cell lines). We hypothesized that PTX exposure of the immune component of our organoid will drive changes in fibroblast behavior and ECM architecture. To test our hypothesis, organoids were prepared with THP-1 (macrophages) and NHLF (fibroblasts) cells. THP-1 cells were treated with 10nM PTX prior to integration into the organoid to induce polarization, then CD206 (M2) and CD197 (M1) expression was measured to assess macrophage phenotype using flow and immunohistochemistry. PTX-treated macrophages differentiated into M2 phenotype and induced more remodeling in collagen architecture of organoids compared to untreated macrophages. Cancer cells cultured in treated organoids are expected to display a more aggressive phenotype. In conclusion, we present a method for simulating PTX-mediated fibrosis *in vitro*, and show that PTX can induce pro-cancer behavior from macrophages, mediated through fibroblast collagen remodeling. Future studies include disrupting this immune-fibroblast axis using small molecule inhibitors or co-therapies for PTX.

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EFFECTS OF MICROBIOTA METABOLITES ON COLON CANCER AND ITS IMMUNE MICROENVIRONMENT IN AN ORGANOID MODEL

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Colorectal cancer is one of the most common cancer types diagnosed in the United States and is expected to have a mortality rate of over 50,000 individuals for 2019[1]. While the death rate has been decreasing for men and women over the past few decades, there is still a need for better models of this disease and development of new therapies. A major challenge of treating colorectal cancers is that the characteristics of each tumor vary greatly based on microenvironment present in each patient. Specific factors that change disease dynamics include diet[2] and genetic disposition[3]. A promising approach to account for individual factors is to use tumor organoids. Tumor organoids create an *ex vivo* 3D tumor microenvironment, recapitulate the ability of the tumor to interact with the surrounding stromal configurations[4] and can be used as predictive models for patient responses to therapeutics[5]. The gut microbiota also changes the way that tumors progress and respond to current therapies. In this study, tumor organoids were made from murine CT26 colon carcinoma cells and lymphocytes. The organoids were used to determine the effect of 3-indolepropionic acid (3-IPA), a tryptophan metabolite secreted by *Clostridium sporogenes,* on tumor and immune cell viability and phenotype.

Tumor organoids were fabricated by suspending cultured CT26 cells, lymphocytes extracted from the tumor draining lymph nodes, or a combination of both in a hydrogel solution made in a 3:1 methacrylated collagen and thiolated hyaluronic acid ratio. Each organoid was then subjected to ~2 seconds of UV irradiation to allow cross-linking to occur, encapsulating the cells within the organoid. Half of the organoids were treated with physiological levels of 3-IPA while the other half were left untreated. After 96-hrs, cell viability was determined using the CellTiter-Glo and cell phenotype was determined by flow cytometry, immunohistochemistry, and RT-qPCR. The CT26 cancer cells also were exposed to varying concentrations of 3-IPA in 2D culture conditions and analyzed using RT-qPCR to evaluate the relative expression of beta-2 microglobulin, a protein segment of the MHC I complex necessary for cytotoxic T-cell interaction.

The tumor organoid system successfully sustained a co-culture of CT26 cancer cells and lymphocytes. Flow cytometry demonstrated two populations of cells in the combined organoids, CD-8 positive cytotoxic lymphocytes and CD-8 negative cells. This supports the prediction that both CD-8 negative cancer cells and CD-8 positive T-cells can be maintained in the organoid system. The viability assay showed that 3-IPA did not affect tumor and immune cell viability. The qPCR data also suggests that the 3-IPA alone does not produce any significant gene expression changes in CT26 cancer cells. There was no significant change in the ratio of CD-8 positive T-cells and CD-8 negative cells for organoids treated with 3-IPA and those left untreated, in accordance with expectations.

Tumor organoids are useful as predictive models for current cancer therapies that can mimic the *in vivo* tumor environment. The viable co-culture of cells demonstrates the ability of organoids to provide an easy and high throughput way to mimic the *in vivo* tumor microenvironment. This verification can lead to tumor organoids being used to model immune checkpoint blockade therapies in conjunction with metabolites for colon cancer as well as other immunogenic cancers.

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DIGITAL ANALYSIS OF FULL-THICKNESS WOUNDS CONFIRMS ENHANCED EPIDERMAL REMODELING THROUGH THE APPLICATION OF BIOPRINTED SKIN

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Full-thickness skin wounds affect over 7 million people in the U.S. annually with current treatments insufficient for covering large area full-thickness wounds. Therefore, bioprinted skin grafts have been proposed as a new treatment option. Epidermal thickness measurements can quantify the efficacy of novel wound treatments, such as bioprinted skin, at promoting normal epidermal remodeling because scar tissue forms during normal full-thickness wound healing, resulting in rete peg loss and a thinner epidermis compared to healthy human skin. Standard epidermal thickness measurement methods include vertical or orthogonal measurements taken at 20 intervals or using ImageJ to measure the epidermal area and divide by its length. These three methods have limitations, including lengthy measurement times, poor reproducibility, and manual pixel to length conversion. The objective of this study was to develop and implement an improved method for measuring epidermal thickness to determine if bioprinted skin promotes enhanced epidermal remodeling and thus further characterize its ability to treat full-thickness wounds.

Skin samples were taken from human donors, pigs, and the backs of mice with tissue-engineered skin, hydrogel only, and untreated wounds after weeks 3, 6, and 12. Samples were histologically processed, sectioned, stained with Hematoxylin and Eosin, and imaged. A MATLAB program was then written to isolate the epidermis and calculate the average thickness by dividing its area by the midline length. To determine the speed and efficacy of the program relative to current methods, 9 raters were timed while measuring the epidermal thickness of the same human skin sample using all four techniques; MATLAB, ImageJ, orthogonal, and vertical. The user's scale bar measurements were also recorded to demonstrate scaling deviation during the measurement process. The reliability of the MATLAB epidermal measurements across different tissue types was tested by performing each method 3 times on human, pig, and tissue-engineered samples. Finally, epidermal thickness was measured using the MATLAB method on human, tissue-engineered, hydrogel, and untreated groups to evaluate improved epidermal remodeling. Statistical analysis was performed using two-way ANOVA.

The MATLAB method measured epidermal depth with no significant difference from the recognized orthogonal measurement standard in human, pig, and tissue-engineered skin, and had decreased standard deviation (48.7±0 vs. 46.1±2.9, 49.1±0 vs. 50.0±1.0, 68.3±0 vs. 64.9±1.2 µm, respectively). The measured scale bar length for the automated MATLAB method was compared to the manual traditional method which showed the automated process had a smaller deviation than the manual process, leading to more reliable, reproducible, and accurate results (311±0 vs. 310.60±9.4 pixels, respectively). Finally, the MATLAB method proved to be significantly faster than all other methods (1.5±0.3 vs. 12.0±2.9, 13.3±4.4, 10.4±3.6 minutes, respectively; $p<0.0001$). All week 3 samples were statistically different from normal human skin due to multiple samples lacking an epidermal layer. The average epidermal thicknesses of bioprinted skin at weeks 6 and 12 were not statistically different from healthy human skin (30.3±5.7 and 56.4±19.5 vs. 55.3±7.0 µm, respectively; $p>0.05$) and were the most similar groups in average thickness to human skin.

Our novel method for measuring epidermal thickness is faster, more reliable, reproducible, and accurate than traditional methods. Using this method, we demonstrated bioprinted skin had the most similar epidermal thickness to healthy human skin, suggesting enhanced epidermal remodeling. Ultimately, our method can be used to measure epidermal thickness for the analysis of other wound treatments and aid dermatopathologists in diagnosing a range of pathologies.

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BIOPRINTING OF MULTIPLE MYELOMA MICROENVIRONMENT TO EVALUATE PATIENT-DERIVED ORGANOIDS

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Multiple Myeloma is a common hematological malignancy, involving B cell differentiation into plasma cells[1]. Plasma cells primary functions involves immunoglobulin production through constant and arbitrary DNA rearrangement; as a result, each multiple myeloma patient has a unique heterogenetic profile[1]. In order to provide precise treatment to each patient we have engineered a humanized ECM-based bioink capable of sustaining patient specific stromal and malignant cells, giving us the capability to recapitulate individual plasma diseases *in vitro*. To scale up the biofabrication of our organoids with consistency and reproducibility standards of high throughput screening, we present a technique to bioprint our myeloma bioink in an automatized 96-well plate set up. Through bioprinted 3D organoids, candidate drugs can be screened in human-based models before human trials, thereby increasing chances of success and reducing costs[2].

A multiple nozzle bioprinter (CellInk BioX) was set up to automatize organoid biofabrication. The personalized G-code first extruded 10µl bioink volumes, followed by a UV light dosage of 600 mJ/cm^2 for 0.25 s, and dispense 50µl of media. The bioink comprised mesenchymal stem cells (MSCs), macrophages, and CD138 positive cells incorporated into a hydrogel composed of thiolated hyaluronic acid, gelatin, and fibronectin mixed with PEGSSDA. The constructs were screened for five days in parallel with a single exposure of dexamethasone or a triplicate therapy exposure of dexamethasone, cyclophosphamide, and bortezomib. ATP assays were performed on days 1, 3, and 5; while total cell quantification and live/dead confocal imaging were performed on days 1 and 5.

We have automated a high throughput bioprinting process to scale up the biofabrication of multiple myeloma organoids for personalize drug screening. A set of control organoids demonstrated steady metabolic activity and maintained viability throughout the five days experiments. As a proof of concept for underlying drug screenings, a contrasting single drug exposure of dexamethasone versus a triplicate drug group was evaluated in the organoids. Dexamethasone exclusive exposed organoids do not demonstrate a drastic decline in metabolic activity or in viability, as the triplicate drug exposed organoids did. Live/dead imaging analysis and metabolism analysis of the triplicate therapy exposed organoids demonstrated a decisive impact in contrast to a single drug exposure.

We present here a system that provides *in vitro* mimicry of *in vivo* environmental and cellular composition of patient-specific multiple myeloma bone marrow. Overall, the demonstrated difference in metabolic and viability data between all three conditions lay down the groundwork for the bioprinted myeloma platform to be adopted for future drug screening purposes. The presented automated high throughput bioprinting process used to create these organoids facilitates coherent biofabrication of multiple myeloma patient derived models. In a relevant future, all of these features will help mitigate the translation from bench to clinical application, allowing for the better prediction of drug treatments in personalized medicine.

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FABRICATION OF ELECTROSPUN PCL SCAFFOLDS FOR REPLICATION OF SMALL INTESTINE SMOOTH MUSCLE TISSUE

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At present, there exist a variety of diseases which possess the ability to inflict severe damage upon the human small intestine (SI). The current standard of treatment for a patient with a severely damaged SI is surgery to remove the damaged portions of the organ; however, post-surgical complications, such as short bowel syndrome, are both common and lethal (1). Therefore, there exists a need for replacement tissue to avoid certain post-surgical complications. In response to this need, current research efforts have placed significant focus on the fabrication of artificial SI tissue. However, human tissue is often complex and hard to replicate, especially the two smooth muscle layers of SI tissue. Each layer features uniaxially aligned smooth muscle cells (SCMs) which are required for each layer to provide the SI with its vital peristaltic function. It is essential that artificial SI smooth muscle tissue replicates this same cellular alignment. One method to obtain alignment of SMCs involves replicating the native extracellular matrix (ECM) of the smooth muscle tissue which contains proteins that promote the alignment of SMCs.

Electrospinning is a relatively straightforward process that has proved itself capable of producing both nanofibers and microfibers to replicate the native ECM proteins found in a variety of human tissues. Furthermore, it is possible to collect these electrospun fibers in an aligned fashion to further mimic tissue with an ECM consisting of aligned proteins, such as that of SI smooth muscle tissue (2). It is also possible to seed certain cell types directly into a sheet of aligned electrospun fibers in vitro. One polymer in particular, polycaprolactone (PCL), has shown promise as a biomaterial capable of being electrospun to produce aligned fiber sheets. In this study, we demonstrate that it is possible to collect aligned sheets of PCL electrospun fibers to mimic the native ECM of human derived SMCs in order to promote their uniaxial alignment and proliferation.

Different solvent systems were prepared with PCL to choose the most suitable solvent system. Microscope imaging revealed that a chloroform solvent system yielded uniform, aligned PCL fibers across a parallel plate collector whereas the other solvent systems did not. This solvent system was used to prepare solutions with different concentrations of PCL to determine which concentration yielded the most consistent fibers. Moreover, several rheological properties were measured for each of these solutions, all of which are known to affect the electrospinning process. Once the suitable concentration of PCL was chosen, additional electrospinning parameters were further optimized to obtain consistent electrospun scaffolds.

The scaffolds were sterilized and seeded with human derived SMCs, after which they were cultured for one week. Samples were taken at days 1, 3, and 7 for metabolic studies and imaging with both confocal microscopy and scanning electron microscope. Cells were visibly aligned on the scaffold after 24 hours. By day 3, the cells had further proliferated and continued to show alignment. After day 7, the cells showed decreased proliferation, suggesting they had obtained confluence, and continued to show alignment. Based on these results, we conclude that the PCL electrospun scaffolds were capable of inducing cellular alignment and promoting proliferation while maintaining cell viability.

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EFFECTS OF PRINTING CONDITIONS ON PRINTABILITY FOR EXTRUSION-BASED BIOPRINTING

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Three-dimensional (3D) bioprinting is a biofabrication strategy that can create complex architectures and patient-specific geometries for various tissue engineering applications (1). Bioprinting often uses biocompatible hydrogels, also known as 'bioinks', to create constructs suitable for cells as tissue scaffolds. However, though the physicochemical properties of various bioinks have been studied, the ability of the bioink to be printed, or its 'printability,' has not been fully characterized. Although previous studies have proposed quantitative definitions of printability, these measurements are often limited in scope, and there is yet to be a standardized method of evaluating bioink printability (2-4). Furthermore, the effects of different printing conditions and cell densities on printability have not been fully studied. Here, we aimed to evaluate these effects using a gelatin methacryloyl (GelMA) – gellan gum bioink and standardize printability measurements using a novel bioink artifact. We hypothesized that cell density would not affect printability or rheology except at the highest density, and that printability would not differ between the same ratios of flowrate-to-feedrate, or speed ratios.

We used a 4% GelMA, 1.2% gellan gum formulation as a representative bioink that has properties that favor printability (5, 6). To test the effects of printing conditions, the bioink was printed using an in-house pneumatic extrusion-based bioprinter at five feedrates, five flowrates, and three nozzle heights. Printability was evaluated by printing specific constructs and measuring the shape of the structures, such as line width, height, and a 'Pr' factor reported in literature (2-4). To test the effects of cell density, chondrocytes and mouse endothelial cells (MS1) were incorporated in the gel at five different cell densities. Constructs were printed at a constant feedrate, flowrate, and nozzle height. Rheological properties and printability measurements were obtained for each construct.

For our printing conditions study, we found constructs to be qualitatively and quantitatively similar among the same speed ratios. Constructs at extreme printing conditions either had failed prints or unfavorable printability. For our cell density study, we found statistically significant differences between storage and loss moduli in between certain cell densities but similar shear thinning behavior and printability. Our results can help define limits of printing conditions that allow for printable GelMA-gellan gum constructs, which can be applied to other similar bioinks. Our results also provide quantitative data using our printability artifact, which can guide the formation of a comprehensive printability value based on the various measurements taken and create a standard that allows for direct comparison of printability among different bioinks and printing conditions.

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A Novel Organoid that is Structurally and Functionally Representative of Human Skin

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Introduction: Transdermal delivery is a common method of administering drugs to patients. Therefore, it is essential to test the ability of drugs to cross the dermal-epidermal junction when evaluating the efficacy of transdermal drug delivery for clinical applications. 2D *in vitro* studies often test the drug on one or two cell types, and are unable to model the dermal-epidermal junction[1]. Similarly, *in vivo* animal testing often fails to predict the effects of a drug on the human body due to the vast genetic and biological differences between species[1]. To overcome these limitations, we have engineered a novel skin organoid that is structurally and functionally representative of real human skin for implementation in the body-on-a-chip technology for testing the effect of transdermal drugs on human organ equivalents[2].

Methods: Skin organoids were engineered using a cell suspension containing keratinocytes, melanocytes, fibroblasts, follicle dermal papilla cells (FDPC), human dermal microvascular endothelial cells, and pre-adipocytes. Samples were taken at 7, 14, and 21 days for histological and molecular analysis. Skin organoid layering was first confirmed using Hematoxylin and Eosin (H&E) staining and Masson's Trichrome staining. Immunohistochemical staining with Pan-Cytokeratin and Vimentin was then used to ensure layer specificity. A series of functional assays was then used to determine skin spheroid utility. First, treatment with UV light ranging from 0mW to 100mW was used to induce melanocyte pigment production, and the pigmented surface area was determined using ImageJ. Second, spheroids were treated with TGF-β or LPS to induce a model of skin fibrosis, which was evaluated using H&E staining. These findings were supported morphologically and structurally using Scanning Electron Microscopy.

Results: Cells in skin spheroids self-organized over time to form appropriate skin layers- with keratinocytes and melanocytes forming the epidermis and FDPC, endothelial cells, and pre-adipocytes forming dermal core, as visualized by immunohistochemistry and Scanning Electron Microscopy. Organoids treated with higher doses of UV light had increased pigmented area over time with the greatest pigmented area in the group treated with 100mW over 21 days. When assessing fibrotic development in TGF-β and LPS treated organoids, H&E staining demonstrated the development of more fibroblast cells in treated organoids when compared to the untreated control. The increased expression of fibroblasts, responsible for producing collagen, is evidence for the greater collagen production that characterizes fibrosis[3]. Additionally, in LPS-treated spheroids, Scanning Electron Microscopy confirmed a more densely-packed extracellular matrix where individual collagen fibrils were indiscernible, as opposed to untreated control organoids, similarly suggesting an increased collagen production. TGF-β treated organoids had similar areas of densely-packed collagen, as well as areas where collagen fibrils were clearly visible, as in the control organoids.

Conclusions: The organoids we developed model human skin in its layered structure and ability to replicate key functions including the development of pigmentation when exposed to UV, and the development of fibrotic characteristics when treated with fibrosis-inducing factors. Further utility testing involves testing epidermal barrier function by treating organoids with lucifer yellow dye, and assessing the permeation of the dye in the organoids[4]. Also, a retinoic acid detection assay will be used to demonstrate the ability of cytochrome p450 in the spheroids to enzymatic metabolize retinol into retinoic acid. These tests will further confirm the ability of the organoids to uphold other vital functions of the skin, and demonstrate how well the organoids can represent human skin.

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CHARACTERIZATION OF HUMAN PRIMARY CELLS GROWN IN REMDO'S CHEMICALLY DEFINED AND XENO-FREE MEDIUM

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Abstract

Background: Animal-derived biological extracts, including animal serum, are incorporated into cell culture medium to provide bioregulatory factors that support the maintenance of cell viability and promote cell proliferation. These biological extracts are not chemically defined, are inconsistent from lot to lot, and carry the risk of disease transmission. As such, these media supplements are not optimal materials for use in clinical manufacturing processes. Our group has developed a medium based on known constituents within the well-characterized biological extract human platelet lysate. This media supported skeletal muscle cells (SkMCs) and several other cell types derived from the mesodermal embryonic germ layer.

Methods: Human sourced or recombinant versions of the major bioregulatory factors present in human platelet lysate were added to a modified DMEM-F12 minimal medium. Growth curves and morphological panels for an extensive selection of commercially available human primary cells were generated using an IncuCyte S3 Live Cell Imager. Cell metabolic characteristics were measured using a Cedex Bioanalyzer. Preservation of cell type specific functional markers were determined using the BD Accuri C6 flow cytometer and immunocytochemical staining techniques.

Results: Proliferation rates for skeletal muscle cells in the chemically defined medium were equivalent or superior to proliferation rates measured in the cell supplier's recommended, chemically undefined medium. Preservation of functional biomarker expression indicated that cell phenotype was maintained across multiple cell passages for each cell type. Conservation of cell morphology was also preserved. Metabolic trends for glucose, ammonia, and lactate showed significant differences for cells grown in ReMDO media against commercial media. However, additional research is needed to better understand the cause and impact of such metabolic trends. When staining for Ki67 to determine the proliferative potential of cells, flow cytometry and ICC results both supported earlier growth curves showing improved growth rates of skeletal muscle cells in ReMDO Media formulation over commercial media.

Conclusion: A chemically defined cell culture medium based on the known constituents of the biological extract, human platelet lysate was formulated using human sourced and recombinant protein bioregulatory factors. This media formulation supported proliferation and preservation of phenotype for skeletal muscle cells. ReMDO's cell culture medium represents a chemically defined and xeno-free medium for supporting various clinical manufacturing processes.

Acknowledgement:

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UTILIZATION OF DECELLULARIZED EXTRACELLULAR MATRIX FOR THE DEVELOPMENT OF BONE TISSUE-SPECIFIC BIOINK FOR CELL-BASED 3D BIOPRINTING

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Tissue engineering is the fabrication of tissues through engineering concepts. The aim of tissue engineering in regenerative medicine is regeneration of damaged tissues over replacement of tissues. Three key aspects are needed in tissue engineering which are cells, signals (growth factors), and scaffolds which give structure and stability for cells to migrate, adhere, and produce tissues. There are certain criteria's set-in place with regards to scaffolds in tissue engineering biocompatibility which looks at adhesion, function, and migration. Biodegradability looks at the ability of the scaffold to eventually be replaced by the tissues of the body. Mechanical properties observe the consistency of the scaffold with regards to the anatomical site of the implantation location and strength during implantation to hold its structure. Scaffold architecture requires that the scaffolds have interconnected spaces and high porosity for cells to easily enter the scaffold. Manufacturing technology looks at cost efficiency and time to be effective. Finally, biomaterials are the final criteria. Biomaterials are non-viable materials utilized to interact with biological systems. Three groups of biomaterials are ceramics, synthetic polymers, and natural polymers (O'Brien, 2011).

In this experiment decellularized bone matrix hydrogel bioink was prepared from pig bones through bone powder creation, demineralization, lipid removal, decellularization, and digestion. Printability and biological properties were evaluated. The decellularized extracellular matrix was methacrylated and bioprinted with the micro-extrusion technique followed by maturation. The hypothesis that use of Decellularized Extracellular Matrix (dECM) for bioink development will produce a tissue-specific micro-environment for induced tissue growth for bioprinting of bone tissue due to the dECM containing collagen type I, ECM components, and bone morphogenetic proteins (BMPs) was tested in this experiment. The native bones and dECM were tested with by utilizing sircol collagen assay s1000, Blyscan sulfated Glycosaminoglycan B1000, DNeasy blood and tissue kit with Piccogreen dsDNA assay kit, and Quantikine enzyme-linked immunosorbent assay to measure BMPs (BMP-2 and BMP-7). The absorbance, optical density, and fluorescence was measured with the spectrometer and the data was analyzed through Microsoft excel. The collagen content was maintained which is great to facilitate a microenvironment for growth of bone tissues. The dECM produced low DNA content which is good to reduce the risks of activating the immune system and prevent an inflammatory response and rejection during implantation. There was no significant loss in BMP-2 and BMP-7 after decellularization which shows decellularization isn't harmful to BMPs. Bioprinting can reduce the need for donors in surgical aspects as an individual's bones can be regenerated with scaffolds that can be 3D printed and implanted for greater chances of acceptance from the body through decellularization. This method can also reduce the amount of medication utilized in immunosuppression after surgery.

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Mathematical and Physical Modeling of GelMA Bioprinting for Optimized Urethra Bioprinting

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Bioprinting technology has the goal of advancing the field of tissue engineering by recapitulating biological tissue formation from spatially organized cells and biomaterials. Gelatin-methacryloyl hydrogels are widely used in the bioprinting due to its tunable characteristics. The three main bioprinting techniques include: inkjet, laser-assisted and extrusion printers. Extrusion-based bioprinting is a preferred method for printing tissues and cell-laden biomaterials. During extrusion-based bioprinting, shear stress and pressure impact cell viability. We hypothesize that the effects of shear stress are more damaging than those of pressure but vary based on viscosity. By decoupling pressure and shear stress using fluid dynamics relationships, we can establish ranges for optimal bioprinting.

Fluid velocities were measured across the cross-section of polyethylene tubing in an extrusion-based printing environment. Using this data, a flow profile can be developed. We created a flow of a 6% GelMA (w/v) and 20 mg/ml gelatin hydrogel mixed with fluorescent microbeads with a syringe pump. Time lapses of the flow were captured using fluorescence imaging and cellSens software. Flow tracks of the particles were developed using the TrackMate plugin on ImageJ. The radial positions of the individual tracks from the center of the tubing were calculated. Flow velocities were measured and displayed on the developed tracks.

From the flow profile developed, shear stress can be calculated and the relationship between shear stress and flow rate is shown. Validations of the fluid dynamic model will next be applied to cell bioprinting of bioinks of disparate viscosities with an assessment of cell viability, which will allow for optimization of urethra bioprinting according to cell type sensitivity.

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Digital Light Projection (DLP)-based 3D Bioprinting for In Vitro Liver Models

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Current drug development techniques can take one billion dollars and around 10 to 15 years but many drugs fail due to unexpected liver metabolism of the drug.[1] The current standard of *in vivo* studies in animals cannot provide an accurate model of drug metabolism since most animal livers function very differently from human livers. An alternative to *in vivo* animal studies are *in vitro* models of human organs.

Bioreactor systems containing models of human organ miniatures (or mimetics) and organ systems have the potential to provide a more accurate model of the human body and thereby provide a better platform for drug testing. This removes the need for laboratory animals and also can provide an accurate representation of human liver function. A suitable *in vitro* model would be able to support liver cells in a perfusion bioreactor system so the liver cells can perform necessary functions. The objective of this study was to create a static control of cell-laden hydrogel constructs of various formulations to identify the formulation which best supports cell viability and function of HepG2 liver cells. HepG2 cells were suspended in PEG-based bioinks and printed into gyroid structures (Dimension ?) using digital light projection (DLP)-based 3D bioprinting. Three formulations were tested; 8% PEG, 8% poly(ethylene glycol) (PEG) conjugated with RGD peptide, and PEG mixed with gelatin methacrylate (GelMA). After printing, the constructs were cultured in high glucose DMEM media for 4 weeks.

Over the course of 4 weeks cell viability and functionality were maintained in most groups. Cells in the PEG and PEG-RGD groups were able to maintain cell viability as seen in the live dead assays. Cell aggregation, a characteristic function of liver cells, was also observed in these groups using the live dead stains. The PEG-GelMA group, however, lost cell viability over time and showed much less cell aggregations. AlamarBlue assays confirmed similar behavior since the cells in the PEG and PEG-RGD groups proliferated whereas the cells in the PEG-GelMA groups did not. The cells were also able to maintain functionality in most groups. The PEG-RGD group produced the most albumin as seen in immunohistochemical staining. Urea concentrations were very similar among the groups although the PEG-GelMA group had slightly higher amounts of urea.

Other studies of these hydrogel structures in prefusion bioreactor systems have been ongoing and have found similar results. Further studies using non-cancerous liver cell lines are necessary for further confirmation of these results and development of a fully representative liver *in vitro* model.

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ENGINEERING AN ALGINATE MATRIX TO MIMIC THE PANCREATIC MICROENVIRONMENT TO ENHANCE ENCAPSULATED ISLET FUNCTION

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Diabetes is an autoimmune disorder in which the patient's immune system adversely targets and destroys the insulin-producing β-cells of the pancreas and renders the body unable to regulate blood glucose. An alternative to the current standard treatment of exogenous insulin administration is islet transplantation, where pancreatic islets are isolated from a donor pancreas and encapsulated in alginate[1]. When mixed with a divalent cationic solution, the polymer capsule rapidly crosslinks to form a hydrogel. Alginate can also be coated with pore-selective polymers to provide immune protection post-transplantation and eliminate the need for immunosuppressants. However, the current approach of using alginate-only microenvironment structurally and mechanically differs from the native pancreas and may contribute to transplanted islet cell loss of function and death. The standard 100mM $CaCl_2$ divalent cation crosslinker forms too stiff a hydrogel and leeches out into the body over time. The use of a lower concentration $SrCl_2$ crosslinking solution can reduce the stiffness and prevent diffusion of cations from the hydrogel resulting in better stability upon implantation. The alginate hydrogel alone also lacks adhesive properties and biological support which addition of extracellular matrix (ECM) material can provide to guide cell adhesion, motility and stem cell fate through biomechanical and biochemical cues.

The objective of this study is to engineer an alginate stiffness that mimics the natural pancreatic scaffold using a lower concentration $SrCl_2$ crosslinker and the incorporation of ECM material and to determine its effect on encapsulated islet viability and function. Capsule stiffness was optimized by comparing complex shear modulus measurements of $SrCl_2$ crosslinked alginate and porcine pancreas through oscillatory shear rheometry. The complex shear modulus of the porcine pancreas was measured to be 1779 Pa. We selected a 25mM $SrCl_2$ crosslinking solution due to its mechanical similarity to the native porcine pancreas.

Islet cells were isolated from a porcine pancreas by selective osmotic shock (SOS) protocol. Islets were encapsulated in two groups of 1.5% LVM alginate either with or without decellularized human pancreatic extracellular matrix material and crosslinked in either 100mM $CaCl_2$ or 25mM $SrCl_2$ divalent cationic solutions. Islets will be assessed for viability through a CFDA and PI live/dead assay and MTT metabolic assay and for glucose response through a glucose-stimulated-insulin-response (GSIR) assay to determine impact of mechanical stiffness and ECM content on encapsulated islets.

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Controlled Delivery of IGF1 from Alginate Microbeads

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Stress urinary incontinence (SUI) is a common medical problem that affects up to 50% of women. The overall goal of this project is to determine the effect of sustained delivery of insulin-like growth factor 1 (IGF1) and nerve growth factor (NGF) from biodegradable Alginate-PLO-Gelatin microbeads on urine-derived stem cells (USC)-mediated repair of SUI. In the present study we are examining the use of alginate microbeads fabricated with a microfluidic chip device for controlled delivery of IGF-1.

A template developed at WFIRM was cut into a microfluidic chip using a Full Spectrum Laser. Mineral oil and Span80 flowed through the first inlet to cut the 1.5% LVG alginate into microbeads. These beads crosslinked with mineral oil and acetic acid through the rest of the chip before being collected. A standard curve to measure IGF-1 concentrations in the range of 0.005µg/mL to 5µg/mL using a modified Lowry assay with 1.5% nano CuO particles in place of 1% $CuSO_4$ is currently being developed.

Alginate microbeads measuring an average size of 160 µm in diameter were created with the microfluidic device. The microbeads showed monodispersity and shape consistency. The newly developed microfluidic chip is efficient at creating large amounts of alginate microbeads that are consistent in size and shape. This allows biocompatible, controlled drug delivery systems to be developed using alginate microbeads. After optimization, the modified Lowry method will be used to measure IGF-1 concentrations released from microbeads loaded with IGF1 by soaking them in 5µg/mL of IGF1. The loading efficiency of IGF-1 in alginate beads will be determined along with IGF-1 concentrations released over a 24-hour incubation period.

At the completion of the present *in vitro* studies, the data generated would provide the basis for future *in vivo* experiments in which alginate microbeads will be implanted with USC in urogenital tissue to assess efficacy in a rat model of SUI.

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REGENERATIVE POTENTIAL OF EXOSOMES FROM URINE-DERIVED STEM CELLS

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As a novel stem cell source, urine-derived stem cells (USC) can be obtained via a non-invasive, simple and low-cost approach. USC display high expansion capacity and express telomerase activity. In addition, these cells can give rise to multiple cell lineages and prompt tissue regeneration (1). However, in patients with chronic disorders, such as uncontrolled diabetes, regenerative function of USC is impaired (2). Therefore, these patients' own cells might not be well suited for tissue regeneration. As a cell-free component, exosomes enriched in proteins, mRNAs and miRNAs characteristic of parental stem cells represent a potential approach for treating various diseases (3). Exosomes secreted from allogenic stem cells from healthy donors do not induce immunoreactions in the host, acting as optimal agents for therapy (4). Our hypothesis is that exosomes secreted from healthy donors' USC possess regenerative properties for neuromuscular regeneration.

The goal of this study is to isolate exosomes from healthy donors' USC and determine their regenerative protein and RNA content, which could potentially promote neuromuscular regeneration in the treatment of urinary incontinence. USC were initially isolated from healthy donor's urine samples (n=2) and cultured up to passage 3. To assess the ideal condition for augmenting pro-angiogenic factors in exosomes, USC were cultured under hypoxia (1% O_2) and compared to those cultured in normoxia (21% O_2). Extracellular vesicles were isolated from the media via high-speed centrifugation. These vesicles were observed under transmission electron microscopy (TEM) for size and scanned for CD63 and CD9 exosomal surface markers via a Western blot. Moreover, the cultured cells were scanned for mesenchymal stem cell markers, CD44, CD73, and CD90, via flow cytometry to verify that they were USC.

For the normoxically treated USC sample, transmission electron microscopy revealed vesicles with characteristic sizes of exosomes in the range of 40-150 nm in diameter. Moreover, western blot analysis confirmed the presence of vesicles with CD63 and CD9 exosomal surface markers. Finally, the Flow NanoAnalyzer N30 verified the presence of cultured cells with CD44, CD73, and CD90. In conclusion, the present study confirms that human USC secrete rich exosomes, potentially with the capacity to regenerate neuromuscular tissue in the treatment of urethral sphincter dysfunction. Future work will involve isolating exosomes from hypoxically-treated USC and comparing their protein and RNA content to exosomes from normoxically-treated USC. Furthermore, the in vivo neuromuscular regenerative capacity of exosomes from hypoxically treated USC will be compared to those from normoxia-treated USC in a rodent model of urethral sphincter injury.

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Extracellular Matrix as a Cell Culture Media Supplement for Long Term Islet Culture

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Tissue engineering is an interdisciplinary field applying the principles of engineering and life science toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ. Our lab developed a detergent free decellularization method whereby animal or human derived organs are decellularized through a process characterized by a surgical dissection and a mechanical and enzymatic ECM extraction. In this study human pancreatic and renal ECM were obtained and used as a cell culture media supplement for human pancreatic islets.

Human pancreases and kidneys not suitable for transplantation were surgically dissected and decellularized according to a new protocol. Briefly, organs were surgically dissected and mechanically shaken at 200rpm at 4C for 24 hours in deionized water and enzymatically digested in a DNAse based solution. Finally, tissue was washed in deionized water for 24hours at 4C.
Human Islets purchased from ProdoLab were cultured with maintenance medium and medium supplemented with pancreatic and renal ECM respectively. Glucose stimulation insulin release assay and DNA quantification were performed at four different time-points.

Pancreatic and renal ECM were successfully obtained through decellularization. Human Islets were successfully cultured up to day 28. Soluble ECM from both pancreas and kidney didn't show beneficial effects in insulin secretion overtime. Preliminary data obtained in our lab shows that increased insulin secretion is observed when the ECM is co-encapsulated with human islets suggesting that the lack of tridimensional structure is not beneficial for long-term culture of human pancreatic islets.

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The Biological Effects of Pancreas Extracellular Matrix Hydrogel in Recapitulating Human Islets' Biological Niche

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According to the American Diabetes Association, it is estimated that 1.25 million Americans are impacted by type 1 diabetes mellitus. Symptoms occur when beta cells, located in the islets of Langerhans, fail to secrete insulin. The cessation of insulin secretion causes effected individuals to possess high blood glucose levels, which can lead to side effects including renal failure, diabetic retinopathy, and neuropathy. The current treatments of type 1 diabetes seek to restore glucose homeostasis by supplying patients with insulin via insulin injections and islet transplantations [1]. Cell-based therapy treatments utilize the exogenous delivery of cells and biomaterials to treat patients and improve islet transplantations. The extracellular matrix (ECM), which is obtained by a process called decellularization, is a biomaterial that is physiologically adaptable to different cell-based therapies providing bioactive and functional growth factors. Alginate, a seaweed-derived substance, is another biomaterial that has also been successful in cell-based therapies and can form a biocompatible gel. The objective of this study is to demonstrate that encapsulation of islets in decellularized pancreatic ECM and alginate preserves the islets and their functionality.

To obtain the ECM hydrogel, a pancreas was harvested from a human, and the fat and connective tissue were removed in a process called delipidation. The ECM was extracted by a process of chemical-free decellularization, and the decellularized ECM was lyophilized and cryomilled into a fine powder. The cryomilled ECM was then enzymatically digested. Once the ECM was liquefied, the pH was adjusted to inactivate the enzymes. The liquefied ECM was incubated at 37˚ Celsius for crossing linking and hydrogel formation. This study consisted of three groups: free islets as the control, islets cultured with the ECM hydrogel, and islets encapsulated in the ECM hydrogel and alginate.

Islets were purchased from Prodo Laboratories and were encapsulated in alginate with the pancreatic ECM hydrogel. Upon alginate cross linkage in calcium chloride, islets were incubated at 37˚ Celsius, forming pancreas-derived ECM capsules that recapitulated the islets' natural microenvironment. To assess the viability of the islets, LIVE/DEAD imaging was performed, and it was shown that the islets encapsulated in the ECM and alginate retained their viability throughout the duration of the study. DNA samples were evaluated for healthy gene expression to confirm that pancreatic ECM was providing a positive physiological environment. Most significantly, a Glucose Stimulated Insulin Release (GSIR) test was performed to ensure the functionality of the islets.

The islets were cultured up to day 28, and the results of the GSIR showed that the insulin secretion of the encapsulated islets was more enhanced than that of the free islets and the islets cultured solely with the ECM hydrogel. The DNA quantification revealed that the viability and gene expression of the islets encapsulated in the ECM and alginate was more improved than that of the other two groups. It is thus concluded that pancreatic ECM hydrogel can be used to improve the encapsulation of human islets to improve and sustain insulin secretion.

Future work will focus on exploring animal-derived pancreatic ECM to obtain consistent function of metabolic effect of ECM on islets, avoiding interhuman variability.

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Notes:

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