Wake Forest Institute for Regenerative Medicine

Improving Patient's Lives through Regenerative Medicine

WELCOME TO:

THE WFIRM 2017 SUMMER SCHOLARS PROGRAM

FINAL RESEARCH DAY & POSTER SYMPOSIUM

CELEBRATING MULTIDISCIPLINARY RESEARCH EXPERIENCES FOR UNDERGRADUATE STUDENTS IN CHALLENGING AREAS OF REGENERATIVE MEDICINE

Venue: Piedmont Triad Community Research Center (PTCRC)
115 S. Chestnut Street, Winston Salem, NC 27101

Thursday, August 3, 2017

Undergraduate research is not only an essential component of good teaching and learning, but research with undergraduates is the purest form of student learning (Gentile, 2008).

http://www.wakehealth.edu/WFIRM/

Wake Forest™
School of Medicine

Institute for Regenerative Medicine

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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 2017

Today we invite you to join us in celebrating the graduation of 21 summer undergraduate scholars, who participated in our annual 10-week summer research program. May 30th 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 interns to present their summer undergraduate research. We offer congratulations to our scholars, their faculty mentors and a team of graduate, postdoctoral fellows and lab technicians for their important contributions and support. Much success is extended to our interns in their future educational and career pursuits. WFIRM is privileged to be a part of your intellectual and professional growth. We thank you all for joining us today and the support you have extended to these fine young adults.

Sincerely,

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

Joan Schanck, MPA
Summer Scholars Program Director

2017 WFIRM Undergraduate Summer Scholars

Summer Scholar	Primary Faculty Mentor(s)
Katherine Bolten Temple University Bioengineering, Sophomore	**Anthony Atala, MD** Professor and Director of WFIRM
Allison Boone Davidson College Biology, Senior	**Sean Murphy, PhD** Assistant Professor
Egil Brudvik Union College Psychology & English, Junior	**Graca Almeida-Porada, MD, PhD** Professor and **Christopher Porada, PhD** Associate Professor
David Cleveland University of Michigan - Ann Arbor Biomedical Engineering, Junior	**In Kap Ko, PhD** Assistant Professor
William Collier Purdue University Biochemistry/Pre-med, Sophomore	**Shay Soker, PhD** Professor
Nancy Contreras-Quinteros Appalachian State University Chemistry, Junior	**Steve J. Walker, PhD** Associate Professor
John Craig University of Texas at Austin Biomedical Engineering, Freshman	**Aleks Skardal, PhD** Assistant Professor and **Thomas Shupe, PhD** Assistant Professor
Williams Dean University of North Carolina – Greensboro, Biology, Freshman	**Baisong Lu, PhD** Assistant Professor
Amelia Hurley-Novatny University of Maryland - College Park, Bioengineering, Sophomore	**James Yoo, MD, PhD** Professor, Assoc. Director, CSO and **Sang Jin Lee, PhD** Associate Professor
John Latimer Stanford University Biology, Junior	**Anthony Atala, MD** Professor and Director of WFIRM
Emily Long Pennsylvania State University Biomedical Engineering, Junior	**Emmanuel Opara, PhD** Professor

Summer Scholar	Primary Faculty Mentor(s)
Nickolas Mundo Texas A&M University – Corpus Christi, Mechanical Engineering, Senior	**James Yoo, MD, PhD** Professor, Assoc. Director, CSO and **Sang Jin Lee, PhD** Associate Professor
Jennifer Paxton Winston-Salem State University Exercise Science, Sophomore	**Yuanyuan Zhang, MD, PhD** Assistant Professor
Hayley Premo Christopher Newport University Neuroscience, Senior	**Tracy Criswell, PhD** Assistant Professor
Caroline Sane Georgia Institute of Technology Chemical and Biomolecular Engineering, Sophomore	**Anthony Atala, MD** Professor and Director of WFIRM
Mark Schwartz Saint Louis University Biomedical Engineering, Freshman	**John Jackson, PhD** Associate Professor
Charles Spong Clemson University Bioengineering, Senior	**Frank Marini, PhD** Professor
Eliot Teal Clemson University Bioengineering, Senior	**Frank Marini, PhD** Professor
Margaret VanSchaayk Wake Forest University Communications, Senior	**Sang Jin Lee, PhD** Associate Professor
Sue Zhang University of Rochester Biomedical Engineering, Junior	**Hooman Sardi-Ardekani, MD, PhD,** Assistant Professor
Suzanne Zhou Virginia Commonwealth University Biology, Sophomore	**Khalil Bitar, PhD** Professor

WFIRM 2017 SUMMER SCHOLARS FINAL RESEARCH DAY

Thursday, August 3, 2017

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

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

SCHEDULE

7:45 am to 8:00 am Summer Scholars Arrival at PTCRC: Photo Session

8:00 am to 8:30 am Guest Arrivals: Registration w/coffee, biscuits and muffins at PTCRC

8:30 am to 8:45 am Welcome and Overview

Anthony Atala, MD, Director, WFIRM and
Joan Schanck, MPA, Summer Scholars Program Director

8:45 am to 10:00 am Summer Scholars' Presentations – Part I
(Note: Group Q&A follows each session)

Session 1

1 Emily Long Pennsylvania State University	*MICROFLUIDIC ENCAPSULATION OF MESENCHYMAL STEM CELLS FOR TREATMENT OF TYPE 1 DIABETES*
2 John Latimer Stanford University	*CHARACTERIZING THE STRUCTURAL AND MORPHOLOGICAL DEVELOPMENT OF A BIOENGINEERED UTERINE TISSUE IN A RABBIT MODEL*
3 Mark Schwartz Saint Louis University	IN VITRO *OVARIAN ORGANOID VIABILITY AND ENDOCRINE FUNCTION IN A 3D-PRINTED PERFUSION CHAMBER*

Session 2

4 Hayley Premo Christopher Newport University	*EFFECTS OF IRRADIATION ON SKELETAL MUSCLE PROGENITOR CELLS*
5 Margaret vanSchaayk Wake Forest University	*EFFECTS OF BIOACTIVE MOLECULES ON SKELETAL MUSCLE DEVELOPMENT IN 3D BIOPRINTED MUSCLE CONSTRUCTS*
6 John Craig University of Texas at Austin	*DIFFERENTIATION OF MONOCYTES TO MACROPHAGES: WOUND INFLAMMATION AND SCARRING*
7 Williams Dean University of North Carolina, Greensboro	*DELIVERY OF CAS9/sgRNA BY LENTIVIRAL SYSTEM*

Session 3

8 David Cleveland University of Michigan, Ann Arbor	*FABRICATION OF VASCULAR RENAL CONSTRUCTS WITH ACCELERATED ANGIOGENIC ACTIVITY*
9 Sue Zhang University of Rochester	*OPTIMIZATION AND CHARACTERIZATION OF 3D HUMAN PREPUBERTAL TESTIS ORGANOID*
10 Eliot Teal Clemson University	*COMPARISON OF NOVEL OPTICAL TISSUE CLEARING TECHNIQUES ON WHOLE MOUSE ORGANS FOR REGENERATIVE IMAGING*
11 Charles Spong Clemson University	*DIFFERENTIAL Z IMAGING (DIFFZ)- AN AUTOMATED METHOD FOR 3D REGENERATIVE IMAGING*

10:00 am to 10:15 am Coffee Break

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

Session 4

12 Amelia Hurley-Novatny University of Maryland	*CO-CULTURE DIFFERENTIATION OF PLACENTAL DERIVED STEM CELLS TOWARDS BOTH OSTEOGENIC AND CHONDROGENIC LINEAGES*
13 Nickolas Mundo Texas A&M, Corpus Christi	*OPTIMIZATION OF BIOINKS FOR 3D BIOPRINTING OF KIDNEY CONSTRUCTS USING GELATIN METHACRYLATE AND FIBRINOGEN*
14 Jennifer Paxton Winston-Salem State University	*NON-INVASIVE CELL TRACKING WITH BRIGHTER AND RED-TRANSFERRED LUCIFERASE FOR STEM CELL THERAPY IN THE TREATMENT OF ERECTILE DYSFUNCTION*
15 Caroline Sane Georgia Institute of Technology	IN VITRO *MODEL OF THE BLOOD BRAIN BARRIER FOR DRUG SCREENING*

Session 5

16 Egil Brudvik Union College	*SIMULATED MICROGRAVITY IMPAIRS DNA DAMAGE REPAIR IN A PRIMITIVE HUMAN LEUKEMIC CELL LINE*
17 Nancy Contreras-Quinteros Appalachian State University	*ISOLATION AND CHARACTERIZATION OF SERUM-DERIVED EXOSOMES AS BIOMARKERS FOR ILEOCOLITIS IN CHILDREN WITH AUTISM SPECTRUM DISORDER*
18 Suzanne Zhou Virginia Commonwealth Univ	*CELL THERAPY FOR GASTROPARESIS*

Session 6

19 Allison Boone Davidson College	*DEVELOPING AN AIRWAY ORGANOID FOR DISEASE MODELING AND DRUG SCREENING*
20 William Collier Purdue University	*ENGINEERING 3D MICROENVIRONMENTS: COLORECTAL CANCER MOTILITY AND ECM REMODELING*
21 Katherine Bolten Temple University	*OPTIMIZING THE BONE-IMPLANT INTERFACE FOR AMPUTEE-RELATED INJURIES*

11:20 am to 11:35 pm Wrap-Up/Certificates of Completion
(**Note:** Walk to WFIRM for Poster Session & Luncheon)

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

12:30 pm to 1:45 pm Lunch with Scholars at WFIRM (2nd Floor Collaboration Area)

1:45 pm to 2:30 pm Lab Tours at WFIRM
(**Note:** Guests sign-up during registration. Scholars assist with demos, overview of their work in lab at stations)

1

MICROFLUIDIC ENCAPSULATION OF MESENCHYMAL STEM CELLS FOR TREATMENT OF TYPE 1 DIABETES

*E. Long, K. Enck, S. Rajan, J. Aleman, A. Hall, E. Opara
*Summer Scholar, Wake Forest Institute for Regenerative Medicine

Background: Type 1 diabetes is an autoimmune disease, which is characterized by an insulin deficiency caused by cytotoxic T-cells destroying the pancreatic β-cells. The standard treatment is daily injections of exogenous insulin, which temporarily controls blood sugar. An alternative is allogeneic islet cell transplantation, which requires immunosuppression of patients (1). To reduce the need for immunosuppressive treatments, islets have been successfully encapsulated in alginate beads to allow transport of insulin and nutrients while preventing immune cells and antibodies (2). Current methods for encapsulation use dripping methods, which use air and gravity to form microbeads (3). However, these technologies form beads with large polydispersity, which could lead to a variable number of cells in each bead.

Study Objectives: The first objective was to optimize a microfluidic device that produces alginate microbeads with greater monodispersity, while maintaining the spherical form at a reasonable rate of production. The device was designed to reduce the bead size for direct implantation into the pancreas without induction of pancreatitis. The microfluidic device combines bead formation with crosslinking, whereas other devices crosslink the beads externally. The second objective was to encapsulate mesenchymal stem cells (MSCs) for inhibition of the autoimmune response associated with Type 1 diabetes. MSCs have been shown to produce immunosuppressive molecules to combat the cytotoxic T-cells responsible for the destruction of pancreatic β-cells (4).

Experimental Design: Four parameters, including monodispersity, shape, size, and rate of production were considered for optimization of the device. Monodispersity, defined by a coefficient of variation less than 5%, is an important parameter for encapsulation and dosing. Decreasing the range of sizes allows for precise calculation of the number of cells being implanted, and the beads' spherical shape should be maintained to avoid promoting an immune response against implanted alginate. To reduce pressure exerted on the pancreas during implantation, the target microbead size achieved was between 80 μm and 180 μm in diameter. Rate of production was examined in order to maintain encapsulated cells' viability. After optimization, MSCs were encapsulated and cell viability was tested on days 0, 4, 7, 14, 21, and 28 using live dead stains. The number of cells per volume of alginate was calculated and standardized to a 150 μm bead to test for proliferation of cells within the beads.

Results & Conclusion: Optimization of the microfluidic device lead to the generation of spherical beads in the desired range by adjusting flow rates of mineral oil and alginate. Increasing the flow rate of mineral oil, decreased the diameter of the microbeads. Changing the alginate flow rate affected the rate of production and dispersity of the bead sizes. Polydispersity was reduced to a coefficient of variance as low as 9.45%. Live dead stains immediately after encapsulation verified the microfluidic method did not harm the cells, and viability was maintained in culture for 28 days until conclusion of the study. Standardized 150 μm beads contained an average of 12.60 ± 4.25 cells at each time point, indicating encapsulated cells do not proliferate. The lack of proliferation ensures the proper dosing of engineered MSCs for therapeutic effects, and suggests the integrity of the alginate bead will not be compromised by expansion once implanted into the pancreas. The viability and proliferation data collected may lead to future studies using encapsulated engineered MSCs aiming to alter the pancreatic environment and shut down autoimmune response.

Acknowledgements: This summer research position was sponsored by the Henry H. and Dorothy Ray Henley Fund.

References:

1. Daneman, D. Type 1 Diabetes. Lancet. 2006; 367:847-58
2. Opara, E. et al. Design of a bioartificial pancreas. J Investig Med. 2010; 58(7): 831–837.
3. Tendulkar, S. et al. A Three-dimensional microfluidic approach to scaling up microencapsulation of cells. Biomed Microdevices. 2012 June; 14(3): 461-469.
4. Nasaf, A. et. al Immunosuppressive Effects of Mesenchymal Stem Cells: Involvement of HLA-G. Transplantation. 2007 July; 84 (2): 231-237.

2 *CHARACTERIZING THE STRUCTURAL AND MORPHOLOGICAL DEVELOPMENT OF A BIOENGINEERED UTERINE TISSUE IN A RABBIT MODEL*

J.M. Latimer*, R. Magalhaes, A. Atala
Summer Scholar, Wake Forest Institute for Regenerative Medicine

The uterus is essential for mammalian reproduction and is one of the most plastic organs in terms of tissue remodeling. Its main functions include nurturing the implanted embryo and carrying the fetus to term. However, many factors, including congenital malformations and acquired diseases, may affect the reproductive potential of women. Despite current advancements in surgical techniques and reproductive technologies, uterine factor infertility (UFI) continues to affect 3-5% of women in the U.S. Recent discoveries in regenerative medicine have opened the door for bioengineering functional tissues using autologous cells to potentially grant fertility to those affected by UFI[1-5]. Previous studies in WFIRM have shown that autologous cell-seeded, biodegradable polymer-based constructs can be fabricated and implanted in a partially removed uterine horn to restore functionality in rabbits[3-4].

This study evaluated uterine tissue samples retrieved at 1, 3, and 6 month time points from four experimental groups: cell-seeded, non-seeded, injured-only, and control. All animals had one uterine horn surgically removed for endometrial and myometrial cell isolation and expansion. A partial hysterectomy was performed on the opposite horn, followed by an implantation of either a cell-seeded construct, a non-seeded scaffold, or no scaffold (injured-only group) controlling for the rabbit's intrinsic regenerative capacity. An *in vitro* study of the seeded scaffold was performed to augment previous data on cell viability and proliferation. Autologous uterine-derived cells were seeded on a poly-glycolic acid/poly-DL-lactide-co-glycolide (PGA/PLGA) scaffold to create an in vitro engineered construct. During an eight day incubation period, samples taken from the constructs showed high levels of cell viability and proliferation. Furthermore, scanning electron microscopy revealed cell attachment and even distribution throughout the scaffold.

To analyze morphological structure, collagen content as a proxy for fibrosis, and expression of specific tissue markers in the regenerated uteri, H&E, Masson's Trichrome, and immunohistochemistry stains were performed, respectively. The seeded group developed better organized smooth muscle and epithelial layers than both the non-seeded and injured groups, while appearing comparable to the control group. According to the Masson's Trichrome stain, the seeded group had lower collagen deposition than the non-seeded and injury groups, with no significant difference compared to the control at 3 months, unlike the injured and non-seeded rabbits. Samples were tested for the expression of specific markers for myometrium (myosin heavy chain II (MHC) and smooth muscle actin (SMA)) and endometrium (cytokeratin (AE1/AE3), uteroglobin), as well as uterine estrogen and progesterone receptors. The stains detected expression of hormonal receptors (estrogen and progesterone) in both the epithelial and the smooth muscle layer in the seeded group, while the non-seeded and injured groups did not express these receptors at comparable levels. The MHC stain clearly shows that the non-seeded and injured groups' smooth muscle is disorganized, which hinders uterine functionality during pregnancy and labor. Overall, from 3-6 months, well defined glandular epithelium and myometrium structures were observed in the seeded group. The data collected demonstrates that a PGA/PLGA scaffold seeded with autologous uterine cells can be used to improve in vivo tissue regeneration of a partially removed horn in rabbits.

Acknowledgements: This summer research position was made possible by the Marrianne and G. Allen Mebane Fund

References:

1. BaoLin, G. & Ma, P.X. Synthetic biodegradable functional polymers for tissue engineering: a brief review. *Science China. Chemistry* **57**, 490-500 (2014).
2. Freed, L.E. et al. Biodegradable polymer scaffolds for tissue engineering. *Bio/technology* **12**, 689-693 (1994).
3. Atala, A. Tissue engineering and regenerative medicine: concepts for clinical application. *Rejuvenation research* **7**, 15-31 (2004).
4. Atala, A., Bauer, S.B., Soker, S., Yoo, J.J. & Retik, A.B. Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet* **367**, 1241-1246 (2006).
5. Hellstrom, M. et al. Bioengineered uterine tissue supports pregnancy in a rat model. *Fertility and sterility* (2016).

3

IN VITRO *OVARIAN ORGANOID VIABILITY AND ENDOCRINE FUNCTION IN A 3D-PRINTED PERFUSION CHAMBER*

Mark Schwartz[1], Sittadjody Sivanandane[2], Anil Kumar[2], Russel Sequeira[2], John Jackson[2]
[1]*Summer Scholar, Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC*
[2]*Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC*

As tissue engineers face higher pressure to up-scale and accelerate the drug pipeline, more cost-effective and physiologically relevant systems for modeling the human body have become even more lucrative. Through novel body-on-a-chip technology, several "organs" arranged in series through a microfluidic network serve as a biomimetic *in vitro* human body. Testing clinical drug candidates in this way would allow researchers to streamline the drug screening and toxicology study process as they analyze the behavior of an organ system, using human cells as a pre-clinical route in lieu of a potentially less accurate, more costly animal model[1]. Testing a drug's effects on fertility is vital, as 6.7% of married women in the US are infertile[2], and chemotherapy drugs are already known to cause ovarian toxicity[3]. Our hypothesis is that ovarian spheroids cultured in a single-organ, perfusion environment would remain viable and have higher levels of endocrine function than those cultured in a well plate. Functional ovarian cells post-treatment would indicate that the ovary could be added into a body-on-a-chip, opening up more possibilities for drug screening.

The project was laid out to engineer a silicone chamber for perfusion culture of ovarian spheroids and assessing the spheroids' viability and endocrine function. The chamber was modified and printed several times as leaks and other design flaws were eliminated. Theca and granulosa cells were isolated and aggregated into spheroids from 21-day old rats. After maturing for five days, the spheroids were seeded in the perfusion chamber, then cultured for 14 days with media aliquots collected every other day. At days 7 and 14, spheroids were removed from the system and assessed for live and dead cells. Culture in basal media without LH and FSH served as a negative control and culture in wells as a positive control. At the end of the study, the media aliquots were used in ELISA assays for 17 β-estradiol and progesterone, and the organoids' endocrine function was evaluated.

Cell viability assessment through LIVE/DEAD stains suggests that the organoids functioned better in the perfusion chamber than in the well culture, as the organoids in the chamber maintained much higher viability than those in wells. From these results, we can draw the conclusion that the chamber's environment was better suited for the cells to stay alive. However, the spheroids in the static well culture exhibited a hormone secretion level nearly tenfold higher than those in the perfusion chamber, indicating poor endocrine function in the chamber culture. Such a weak hormone signal could be due to two factors. Firstly, the chamber seeding process may have led to much fewer organoids in the chamber culture than in the well culture, making the chamber organoids' hormone secretion seem comparably much weaker. Secondly, it was noted that many organoids clumped together in the chamber's channels, which blocked media flow. Although these clumped cells could not be retrieved from the chamber, they were probably dead or had reduced function due to a lack of nutrients, which could also have affected the media's hormone levels. Curiously, there was a statistically significant drop in hormone secretion in the well culture comparing day 2 to day 14, but no significant change in the chamber culture over the two week period. This shows promise for future iterations of the study if the organoid seeding procedure and chamber are further optimized.

Acknowledgements: This summer research position was made possible by the Frank M. Parrish Jr. Fund for Regenerative Medicine

References:

1. Perestrelo, A., Águas, A., Rainer, A., & Forte, G. (2015, December 10). Microfluidic Organ/Body-on-a-Chip Devices at the Convergence of Biology and Microengineering. Retrieved July 25, 2017, from http://www.mdpi.com/1424-8220/15/12/29848/htm
2. Infertility. (2016, July 15). Retrieved July 23, 2017, from https://www.cdc.gov/nchs/fastats/infertility.htm
3. Singh, K. L., Davies, M., & Chatterjee, R. (2005, January 01). Fertility in female cancer survivors: pathophysiology, preservation and the role of ovarian reserve testing | Human Reproduction Update | Oxford Academic. Retrieved July 23, 2017, from https://academic.oup.com/humupd/article/11/1/69/609608/Fertility-in-female-cancer-survivors

4

EFFECTS OF IRRADIATION ON SKELETAL MUSCLE PROGENITOR CELLS

*H Premo, J Poteracki, Y Zhou, K Moschouris, S Barlow, S Soker, T Criswell
*Summer Scholar, Wake Forest Institute for Regenerative Medicine

Radiation therapy (RT) is a common treatment for many types of cancer, with 50% of cancer patients receiving RT as a single or multi-methodological approach to treatment.[1] Although RT is an effective treatment for killing cancerous cells, it also damages healthy tissue, leading to a condition called radiation-induced fibrosis (RIF). RT affects skeletal muscle by interfering with the activity of muscle progenitor cells (MPCs), the stem cells in muscle tissue that fuse to produce new muscle fibers in response to injury. Upon exposure to RT, MPCs can become fibrogenic instead of myogenic, producing excess collagen instead of fusing into new myofibers. This leads to muscle weakness, decreased muscle elasticity, and an overall decrease in functionality of the muscle in the area exposed to RT. It was hypothesized that muscadine grape extract (MGE) may serve as a potential treatment for RIF. Due to the anti-inflammatory properties of the drug, MGE could reduce the impact of RT on satellite cells by decreasing the inflammatory response to tissue injury. This study will focus on the effects of gamma-radiation and MGE treatment on the morphological and molecular changes in MPCs.

C2C12 cells, a commonly used myogenic cell line, were used for these experiments. Initial experiments examined dose and time dependent changes on C2C12 cells. *Irradiation:* Cells received one dose of radiation at 0,1,5,10,15, or 20 Gy and were then placed in either growth media (DMEM with 10% FBS), to maintain them as single cells, or differentiation media (DMEM with 2% horse serum), to promote cell fusion into fibers. Cells were harvested 2, 4, or 7 days after irradiation. Incucyte was used to generate growth curves and daily images in order to observe the morphological changes of the cells over time. At each time point, RNA was harvested to perform qPCR to analyze markers of proliferation (Ki67), differentiation (Myf5 and MyoG), and fibrosis (CTGF, TGF-β1, and NFKB). *MGE treatment*: C2C12 cells were treated with 10µg/ml of MGE at the time of irradiation and cells were isolated at 2, 4, and 7 days later.

Overall, cells subject to higher doses of radiation demonstrated a decreased amount of myofibers, decreases proliferation, and higher expression of fibrotic markers.
In response to these observations, a second experimental phase was implemented, where half of the irradiated cells were dosed with MGE in an attempt to prevent the phenotypical changes caused by irradiation. However, MGE had a detrimental effect on the cells, resulting in increased cell death and decreased myofiber quantity. This data suggests that MPCs exposed to high levels of irradiation may switch from a myogenic to a fibrogenic phenotype. This could result in higher accumulation of fibrosis in tissues treated with RT. Additionally, our data indicated that MGE sensitized the C2C12 cells to radiation, opening up the possibility that the drug could be used in conjunction with cancer treatments rather than reducing the effects of RIF. Further experiments will need to be done to confirm these results.

Acknowledgments: This summer research position was made possible by the Marianne and G. Allen Mebane Fund for Regenerative Medicine. Additional special thanks to the Joshua Frase family.

References:

1. Baskar R., Lee K.A., Yeo R., Yeoh K.W. Cancer and Radiation Therapy: Current Advances and Future Directions. *Int J Med Sci* 2012; 9(3):193-199. doi:10.7150/ijms.3635

5 *EFFECTS OF BIOACTIVE MOLECULES ON SKELETAL MUSCLE DEVELOPMENT IN 3D BIOPRINTED MUSCLE CONSTRUCTS*

Margaret vanSchaayk*, Ji Hyun Kim, James J. Yoo, Anthony Atala, Sang Jin Lee
*Summer Scholar, Wake Forest Institute for Regenerative Medicine

Volumetric muscle loss (VML) involves defect to at least 20% of a given muscle's mass and results in profound structural and functional impairment. Current standard of care is an autologous muscle graft. However, in many instances, a suitable host muscle flap is not available and poor grafting efficacy limits functional restoration of muscle mass. Being able to bioengineer a functional skeletal muscle construct could potentially fill this clinical void.

Several biofabrication techniques have been used to engineer skeletal muscle tissue, each accompanied by limitations such as inadequate fiber alignment for functionality and low cell viability long term. To overcome these limitations, a previous study applied 3D bioprinting technology to fabricate skeletal muscle constructs. 3D bioprinting enables the fabrication of volumetric tissue constructs with complex structure by controlling the spatial arrangement of cells, biomaterials, and biomolecules. 3D bioprinted skeletal muscle constructs fabricated using human muscle progenitor cells (hMPCs) laden in fibrin-containing hydrogel exhibited a high degree of alignment, high cell viability, and partially restored function to a volumetric muscle injury in rats. In order to fabricate constructs that provide increased restored functionality, further development of the construct is necessary. Paracrine factors associated with human neural stem cells (hNSCs) improve the development of skeletal muscle. However, hNSCs are unable to be obtained somatically and therefore in order to obtain clinically relevant data, they must be replaced while maintaining the same developmental effect. We expected to observe that some indirect effects of hNSCs effect the development of skeletal muscle.

In this study, we investigated the effects of bioactive molecules on skeletal muscle development in 3D bioprinted muscle constructs. Upon analysis of the culture mediums, FGF-2, IGF-2, HGF, CNTF, and GDNF were selected because of their increased presence in the coculture medium compared to the hMPC culture medium. These factors were added to hMPC cultures in 2D and 3D bioprinted muscle constructs. After 5 days of differentiation, muscle development was evaluated by immunostaining with myosin heavy chain, desmin, and acetylcholine receptors. In the 2D culture, FGF-2-, IGF-2- and HGF-treated groups had significantly improved muscle development compared to the non-treated group. The muscle development of these groups was comparable to the coculture of hMPCs and hNSCs. Based on the 2D culture results, skeletal muscle constructs were 3D bioprinted with these added factors. The FGF-2 and HGF treated constructs showed significantly increased muscle development compared to the constructs without added factors, and were statistically comparable to the constructs containing hMPCs and hNSCs.

In summary, FGF-2 and HGF improve the development of skeletal muscle. This finding may be beneficial in working toward creating skeletal muscle constructs with increased development and functionality. In the future, studying the synergistic effects of these factors in combination with other factors may produce an even more pronounced effect on muscle development.

Acknowledgements: This summer scholar's research was supported by the NIH Biotechnology Resource Grant (P41), Center for Engineering Complex Tissues (#P41EB023833).

References

1 Grasman, J. M., Zayas, M. J., Page, R. L., & Pins, G. D. (2015). Biomimetic scaffolds for regeneration of volumetric muscle loss in skeletal muscle injuries. *Acta biomaterialia*, *25*, 2-15.
2 Kang, H. W., Lee, S. J., Ko, I. K., Kengla, C., Yoo, J. J., & Atala, A. (2016). A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nature biotechnology*, *34*(3), 312-319.
3 Ostrovidov, S., Hosseini, V., Ahadian, S., Fujie, T., Parthiban, S. P., Ramalingam, M., ... & Khademhosseini, A. (2014). Skeletal muscle tissue engineering: methods to form skeletal myotubes and their applications. *Tissue Engineering Part B: Reviews*, *20*(5), 403-436.
4 Smith, A. S., Passey, S. L., Martin, N. R., Player, D. J., Mudera, V., Greensmith, L., & Lewis, M. P. (2016). Creating interactions between tissue-engineered skeletal muscle and the peripheral nervous system. *Cells Tissues Organs*, *202*(3-4), 143-158.

6

DIFFERENTIATION OF MONOCYTES TO MACROPHAGES: WOUND INFLAMMATION AND SCARRING

*J. Craig, R. Nelson, T. Shupe
*Summer Scholar, Wake Forest Institute for Regenerative Medicine
University of Texas at Austin, Austin, TX, 78705

Life's ability to adapt to its environment and survive has allowed evolution to take place over millions of years. Whether it has been the first organisms to carry out cellular respiration, possess membrane bound organelles, or the first multi-cellular organisms, evolution has continued throughout history. However, as many of these species have continued to evolve various adaptations have become obsolete. These adaptations are referred to as vestigial (1) – they no longer maintain purpose.

Over the past millennia, a fraction of evolutionary history, modern medicine was not only born, but also has advanced further than any academic field. Evolution has yet to compete and has fallen behind. This process may be the beginning of a new vestigial bodily function: scarring.

In this study, a new material will be tested on its effect on wound inflammation and scarring. This material is silicon dioxide fiber disks produced through proprietary chemistry and machinery by SpyderFiber LLC. These disks consist of microscopic SiO_2 fibers. These fibers are extremely small, allowing for a large surface area for interactions. SpyderFiber LLC has tested these SiO_2 fiber disks in an uncontrolled manner on pets and livestock and has proven extremely effective at healing skin wounds. In one case described by the producer, a fifteen-year-old matted dog had an ongoing skin wound to which the silicon dioxide was applied. Within a month of treatment not only had the wound fully recovered, but the dog's hair over the wounded area was the consistency of a puppy's.

Peripheral blood mononuclear cells (PBMCs) were isolated through the proper centrifugation of blood. All red blood cells were immediately lysed with a ammonium chloric solution, and the targeted monocytes were isolated through immunomagnetics. The monocytes were plated at a seeding density of 75,000 cells/cm^2 and allowed to naturally differentiate into inactive macrophages over 7 days. On the 7^{th} day the macrophages were transferred into 12 wells and separated into 4 experimental groups – control media, M1 growth factor (IFNy), M2 growth factor (Dexamethasone), and SiO_2.

At 12, 24, and 48 hours samples were collected from the wells for cytokine analysis through an ELISA array. Each of the three variant medias (control plus 2 growth factors) were measured as well. Standards were provided for each cytokine tested – IL-1a, IL-1B, IL-4, IL-6, IL-8, IL-10, IL-13, TNF-a, CCL2, and IFNy in order to observe the relative amount of M1 vs. M2 cytokines in each treated of the 12 treated wells.

Finally, at the 72 hour mark the macrophages from each cell were lysed. The RNA was isolated and the amount in each well was measured. From the macrophages' RNA cDNA was synthesized and tested through a qPCR, where the relative amount of the following isolated genes was relatively measured. The genes tested were CD206, IL-10, IL-1B, and TNF-a through a qPCR. This data is mostly relative, and the data was compared relatively to each well based on cytokine and RNA concentrations.

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DELIVERY OF CAS9/sgRNA BY LENTIVIRAL SYSTEM

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The CRISPR/Cas9 system was originally found as a defense mechanism against invading foreign DNA in bacteria. Consisting of the Cas9 protein and a guidance RNA (gRNA), this system can be modified to cut genomic DNA at specific sequences where mutations that may cause defective genes can be corrected or edited. Application of the CRISPR/Cas9 system in medicine has the potential to be used in gene therapy (e.g. gene editing) for conditions such as sickle cell disease and hemophilia. Currently, many methods of delivery, such as lipofection and electroporation, have low efficiency and may have undesired long term Cas9 gene expression in cells. In this study we aimed to develop a system by using RNA Lentivirus for efficient delivery of Cas9 mRNA/gRNA for short-term expression of Cas9.

Lentiviral expression vectors were developed for both Cas9 and gRNA. The lentiviral viruses that were generated after transfecting 293T cells were packaged with Cas9 mRNA and gRNA. The addition of a stem loop to both Cas9 mRNA and gRNA were used to serve as a site for the MS2 protein to bind, which assists the packaging of RNAs inside the virions. The MS2 stem-loop was added to the 3' UTR of Cas9. To determine the best location for MS2 stem loop addition in gRNA, modified gRNA was transfected with MS2 stem loop added at various locations. Cas9 and modified gRNA were co-transfected into a GFP-reporter cell line developed to show the presence of functional Cas9/sgRNA in the cell by GFP expression, whose reading frame may only be restored after editing. Using flow cytometry, it was shown that the 3' end of the sgRNA is the best location for MS2 stem loop addition.

Further studies showed that the addition of stem loop structures to Cas9 mRNA and gRNA, and binding of MS2 to these stem loop structures, greatly facilitate the packaging of the Cas9 mRNA/sgRNA to the viral particles. We found that one copy of MS2 protein outperforms two copies of MS2 protein in binding to stem loop structures in viral packaging when fused to nucleocapsid (NC) protein. Comparison of different combinations of MS2 and stem loop structures showed that without addition of stem loops to RNA or fusion of MS2-RNA binding protein to NC, the packaging of mRNA of Cas9 or gRNA was less efficient. As a consequence of these findings, we have identified an improved method for a lentivirus delivery of Cas9/gRNA for gene editing/therapy.

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References:

1. Javidi-Parsijani P, Niu G, Davis M, Lu P, Atala A, Lu B (2017) No evidence of genome editing activity from *Natronobacterium gregoryi* Argonaute (NgAgo) in human cells. PLoS ONE 12(5): e0177444. https://doi.org/10.1371/journal.pone.0177444
2. Anne Prel, et. al, Highly efficient and delivery of functional RNAs using new versatile MS2-chimeric retrovirus-like particles, Molecular Therapy - Methods & Clinical Development, Volume 2, 2015, Page 15039, ISSN 2329-0501, http://dx.doi.org/10.1038/mtm.2015.39.

8
FABRICATION OF VASCULAR RENAL CONSTRUCTS WITH ACCELERATED ANGIOGENIC ACTIVITY

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Vascularization of bioartificial constructs is one of the major complications in the creation of tissue-engineered organs. Avascular implants at whole-organ scales die from a lack of necessary nutrients before adequate vascular ingrowth can occur (1, 2). To address this challenge, strategies for pre-vascularization of engineered constructs are suggested and such pre-vascularization approaches include pre-vascularization through the establishment of "vascular routes" within constructs. We have previously developed a biomimetic method for creating a vascular scaffold based on a vascular corrosion casting (2). Preliminary *in vitro* and *in vivo* studies revealed that the vascularized scaffold with kidney architectures was successfully used to create an implantable renal construct *in vitro*, and the engineered kidney construct facilitated host integration after implantation (2). However, the rate of vascularization into the implant is still a challenge and acceleration of angiogenesis following implantation is critically important for cell survival within the implant. Therefore, this study aims to develop a fabrication method that provides the vascular scaffold with appropriate angiogenic signaling with the goal of promoted vascular integration with the host. To this end, we hypothesized that controlled release of angiogenic factor signals such as vascular endothelial growth factor (VEGF) from the scaffold would efficiently stimulate angiogenic activities *in vitro*.

To facilitate the maximized angiogenic outcomes by VEGF gradient, we designed a sustained release system of VEGF from the scaffold via heparin incorporation. In the release kinetics study, the incorporated VEGF showed sustained release from the scaffold. To investigate effects on the improved angiogenic properties, we determined the endothelial cell viability, proliferation, and migration using human umbilical vein endothelial cells (HUVECs). In the cell migration experiment using a transwell migration assay, VEGF stimulation using as a soluble state (student t-test, n=3, P= 0.022) or release VEGF from the scaffold (n=3, P= 0.03) significantly increased HUEVC migration compared with the control without VEGF, indicating that the released VEGF from the scaffold is effective to facilitate VEGF-induced cell migration.

As another parameter of the angiogenic capability, endothelial cell proliferation by VEGF stimulation was determined. HUVEC were incubated with VEGF moieties collected at 1, 3, and 7 days during release test and the cell proliferation ability was measured using MTS and Live/Dead assay. The cell proliferation results indicate that the VEGF released from the scaffold was biologically active to significantly increase cell proliferation compared with the control (n=8, P = 0.015 for 1 day, n =4, P = 0.028 for 3 day). Particularly, the released VEGF obtained by degrading the scaffold at 7 day significantly increased cell proliferation (n=12, P <0.0001), indicating bioactivity of the released VEGF under an *in vivo*-like environment with scaffold degradation.

To closely simulate the cell viability and migration behavior within an *in vivo* system, we examined whether the released VEGF from scaffold within the gel will facilitate endothelial cell viability and migration into the scaffold. For this experiment, we developed a cell migration chamber system that allows the cell migration into a collagen gel containing the scaffold. Live/dead staining results demonstrated that significant HUVEC viability and migration was found in the VEGF-scaffold within the gel at 1-day culture (n=9, P <0.0001). Overall, our results demonstrated that sustained release of VEGF from our microvascular scaffolds increases cell migration, proliferation and viability *in vitro*, demonstrating the possibility of accelerating of angiogenesis into engineered renal constructs *in vivo*.

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References:

1. Miyagi Y, Chiu LLY, *et al.* Biodegradable collagen patch with covalently immobilized VEGF for myocardial repair, Biomaterials 2011 Feb;32(5):1280-90 Epub 2010 Oct 29
2. Huling J, Ko IK, *et al.* Fabrication of biomimetic vascular scaffolds for 3D tissue constructs using vascular corrosion casts, Acta Biomaterialia 2016 Mar 1;32:190-7 Epub 2016 Jan 6

9 *OPTIMIZATION AND CHARACTERIZATION OF 3D HUMAN PREPUBERTAL TESTIS ORGANOID* SYSTEM

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Recent advances in cancer treatments have increased the childhood cancer survival rate, creating urgency for strategies which address fertility preservation in prepubertal males undergoing gonadotoxic treatments such as chemotherapy. Cryopreservation of immature testicular tissue can be a potential option, from which spermatogonial stems cells (SSC) can be isolated and propagated *in vitro*. Using SSCs, along with Leydig, Sertoli, and peritubular cells of adult (mature) human testicle, we developed a 3-dimensional (3D) testis organoid culture system. The aim of this study is to optimize the 3D testis organoid culture system and assess its feasibility for prepubertal (immature) human testis organoids.

This study was performed in three consequent phases. Testicular cells were isolated from cryopreserved testicular tissue of a 10-year-old brain dead subject from National Disease Research Interchange. In phase I, isolated and 2D cultured testicular cells were seeded in ultra-low attachment round-bottom plates to form organoids of different concentrations: 10000, 7500, 5000, 2500 and 1250 cells per organoid. Half the organoids in each group were centrifuged at 150xg for 30 seconds ("with spin") and the other half were not centrifuged ("without spin"). After 48 hours, the 3D-formed organoids were imaged and their diameters were measured. Organoids formed with both methods were dissociated into single cells using collagenase and cells were counted. In phase II, organoids were formed using the same series of cell concentrations "with spin". After formation, organoids were dissociated and cells were counted. Over three weeks of 3D culture, organoid diameters were measured and their viability was evaluated using ATP production assay. In phase III, each organoid was formed using 10000 cells "with spin". At days 0, 7, 14 and 21 of culture in differentiating medium containing human testis extracellular matrix, organoids were harvested and evaluated using light microscopy, live/dead cell staining, and ATP production assay. Organoids were also evaluated for testosterone production and specific gene expression levels including ZBTB16 (PLZF) for spermatogonia; STAR and CYP11A1 for Leydig cells; CYP19A1 for Sertoli cells; ACTA2 for peritubular cells; and PRM1 for post-meiotic germ cells. At each time point, organoids were dissociated and cells were counted.

Phase I results showed no significant difference between the diameters of organoids using either "with spin" or "without spin" methods; however, centrifugation helped retain 13% more cells per organoid. Therefore, we used centrifugation in phase II. Phase II showed that average cell loss per organoid was comparable for all seeding concentrations (67-69%) except 2500, which had the highest percentage of cell loss at 83%. ATP production assay showed that seeding concentrations of 1250 and 5000 had the highest fold decrease in ATP production over 3 weeks. The seeding concentration of 10000 retained the highest average number of cells per organoid (3181), and was chosen as the optimal seeding concentration for phase III. In phase III, organoids maintained their structure, viability, metabolic activity and produced testosterone over 3 weeks of culture. qRT-PCR results confirmed the presence of spermatogonia, Leydig, Sertoli and peritubular cells throughout 3 weeks of culture. Up-regulation of specific post-meiotic germ cell marker (PRM1) confirmed the formation of spermatid-like cells in this system. In summary, *in vitro* 3D testis organoid system using prepubertal testicular cells was formed and maintained. Similar to mature testis *in vivo*, this system produced testosterone and differentiated spermatogonial cells to post-meiotic stages *in vitro*.

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References

1. Giudice, M.G., et al., Update on fertility restoration from prepubertal spermatogonial stem cells: How far are we from clinical practice? Stem Cell Res, 2017. 21: p. 171-177.
2. Hussein, A.A., et al., Fertility preservation options for prepubertal boys facing gonadotoxic therapies. Minerva Ginecol, 2016. 68(6): p. 668-74.
3. Sadri-Ardekani, H., et al., Propagation of human spermatogonial stem cells in vitro. JAMA, 2009. 302(19): p. 2127-34.
4. Pendergraft, S.S., et al., Three-dimensional testicular organoid: a novel tool for the study of human spermatogenesis and gonadotoxicity in vitro. Biol Reprod, 2017. 96(3): p. 720-732.

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COMPARISON OF NOVEL OPTICAL TISSUE CLEARING TECHNIQUES ON WHOLE MOUSE ORGANS FOR REGENERATIVE IMAGING

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When imaging tissues in regenerative medicine, it is of great importance to be able to visualize the entire, intact structures of the tissues of interest. While traditional thin slicing of tissue and 2D imaging is effective to some degree, three dimensional detail is lost in this process. In order to draw the most accurate conclusions on the efficacy of a treatment, 3D optical imaging and analysis can be used to overcome the limits of standard histology. However, to implement 3D imaging, there are major difficulties in this process due to light scattering. Light scattering is a physical phenomenon that occurs as light rays travel through different mediums[1]. Instead of traveling in straight lines, light rays are reflected off of membranes, cells, and molecules throughout the tissue sample. This results in the natural opaque appearance of thick tissue samples. Due to the light scattering effects, it becomes more difficult for microscopes to image deeper into thick tissues. In order to overcome this light scattering problem, optical tissue clearing methods have been developed that expose the tissues to a series of chemicals which match the refractive indices (RI) throughout the sample, reducing the effects of light scattering. Optical tissue clearing can make opaque tissue appear transparent, and allows 3D imaging of samples at 4-8x the traditional imaging depth of 2 mm^2.

The objective of this study was to evaluate two recently developed clearing protocols, Visikol and uDISCO, and compare their efficacy to the in-house clearing technique termed inCITE. Visikol and uDISCO both make use of alcohols as their primary clearing reagents, using tricholoroethanol and benzyl alcohol-benzyl benzoate solutions respectively. Alternatively, inCITE uses a detergent based approach for clearing. Whole mouse organs were stained with tomato-lectin and removed after cardiac perfusion of a fixative. A set of whole mouse organs including brain, heart, lung, liver, kidney, and spleen were used for each of the three tissue clearing techniques under evaluation. Each protocol required exposure of the tissue to a series of chemical steps, each for varying periods of time. Photographs of each organ sample were taken at various time points during the clearing process. Photos taken at the conclusion of each clearing protocol were compared visually for optical clarity. We observed that the Visikol treatment resulted in the least tissue clearing, maintaining a mostly opaque appearance in each tissue type. The uDISCO samples showed much higher tissue clarity, with transparent brain and lung samples. inCITE samples showed a tissue clarity in between the two previously mentioned methods.

Our data suggests that uDISCO produced the highest clarity in whole mouse organs, followed by the inCITE technique. These optically cleared samples will be imaged using two-photon microscopy, and quantitated using an intensity analysis program to validate our observations. Future studies will likely include variations and combinations of the different techniques in order to optimize optical clarity. Other factors such as clearing time, ease of use, and maintaining protein structure will be used in addition to optical clarity. These studies will be used to ultimately obtain an optimized clearing technique that can be used for the evaluation of large, intact tissue samples that have undergone a variety of treatments in regenerative medicine.

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References:

1. Richardson, D. S., & Lichtman, J. W. (2015). Clarifying Tissue Clearing. *Cell*, *162*(2), 246–257.
2. Cowdrick, K. R., Zarifpour, M., Booth, C., Stumpf, K., Mutkus, L., Patolia, H., …, Marini F. “Cleared Tissue Affords Whole Organ Morphometric Analysis in Murine Bladder Regeneration Model.” N.d. MS. Wake Forest Institute for Regenerative Medicine, np.

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DIFFERENTIAL Z IMAGING (DIFFZ)- AN AUTOMATED METHOD FOR 3D REGENERATIVE IMAGING

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Differential Z Scanning [DiffZ] is a novel imaging optimization method that is very effective for large tissue imaging. The process eliminates the need for rectangular bounding boxes in which to direct the microscope in a 90-degree fashion, essentially allowing the microscope to follow the curvature of each sample. DiffZ provides regenerative medicine imaging with a larger field of view while also being able to identify smaller regions of interest in very high resolution. Differential Z is a MATLAB based GUI program designed to save large amounts of imaging time by altering the microscopy data to image along a gradient of signal. The program "talks" with the Olympus FV1000 microscope by taking the initial imaging data acquired through an Atlas Scan, finding a gradient threshold, and generating a defined image mask which follows the contours of the sample. In practice, DiffZ can theoretically save around 80 to 90% of the theoretical image acquisition time and reduce data acquisition.

Until this summer, the DiffZ program was on the cusp of completion. In addition, process improvements and code improvements were necessary, and were implemented throughout the summer. The main objective was to bring the idea behind the DiffZ program to completion and facilitate end use. My work improved the work flow, reduced data computations and simplified the ideas and processes behind DiffZ Scanning. Technical instructions were developed so that even an inexperienced user could effectively use the program. Importantly, the program was pushed towards full automation. This included the elimination of unnecessary MATLAB output, a simple step towards making the process work faster. Next, one of the main objectives, we automated the thresholding of the program, thus enabling the computer to automatically generate the threshold gradient across which the microscope will image. By graphing the coordinate data from each image tile, the data could then be manipulated so that a threshold number is given to the user which the user can implement to eliminate as much background noise as possible, thus decreasing the overall imaging time. Each graph showed the slices of each tile versus its arbitrary, relative intensity. The data was manipulated in multiple different ways, but the most effective method was developed by rounding each intensity value to the nearest five, taking the mode intensity of each tile, and then implementing that rounded value into the DiffZ GUI.

Moving forward, our work demonstrated that DiffZ can be implemented by novice microscope users to image large tissue and organs. This work included simplifying the workflow and data processing so that anyone with a compatible microscope can utilize this program. Through generating the code, it is clear that there are still additional opportunities for automation, with an ultimate goal of being as simple as pressing one button, thus allowing the computer to execute all of the work-flow. With the advancements made to DiffZ over the past few years and over this summer, the program could be useful enough to implement across the tissue engineering and regenerative medicine community.

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CO-CULTURE DIFFERENTIATION OF PLACENTAL DERIVED STEM CELLS TOWARDS BOTH OSTEOGENIC AND CHONDROGENIC LINEAGES

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Articular cartilage damage can result from trauma, wear, and several other underlying diseases. Due to low vascularization, the tissue has limited ability to heal and regenerate without medical intervention. Current treatments include arthroplasty, microfracture, and autologous chondrocyte implantation[1]. However, these treatments are not ideal because they are invasive and have relatively high failure rates[1]. Tissue engineering is a potential solution for more effective treatments for articular cartilage. However, engineered cartilage alone also has high failure rates *in vivo* due to poor integration at the bone interface[2]. The interface of bone and cartilage is an area of both high shear and compressive stresses[3]. Meanwhile, many previous studies have demonstrated the successful integration of engineered bone constructs *in vivo*[4]. A tissue engineering approach which integrates with native bone tissue and includes all regions of cartilage would provide a more successful treatment for articular cartilage.

Incorporation of multiple cell and tissue types into a single construct remains a great limitation hindering tissue engineering. Typical differentiation media cannot be used because of potential adverse effects on the various cell types. Gelatin methacrylate (GelMA) has been conjugated with growth factor mimicking peptides to drive differentiation. The objective of this study was to evaluate the potential of GelMA modified with bone morphogenic protein (BMP-2) and TGF-β mimicking peptides to differentiate stem cells into both osteoblasts and chondrocytes in co-culture. Physical characteristics of the modified hydrogels were also obtained to evaluate the potential effects on gel characteristics. In this study, human placental mesenchymal stem cells (pMSCs) were used because of their rapid proliferation, non-invasive origin, and reduced rejection potential[5]. Cells were suspended in a hydrogel composed of hyaluronic acid, glycerol, gelatin, GelMA, and photoinitiator in media. To induce differentiation, GelMA was modified with a peptide which mimics natural BMP-2 and a synthetic TGF-β known as Cytomodulin-10 (CM-10) in concentrations previously determined to induce differentiation towards osteoblasts and chondrocytes, respectively. Spatial control over the distribution of growth factors was accomplished via bioprinting on the Integrated Tissue-Organ Printer (ITOP). Using the ITOP, three control groups (peptide free, BMP-2 only, and CM-10 only) were printed along with two experimental groups (a split group of half BMP-2 modified hydrogel and half CM-10 modified hydrogel and a homogenous group containing both BMP-2 and CM-10). To characterize the conjugated hydrogels, physical characteristics were identified using swelling tests, rheological data, release kinetics, and dimensional stability.

The data regarding the printed constructs is still forthcoming; each will be analyzed using histological stains to identify cell morphology and ECM deposition and quantitative PCR to evaluate extent of differentiation. It is expected that co-culture of pMSCs in BMP-2 and CM-10 will lead to differentiation into osteoblasts and chondrocytes, respectively. Cells at the interface and in homogenously conjugated constructs are expected to present a mixed expression, potentially similar to that of calcified chondrocytes. Higher swelling ratio of conjugated hydrogels indicate that BMP-2 interferes with the formation of the network. Rheological data indicates that the hydrogel used for printing has a low storage modulus compared to natural tissue, but can be modified to be more physiologically relevant. In conclusion, peptide-conjugated hydrogel may be useful in engineering a physiologically relevant articular cartilage repair. In future studies, the mechanical characteristics, medium composition, cell density, and peptide concentration may also be optimized to aid co-culture differentiation.

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References:

1. Makris, E. A., Gomoll, A. H., Malizos, K. N., Hu, J. C., & Athanasiou, K. A. (2015). Repair and tissue engineering techniques for articular cartilage. *Nature Reviews. Rheumatology*, *11*(1), 21–34. http://doi.org/10.1038/nrrheum.2014.157
2. Hollander, A. P., Dickinson, S. C., & Kafienah, W. (2010). Stem Cells and Cartilage Development: Complexities of a Simple Tissue. *Stem Cells (Dayton, Ohio)*, *28*(11), 1992–1996. http://doi.org/10.1002/stem.534
3. Zhang, T., Zhang, H., Zhang, L., Jia, S., Liu, J., Xiong, Z., & Sun, W. (2017). Biomimetic design and fabrication of multilayered osteochondral scaffolds by low-temperature deposition manufacturing and thermal-induced phase-separation techniques. *Biofabrication*, *9*(2), 25021. https://doi.org/10.1088/1758-5090/aa7078
4. Schantz, J.-T., Hutmacher, D. W., Lam, C. X. F., Brinkmann, M., Wong, K. M., Lim, T. C., … Teoh, S. H. (2003). Repair of calvarial defects with customised tissue-engineered bone grafts II. Evaluation of cellular efficiency and efficacy in vivo. *Tissue Engineering*, *9 Suppl 1*, S127-139. https://doi.org/10.1089/10763270360697030
5. Antoniadou, E., & David, A. (2016). Placental stem cells. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *31*, 13–29.

13 OPTIMIZATION OF BIOINKS FOR 3D BIOPRINTING OF KIDNEY CONSTRUCTS USING GELATIN METHACRYLATE AND FIBRINOGEN

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Kidney diseases are a global health problem where renal failure progresses through series of stages. In time, this leads to acute and chronic symptoms subsequently ending in end-stage renal disease (1). Current treatment methods are dialysis and kidney transplantation, limited by donor organ shortage and graft failure. These limitations can be addressed by replacing damaged cells with functional kidney cells and restoring normal kidney function through tissue engineering. 3D bioprinting has the potential to fabricate complex, multicellular structures precisely modeled from a patient's own kidney. In order to do this, a bioink must be optimized to meet the mechanical and biological requirements for 3D bioprinting using kidney cells. Two hydrogels, Gelatin Methacrylate (GelMA) and fibrinogen, were selected for bioprinting kidney constructs for their respective unique properties. GelMA is capable of being printed into sturdy, long-lasting constructs when crosslinked using UV light, but kidney cells favor a softer gel environment. Fibrinogen constructs, after being crosslinked into fibrin using thrombin, are soft and fibrous in nature, but degrade in media within a few days (2). The objective of this study was to evaluate combinations of GelMA and fibrinogen at different concentrations to be used as a bioink for 3D bioprinting of kidney constructs.

This study included five groups with GelMA and fibrinogen at different weighted percentages from two distinct stocks of 3.5% GelMA with 0.5 % Irgacure 2979 and 1.7% Fibrinogen prepared, both in Dulbecco's Modified Eagle's Medium (DMEM), high glucose. Ratios of 3:0, 2:1, 1:1, 1:2, and 0:3 respectively for GelMA:Fibrinogen were selected. All formulations contained 0.3% of Hyaluronic Acid, 3.5% Gelatin, and 10% glycerol per mL to improve printability. The bioinks were characterized by rheology, water contact angle, and fiber strength analysis of the extruded fibers. Each bioink was suspended with Human Umbilical Vein Endothelial Cells (HUVEC) or human kidney cells at a density of 1 and 6 million respectively. Multiple 6 mm by 6 mm, 1.2 mm high construct were printed using an Integrated Tissue-Organ Printing system followed by crosslinking. Constructs were evaluated by histoanalysis and viability staining. Rheology of the crosslinked gels showed inverse relation of storage modulus to the fibrin concentration. The tan (delta) values showed the elastic nature of the gels. Water contact angle revealed all bioink groups showed hydrophilicity suitable for cell culture. The fiber collapse analysis confirmed the strength of the extruded filaments after 3D printing. H&E staining showed varying bioink morphologies from different concentrations of GelMA and Fibrin. It was observed that GelMA and fibrin dissociated from one another and fibrin formed hollow ringlets in the 2:1 and 1:1 bioinks. Bioprinted kidney constructs with mixed GelMA and fibrin demonstrated structural stability with slight dimensional change, while fibrin bioink 0:3 showed pronounced dimensional change. All bioink groups showed high cell viability.

In conclusion, the different concentrations of GelMA and fibrin have an impact on bioink physical properties. All bioinks were printable as demonstrated by the fiber collapse test. However, samples containing larger amounts of fibrinogen (1:2 and 0:3) were found to degrade at a faster rate than the other samples (3:0, 2:1, and 1:1) post printing. By combining GelMA and fibrin the elasticity of the bioinks were found to decrease compared to GelMA alone. Further, the longevity of GelMA/fibrin constructs were showed to increase compared to fibrin alone. Thus, bioinks containing both GelMA and fibrin were found to express the beneficial characteristics of both GelMA and fibrin and are suitable for bioprinting of kidney constructs.

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References:

1. Hospodiuk, M., Dey, M., Sosnoski, D., & Ozbolat, I. T. (2017). The bioink: A comprehensive review on bioprintable materials. *Biotechnology Advances, 35*(2), 217-239. doi:10.1016/j.biotechadv.2016.12.006
2. National Institute of Diabetes and Digestive and Kidney Diseases. (2016, December 01). Kidney Disease Statistics for the United States. Retrieved July 27, 2017. from https://www.niddk.nih.gov/health-information/health-statistics/kidney-disease

14 *NON-INVASIVE CELL TRACKING WITH BRIGHTER AND RED-TRANSFERRED LUCIFERASE FOR STEM CELL THERAPY IN THE TREATMENT OF ERECTILE DYSFUNCTION*

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Introduction: Despite great progress in the treatment of erectile dysfunction (ED) with stem cell therapy, the exact therapeutic mechanism of action of stem cells remains undefined. Our previous and other studies demonstrated that stem cell therapy significantly improved erectile function in an ED rodent model but no cells were found in the penile tissue after the cells were implanted. However, it is unknown how many and how long the grafted cells survive after implantation of stem cells in penial tissue in the treatment of erectile dysfunction[1,2]. Therefore, it is critical to monitor the fate of implanted stem cells for the treatment of erectile dysfunction and to examine the progress of these cells over time for improvement of cell therapy efficacy. Firefly luciferase is the most commonly used in cell tracking, however it generates relatively low bioluminescent signal, which faces challenges in the detection of low numbers of luciferase-expressing cells in vivo. In addition, this low signal makes it difficult to detect expressing cells located in deep tissues, a problem for cell tracking in preclinical research of stem cell therapy. To improve luciferase brightness, a new codon-optimized luciferase from Renilla reniformis (renLUC) develops with red-shifted emission peak wavelength of 617 nm (as compared to 550 nm [Luc] and 590 nm [Luc2]) and approximately 100-fold higher signal intensity compared to firefly luciferases. The bioluminescence of renLUC can be monitored using IVIS imaging system, a non-invasive approach with quantitative analysis. In addition, cells labelled with mKATE ^{can} be checked with a confocal microscope. Therefore, to label cells with both a red fluorescent protein variant mKATE and a new codon-optimized luciferase from Renilla Reniformis (mKATE-renLUC) using a lentivirus vector would be an optimal approach to monitor the fate of the implanted stem cells in the treatment of ED in a rodent model.

Objective: The goal of this study is to determine the effect of this dual labeling technology with mKATE-renLUC on properties and function of hPSCs in cell viability, proliferation, and migration.

Methods: hPSCs were isolated from term placenta. To optimize the transfection protocol, transfection efficiency with different cell concentrations of hPSCs and titers of mKATE-renLUC lentivirus (hPSCs$^{mKATE\text{-}renLUC}$) were evaluated. The cell morphology, viability, proliferation, and migration of hPSCs $^{mKAT02E\text{-}renLUC}$ were examined, compared with non-labeled hPSCs. In addition, the density and duration of bioluminescence with different cell concentrations was monitored by IVIS imaging system in vitro.

Results: Cell morphology, viability, proliferation, and migration remain similar between hPSCs$^{mKATE\text{-}renLUC}$ and non-labelled hPSCs. The bioluminescence of mKATE-renLUC remained brightest for up to 120 minutes after activation after coelenterazine addition.

Conclusions: Dual labelling technology with mKATE-renLUC can be efficiently used to monitor the fate of hPSCs in the treatment of ED in a rat ED model.

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References:

[1] Murphy MB, Moncivais K, Caplan AI: Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med 2013, 45: e54.c [2] Li L, Zhang D, Li P, Damaser M, Zhang Y: Virus integration and genome influence in approaches to stem cell based therapy for andro-urology. Adv Drug Deliv Rev 2015, 82-83:12-21. [3] van der Bogt KE, Sheikh AY, Schrepfer S, Hoyt G, Cao F, Ransohoff KJ, Swijnenburg RJ, Pearl J, Lee A, Fischbein M, Contag CH, Robbins RC, Wu JC: Comparison of different adult stem cell types for treatment of myocardial ischemia. Circulation 2008, 118:S121-9.[4] Li Z, Wilson KD, Smith B, Kraft DL, Jia F, Huang M, Xie X, Robbins RC, Gambhir SS, Weissman IL, Wu JC: Functional and transcriptional characterization of human embryonic stem cell-derived endothelial cells for treatment of myocardial infarction. PloS one 2009, 4:e8443.

15 *IN VITRO MODEL OF THE BLOOD BRAIN BARRIER FOR DRUG SCREENING*

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Introduction: Neurodegenerative disease accounts for approximately one million new diagnoses[1] in the United States every year, with 50 million pre-existing cases.[2] With novel technologies promoting longevity, neurodegenerative diseases are only increasing. Currently, there are limited treatment options, as drugs are ineffective due to the selective nature of the blood brain barrier (BBB) and lack of a physiologically relevant in vitro model for screening. Extant in vitro BBB models use animal components, only two or three cell types, and 2D transwell systems.[2] We propose that our three-dimensional models containing all six major human brain cell types (astrocytes, pericytes, microglia, endothelial cells, oligodendrocytes, and neurons) will account for this issue.

Objective: The purpose of this research is to develop a human-based, physiologically relevant blood brain barrier model that can be utilized in drug screening. Specific aims include (1) assessment of model viability, (2) testing for the presence and physiological relevance of components utilized in the model, including junction markers, and (3) testing for BBB functionality.

Methods: Principle models include self-assembly spheroids cultured in a Hanging Drop system and bioprinted constructs consisting of cultured cells printed in a fibrin-hyaluronic acid hydrogel. Viability was determined via Live-Dead analyses taken on regularly spaced intervals. Immunofluorescent staining was utilized to determine the presence of intercellular junction markers (Beta-Cateinin, ZO-1, Claudin-5) as well as markers for neuronal maturity (MAP2, PSD-95, Vimentin, Sox-1).
Hypoxia Experiment: A group of spheroids were incubated in a hypoxic chamber for 24 hours, after which staining for intercellular junction markers was conducted and compared to spheroids stained for the same markers under normal growth conditions.
MPTP Neurotoxicity Experiment: Following lowering on day 4, neuronal spheroids and spheroids containing all cell types were treated with neurotoxins MPTP, MPP+, and $HgCl_2$ (10µM) with media changes made every two days. On day 6 of treatment, an ATP fluorescent assay was conducted to determine cell viability using a luminometer.
All images for viability and immunofluorescence were obtained via confocal microscopy.

Results, Discussion, & Conclusion: Live-Dead analysis of spheroids consisting of all cell types and bioprinted constructs demonstrated the maintenance of viability for up to 21 days. Additionally, increased cellular interaction was observed to occur for the bioprinted constructs over time; cellular differentiation was observed for bioprinted constructs with the formation of axon extensions and dendrites. These findings indicate the potential for long-term culture applications. Thorough distributions of junction and neuronal maturation markers indicate physiological relevance, which was upheld by the major disruption of junction interaction in hypoxic spheroids compared to those cultured under normal conditions. In the MPTP experiment, significant cell death occurred for neuronal spheroids treated with $HgCl_2$ ($p < 0.0001$) and MPP+ ($p < 0.01$). Treatment with MPTP resulted in a minor loss of viability. Treatment with $HgCl_2$ and MPP+ for the spheroids of all cell types resulted in no loss of viability and a minor loss of viability, respectively. Treatment with MPTP resulted in the highest loss of viability, due to its lipophilicity and conversion to MPP+, which cannot penetrate the BBB. This experiment demonstrates charge selectivity is a feature of our model resembling the BBB in vivo. Overall, the BBB in our model possesses functional relevance with charge selectivity mirroring the BBB *in vivo*, holding potential as a platform for understanding BBB mechanisms and as an agent for drug screening and modeling disease.

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References:

1. Brown, R.C., Lockwood, A.H., Babasaheb, R. S. Neurodegenerative Diseases: An Overview of Environmental Risk Factors. NCBI. Environmental Health Perspectives. 2005; 113(9): 1250-1256. doi: 10.1289/ehp.7567. PMCID: PMC1280411.
2. Fei, E. Xiong, Wen-Cheng, & Mei, L. Ephrin-B3 recruits PSD-95 to synapses. Nature Neuroscience 18, 1535-1537 (2015). doi: 10.1038/nn.4147.
3. Nzou, Goodwell. Human Neurovascular Unit Models for High-Throughput Neurotoxicity Screening and Drug Discovery. 2017.

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SIMULATED MICROGRAVITY IMPAIRS DNA DAMAGE REPAIR IN A PRIMITIVE HUMAN LEUKEMIC CELL LINE

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The National Aeronautics and Space Administration (NASA) is working hard to expand human presence beyond the moon. Goals outlined in the 2010 U.S. National Space Policy state the agency intends to send humans to Mars by the mid-2030s[1]. One of the major factors limiting manned spaceflight beyond low-Earth orbit is the poorly defined health risks of exposure to space radiation in the form of solar energetic particles (SEP) and galactic cosmic rays (GCR), which could potentially increase cancer morbidity/mortality in astronauts. We have recently demonstrated the seriousness of this risk by showing that exposing human hematopoietic stem cells (HSC) to simulated GCR results in the generation of leukemia when these cells repopulate the hematopoietic system of mice[2]. Astronauts are also subjected to conditions of microgravity (uG) during spaceflight, which exerts many untoward effects on the body[3], including altering the response to radiation[4]. We hypothesized that conditions of uG present during spaceflight may alter HSC's ability to carry out DNA damage repair in response to the harmful effects of space radiation, and may thereby increase the risk of leukemogenesis during prolonged missions.

To test this hypothesis, we treated the HSC-like cell line, KG1a, with an acute dose of bleomycin to mimic the damaging effects of solar and cosmic radiation. We then took the treated cells, in addition to an identical batch of untreated cells, and split them in half. Half of the cells from each lot were cultured under conditions of normal gravity (1G), and the other half was cultured in the Synthecon Rotary Cell Culture System (RCCS) at 22.6 RPM to create a state of continual freefall, and thereby mimic uG. At 1 hour and 4 hours, half of the cells were harvested from the 1G and uG cultures, and we performed immunofluorescence staining for *γ*-H2AX – a commonly targeted DNA damage response protein[5] – to quantitate the extent of double-strand breaks and the kinetics of repair. The cells were analyzed using flow cytometry and confocal imaging to monitor the formation and disappearance of *γ*-H2AX foci.

When we analyzed the flow cytometry data, we saw an increase in the median fluorescence intensity (MFI) of bleomycin-treated and untreated cells cultured in uG, due to a rise in the number of *γ*-H2AX foci. This indicates an increase in DNA damage. To determine the impact of uG conditions on the ability of KG1a cells to repair the induced DNA damage, we calculated the ratio of bleomycin-treated cells' MFI to untreated cells' MFI under conditions of 1G vs. uG between our two time points. In normal DNA repair, this ratio should decrease over time, since *γ*-H2AX foci disappear as DNA is repaired. Our calculations show that this ratio decreased by 26% from the 1-hour to the 4-hour time point for the cells in 1G, demonstrating successful DNA damage repair. In marked contrast, cells maintained in uG experienced a 20% increase over the same period. This suggests that they were unable to repair the bleomycin-induced DNA damage, and this damage accumulated during the 4-hour incubation. We conclude that HSC DNA damage repair is compromised in conditions of uG, leading to an accumulation of double-strand breaks that cannot be resolved over time. These findings thus support our hypothesis that conditions of uG may enhance the genotoxic effects of space radiation, and increase the risk of leukemogenesis.

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References:

1. Executive Office of the President (2010). *National Space Policy of the United States of America*. Washington, DC: Author. Retrieved July 26, 2017, from https://obamawhitehouse.archives.gov/sites/default/files/national_space_policy_6-28-10.pdf.
2. Rodman, C., Almeida-Porada, G., George, S. K., Moon, J., Soker, S., Pardee, T., . . . Porada, C. D. (2016). In vitro and in vivo assessment of direct effects of simulated solar and galactic cosmic radiation on human hematopoietic stem/progenitor cells. *Leukemia, 31*(6), 1398-1407. doi:10.1038/leu.2016.344
3. Rea G, Cristofaro F, Pani G, Pascucci B, Ghuge SA, Corsetto PA, Imbriani M, Visai L, Rizzo AM. Microgravity-driven remodeling of the proteome reveals insights into molecular mechanisms and signal networks involved in response to the space flight environment. *J Proteomics*. 2016 Mar 30;137:3-18. doi: 10.1016/j.jprot.2015.11.005. Epub 2015 Nov 10.
4. Girardi C, De Pittà C, Casara S, Sales G, Lanfranchi G, Celotti L, Mognato M. Analysis of miRNA and mRNA expression profiles highlights alterations in ionizing radiation response of human lymphocytes under modeled microgravity. PLoS One. 2012;7(2):e31293. doi: 10.1371/journal.pone.0031293. Epub 2012 Feb 9.
5. Bonner, W. M., Redon, C. E., Dickey, J. S., Nakamura, A. J., Sedelnikova, O. A., Solier, S., & Pommier, Y. (2008). γH2AX and cancer. *Nature Reviews. Cancer*, *8*(12), 957–967. doi: 10.1038/nrc2523

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ISOLATION AND CHARACTERIZATION OF SERUM-DERIVED EXOSOMES AS BIOMARKERS FOR ILEOCOLITIS IN CHILDREN WITH AUTISM SPECTRUM DISORDER

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Autism is a neurodevelopmental disorder affecting about 200,000 patients each year in the U.S alone. Statistics show that children with ASD are 1.3 to 2.4 times more likely to be diagnosed with gastrointestinal (GI) inflammation than typically-developing children. The increased likelihood of GI symptoms in children with ASD has been attributed in part to a unique variant of GI inflammation seen only in this population. Current diagnostic procedures include an upper endoscopy as well as a colonoscopy, both of which are highly invasive. The discovery of a reliable biomarker for GI inflammation in ASD patients from a serum sample could replace the need of current invasive diagnostic procedures in some individuals.

The objective of this research is to identify a biomarker using microRNA (miRNA) isolated from serum-derived exosomes. Exosomes are microvesicles, found in most biological fluids, which are known to encapsulate and release miRNAs from surrounding cells. MiRNAs are often targeted for biomarker research because it is known that they have important roles in the regulation of transcription and translation of signaling pathways in the body.

To achieve this research objective, exosomes were isolated from serum samples collected from patients with ASD and GI inflammation and from patients without ASD and without GI inflammation using ExoQuick per the user manual protocol. The quality of the exosomes was evaluated using transmission electron microscopy (TEM). Exosome pellets were lysed and total RNA, including miRNA, was extracted by testing the following three methods: (1) Direct-ZOL, (2) TRIzol and, (3) RNeasy mini kit (With Qiazol and without Qiazol). Quantity and quality of total RNA samples were measured using a NanoDrop spectrophotometer. The three protocols for RNA isolation resulted in highly variable RNA quantity and quality, with no single protocol demonstrating clear suitability over the others. To proceed, each of the total RNA samples was tested using a PCR starter kit that contains a “house-keeping” miRNA (miR-15a) to test for the presence of miRNA in the samples. No amplification was observed in any of the samples, however it is possible that the house-keeping miRNA tested is not regularly found capsuled in exosomes.

In conclusion, more research must be done to identify a total RNA isolation method that provides quality miRNA that is compatible with PCR amplification. Once this is achieved all the serum samples will be treated with ExoQuick for exosome isolation and miRNA extraction. The miRNA will be converted to cDNA and assayed on human microarray PCR plates that contain hundreds of miRNAs commonly found in human serum. The data will be analyzed to identify significant up- or down-regulation of miRNAs from the experimental samples versus the controls in order to identify a unique and reliable biomarker for GI inflammation in patients with ASD.

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CELL THERAPY FOR GASTROPARESIS

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Background: Gastroparesis is delayed gastric emptying without known mechanical obstruction (1). Symptoms and pathologies are heterogeneous among cases; however, most patients diagnosed have a reduction of gastric neurons and interstitial cells of Cajal (ICCs) upon histological investigation. Without neuronal signaling to the smooth muscle cells (SMCs), the pyloric sphincter cannot relax and allow normal passage of food. ICCs are known as the pacemakers of the gut and help enteric motor neuron signaling (2-3). Loss of both leads to dysfunctions such as pyloric stenosis, thus decreasing gastric motility. The current treatments are primarily transient symptom alleviators such as dietary changes and antibiotics (4). Cell therapy may offer a long-term solution. Neural progenitor cells (NPCs) injections have previously been shown to re-establish partial functionality of the diseased pylorus. Replenishing both ICCs and NPCs may improve functionality restoration (5).

Aim: We aim to create an *ex vivo* tissue model mimicking gastroparesis condition and to determine the efficacy of cell therapy, using ICCs and NPCs, at restoring functionality to the disease model.

Methods: Isolated murine small intestine NPCs and ICCs were expanded in culture and fluorescently tagged with cyan fluorescent protein (CFP) and green fluorescent protein (GFP), respectively. Rat stomach was harvested; benzalkonium chloride (BAC) and imatinib mesylate were used to ablate neurons and ICCs, respectively, from one part of pylorus. The rest of the pylorus was treated with phosphate-buffered saline (PBS) as positive control. The BAC/imatinib treated tissues were divided into two groups; one group was injected with fluorescently tagged NPCs and ICCs and the other was kept as negative control (BAC/imatinib treated). Tissues were evaluated using qPCR and immunohistochemistry (IHC) while functionality was analyzed using organ bath studies.

Results: Chemical treatment successfully depleted the tissues of ICCs and neural cells as confirmed by qPCR, IHC, and functionality tests. The BAC/imatinib treated tissues showed a 70% decrease in ANO1 (ICC marker) and 83% decrease in beta-III tubulin (B3T, neuron marker) expression compared to the PBS-treated tissues. IHC confirmed reduction of ICCs and NPCs when tissues were stained for ANO1 and B3T specific antibodies. Similarly, contractile force generated by the negative control was significantly less than the positive control in the presence of acetylcholine (decreased by 51%). ICCs and NPCs were isolated and fluorescently tagged without affecting cell phenotype. ICCs and NPCs were successfully delivered and restored functionality to the BAC/imatinib treated tissue. IHC confirmed ICCs and NPCs presence in the diseased tissue model. NPCs/ICCs injected tissue showed a 225% increase in ANO1 expression and a 384% increase in B3T expression when compared to the negative control. Contraction in the presence of ACh indicated ICCs/NPCs injected tissue had 84% contractile force of positive control which is a 35% improvement from the negative control. This indicates that injected NPCs and ICCs established connections to the SMCs in the tissue. Electrical field stimulation showed 57% regain of relaxation in the ICCs and NPCs injected tissues compared to negative control.

Conclusion: An ICCs and NPCs depleted *ex vivo* gastroparesis pylorus model was developed. NPCs and ICCs implanted into disease tissue restored the neuromusculature and functionality of the pylorus. These findings suggest that cell therapy using injection of NPCs and ICCs can be used to treat neurodegenerative disorders.

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References: [1] Waseem, S., Moshiree, B., Draganov, P. V. Gastroparesis: Current diagnostic challenges and management consideration. World J. Gastroenterol 2009;15(1):25-37. [2] Burns, A. J., Lomax, A. E., Torihashi, S., Sanders, K. M., Ward, S. M. Interstitial cells of Cajal mediate inhibitory neurotransmission in the stomach. Proc Natl Acad Sci U S A 1996;93(21):12008-12013. [3] Ward, S. M., Beckett, E. A. H., Wang, X., Baker, F., Khoyi, M., Sanders, K. M. Interstitial Cells of Cajal Mediate Cholinergic Neurotransmission from Enteric Motor Neurons. Journal of Neuroscience 2000;20(4)1393-1403. [4] Zárate, N., Mearin, F., Wang, X-Y., Hewlett, B., Huizinga, J. D., & Malagelada, J-R.. Severe idiopathic gastroparesis due to neuronal and interstitial cells of Cajal degeneration: pathological findings and management. *Gut* 2003;52(7):966-970. [5] Micci, M. A., Kahrig, K. M., Simmons, R. S., Sarna, S. K., Espejo-Navarro, M. R., Pasricha, P.J. Neural stem cell transplantation in the stomach rescues gastric function in neuronal nitric oxide synthase-deficient mice. *Gastroenterology* 2005;129(6):1817-24.

19 DEVELOPING AN AIRWAY ORGANOID FOR DISEASE MODELING AND DRUG SCREENING

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Animal models and 2D cell culture have been instrumental in studying disease pathogenesis and drug efficacy; however, these models have also contributed to declining productivity within the pharmaceutical industry due to their inability to accurately mimic human tissue function.[1] A possible solution to this issue is the development of organoids, which are 3D cell cultures that more closely replicate the shape and function of human tissues. Organoids can be integrated into microfluidic devices that emulate circulation by providing cells with a constant flow of nutrients. These systems, referred to as organ-on-a-chip, are providing new methods of testing tissue physiology and function. In particular, lung-on-a-chip has shown promise for future use in high-throughput screening, facilitating personalized medicine, and developing models for respiratory infection.[2]

This study sought to optimize a lung-on-a-chip design with the goal of eventually using this system to model respiratory infections. Chips were built onto a glass slide and were composed of layers of 3M Extreme Sealing Tape, polymethyl methacrylate (PMMA), and polydimethylsiloxane (PDMS). Cell chambers and channels were cut into the tape and PMMA using an H-Series Full Spectrum Laser Cutter. Initially, the chips included a polyvinylidene fluoride (PVDF) membrane on which cells were seeded. After coating the membrane with collagen IV, normal human lung fibroblasts (HLF) and human alveolar epithelial cells (A549) were seeded onto the membrane. The system was sealed with PDMS and allowed to rest 1 – 2 days before initiating flow via a peristaltic pump operating continuously. Organoids were maintained for up to three weeks. Each week, the organoids in one of the chips were stained using a LIVE/DEAD Viability/Cytotoxicity kit in order to track cell viability over time.

Images from the LIVE/DEAD stain revealed considerable cell detachment and poor cell viability within the chips, possibly due to either the formation of bubbles within the cell chamber or poor cell adhesion to the collagen IV coating. The latter issue was addressed by replacing the collagen IV coating with a hydrogel containing lung extracellular matrix proteins. HLFs were suspended in the hydrogel and seeded onto the chip, followed by A549 cells. Although the hydrogel promoted enough cell viability to justify its use in subsequent experiments, the hydrogel also obstructed channels within the chip. A new chip was designed with a deeper cell chamber and outlet channels in order to accommodate the hydrogel and prevent bubbles from interfering with media flow within the chip. In this new design, bubbles were able to float to the top of the chip without disturbing the cells. After a day under flow, the new chips showed some cell detachment, indicating that the hydrogel had not solved the issue of cell attachment. Current experiments underway are assessing the possible effects of continuous media flow on cell detachment. Although this system shows promise, further experimentation will be necessary to obtain a fully functional lung-on-a-chip.

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References:

1. Pampaloni, F., Reynaud, E. G. & Stelzer, E. H. K. The third dimension bridges the gap between cell culture and live tissue. *Nat. Rev. Mol. Cell Biol.* **8,** 839–845 (2007).
2. Murphy, S. *et al.* Lung-On-A-Chip Technologies for Disease Modeling and Drug Development. *Biomed. Eng. Comput. Biol.* 17 (2016). doi:10.4137/BECB.S34252

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ENGINEERING 3D MICROENVIRONMENTS: COLORECTAL CANCER MOTILITY AND ECM REMODELING

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Introduction: Colorectal cancer is the second most common cancer in the United States, killing over 50,000 people each year[1]. Although early stage 5-year survival rates are high (92%), these rates significantly drop (11%) when the cancer begins to metastasize[1]. Furthermore, the chances of developing intestinal cancer increases in patients with inflammatory bowel diseases, such as Crohn's disease[3], in which the intestinal extracellular matrix (ECM) is uniquely remodeled. If we can better understand colorectal cancer cell motility, the main mechanism behind metastasis, as well as how colorectal cancer changes within a remodeled ECM, then we may be able to produce better treatment and predictive measures. In this study, we examine the inhibition of three pathways associated with cell migration and also produce organoids made with mildly activated stellate to mimic *in vivo* tissue. Previously, researchers have utilized two-dimensional (2D) cell cultures to study cancer. These models present limitations when it comes to studying cancer progression and potential therapeutic drugs. Instead, three-dimensional (3D) *in vitro* systems can allow cells to grow, migrate, and interact with the surrounding matrix, resulting in more realistic constructs[2].

Materials and Methods: 3D microenvironments were fabricated by suspending colorectal cancer spheroids within a type 1 collagen (Col 1) hydrogel. Spheroids were generated by culturing HCT-116, colorectal carcinoma, cells in ultra-low attachment plates at 10,000 cells per spheroid. After spheroids were embedded in 1mg/mL Col 1 hydrogel, Lysyl Oxidase (LOX), Matrix Metalloproteinases (MMP), and Rho-Associated Protein Kinase (ROCK) pathways were inhibited using β-aminopropionitrile (BAPN), Marimastat, and H-1152 respectively. At 1, 4, and 7 days following spheroid embedding, outward cellular migration from the spheroid body was measured using ImageJ. Immunohistochemistry was performed after day 7 to detect expression levels of epithelial-to-mesenchymal transition (EMT) markers E-cadherin, N-cadherin, and β-catenin. Enhanced expression of E-cadherin denotes an epithelial phenotype whereas enhanced N-cadherin expression denotes a mesenchymal phenotype. β-catenin is the main signaling protein for the WNT pathway; it is localized around the membrane in normal cell phenotype and accumulates within the nuclei in aggressive cancer cells where it causes upregulation of oncogenic genes. In order to model colorectal cancer within a tissue-like ECM, we embedded HCT-116 spheroids into organoids containing Col 1 and LX2, liver stellate, cells. These were compared to spheroids embedded in bare Col 1 hydrogels. Organoids were prepared by suspending LX2 cells in 2mg/mL Col 1 at a density of 5 million cells/mL.

Results: After 7 days of culture, migration of the MMP and ROCK inhibited spheroids in Col 1 hydrogels was significantly decreased compared to control. The LOX inhibited spheroids showed no statistical difference in outward migration compared to the control. LOX and MMP inhibited spheroids both showed enriched expression of E-cadherin at the spheroid center and enriched expression of N-cadherin around the spheroid perimeters, similar to the control. Conversely, the ROCK inhibited spheroid showed enriched expression of E-cadherin and decreased expression of N-cadherin throughout the spheroid body. Spheroids embedded in LX2 organoids showed decreased expression of E-cadherin and enriched expression of N-cadherin. In contrast, the control spheroids displayed enriched expression of E-cadherin and decreased expression of N-cadherin. β-catenin can be seen localized within the nuclei of the spheroids cultured in LX2 organoids; in contrast to the control where it is localized around the membranes.

Conclusions: Our results indicate that inhibiting the MMP and ROCK pathways may serve to decrease migration. Furthermore, inhibiting the ROCK pathway causes colorectal cancer cells to develop a more epithelial phenotype. Finally, LX2 remodeled ECM causes colorectal cancer to express a more malignant phenotype as evident by the β-catenin localization. Taken together, this study sheds light on the ROCK pathway as a possible target for new therapeutic drugs as well as exhibits how ECM remodeling can induce a more pronounced mesenchymal phenotype in colorectal cancer cells.

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References: [1] American Cancer Society. Colorectal Cancer Facts & Figures. *American Cancer Society* 2014. Web. [2] Devarasetty, M et al. Mesenchymal stem cells support growth and organization of host-liver colorectal-tumor organoids and possibly resistance to chemotherapy. *Biofabrication* 2017. [3] Chang, M et al. Intestinal and Extraintestinal Cancers Associated with Inflammatory Bowel Disease. *Clin Colorectal Cancer* 2017

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OPTIMIZING THE BONE-IMPLANT INTERFACE FOR AMPUTEE-RELATED INJURIES

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There is a need for the improvement of current prosthetic devices designed for combat-related amputees. Current devices result in issues such as heterotopic ossification, bone hypertrophy, and low durability. Heterotopic ossification, the transformation of soft tissue into bone-like tissue, forms as a result of shear stress from the prosthetic on the surrounding tissue. Additionally, bone hypertrophy often occurs at the socket base, causing both pain and discomfort. Current solutions include the Compress® Compliant Pre-Stress Implant which is designed to direct force of impact to the bone rather than surrounding tissue, thus eliminating heterotopic ossification and hypertrophy at the interface. However, osseointegration and infection at the transdermal interface are still issues that must be addressed. WFIRM is working with the Naval Medical Research Center to optimize osseointegration at the interface where bone and skin meet the implant. A porous titanium collar with polished transdermal post has been designed to support the device's interface. Due to its osteoconductive properties, a polycaprolactone-tricalcium phosphate (PCL-TCP) and cell-laden hydrogel cap has been designed to fit over the collar to better integrate both native and regenerated tissue into the construct, with the intention of reducing complications associated with traditional prosthetics.

The purpose of this study was to determine the effect of hydrogel stiffness on the osteogenesis of bone marrow derived stem cells (BMSCs) within 3D-printed constructs. Two hydrogels (GelMA 50 and GelMA 80) were prepared with 37.5 mg/mL porcine gelatin, 3 mg/mL hyaluronic acid, 50 mg/mL gelatin methacrylate (50% or 80% methacrylation), 10% glycerol, and 0.2% photoinitiator. Rheology was conducted to analyze the storage and loss moduli at varying cross-linking times (0, 60, 90, 120 seconds) in order to optimize the difference in stiffness between hydrogels. GelMA 50 at 60 seconds and GelMA 80 at 120 seconds yielded storage moduli of 8 and 15 kPa, respectively. It is hypothesized that GelMA 80, a stiffer hydrogel, will better induce osteogenesis because it mimics the environment of bone tissue.

BMSCs were suspended in hydrogels and bioprinted with PCL-TCP (1:1) in a layer-by-layer fashion. After printing, the constructs were cross-linked and cultured for seven days in growth media to allow the cells to recover from the stress of the printing process. In the week after printing, live-dead assays showed 95% cell viability. After a week of recovery, half of the constructs were changed to osteogenic media. Alkaline phosphatase assays of media samples and hydrogels were completed to compare the constructs at days 3, 7, and 14 to measure osteoblast activity. GelMA 80 outperformed GelMA 50 in samples from days 3 and 7 in growth media and day 7 in osteogenic media with a statistically significant difference in ALP activity. Hydrogel ALP data revealed that constructs grown in osteogenic media have higher rates of osteogenesis, but future standardization by cell number must be performed in order to conclusively determine that GelMA 80 causes a higher rate of osteogenesis than GelMA 50. Further analysis of ALPL expression using qPCR supported day 14 ALP assays in that stiffer hydrogels better induce osteogenesis. Ultimately, it is evident that the stiffer hydrogel induced osteogenesis at a higher rate, and the presence of osteogenic media further increases this rate. By determining the ideal differentiation conditions for printed BMSC constructs, the bone-implant interface may be optimized to improve prosthetic devices by limiting the risk of infection.

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References:

1. Kang, Hyun-Wook, Sang Jin Lee, In Kap Ko, Carlos Kengla, James J. Yoo, and Anthony Atala. "A 3D Bioprinting System to Produce Human-scale Tissue Constructs with Structural Integrity." *Nature Biotechnology* 34.3 (2016): 312-19.

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WFIRM High School Summer Research Exposure Program Participants

Program Period: June 5, 2017 to July 7, 2017

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- Heighten and inspire interest and academic confidence in STEM disciplines
- Become more competitive when applying to undergraduate schools in the STEM disciplines

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2017 High School Participants

Summer Scholar	Primary Faculty Mentor(s)	Broad Research Focus
Sarah Barlow RJ Reynolds High School Junior	**Tracy Criswell, PhD** Assistant Professor	*Skeletal Muscle Regeneration*
Alice Carroll West Forsyth High School Sophomore	**In Kap Ko, PhD** Assistant Professor	*Kidney Regeneration*
Bronson Gatts Christ School Junior	**Graca Almeida-Porada, MD, PhD** Professor and **Christopher Porada, PhD** Associate Professor	*Stem Cells to Measure Effects of Deep Space Radiation*
Lillian Hiser Bishop McGuinnes Catholic High School, Senior	**Frank Marini, PhD** Professor	*Novel Imaging in Regenerative Medicine – Specific focus TBC w/Scholar*
Alexander Marshall West Forsyth High School Junior	**Steve J. Walker, PhD** Associate Professor	*Understanding Clinical Studies in Regenerative Medicine*
Ali Mirzazadeh Atkins High School Junior	**Aleks Skardal, PhD** Assistant Professor and **Thomas Shupe, PhD** Assistant Professor	*Body-on-a-Chip Technologies or Special Topics in RM Manufacturing (Bioinks)*
Kelly Chen Forsyth Country Day School Junior	**John Jackson, PhD** Associate Professor	*Regenerative Medicine for the Inner Ear and other RM Projects*
Demetri Hodges Forsyth Country Day School Sophomore	**Hooman Sardi-Ardekani, MD, PhD,** Assistant Professor	*Male Reproduction and Regenerative Medicine*

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