A NOVEL BIOREACTOR FOR VOLUMETRIC MUSCLE LOSS TISSUE ENGINEERING

THE USE OF RACE BASED CATEGORIES IN PRECISION MEDICINE

A Thesis Prospectus
In STS 4500
Presented to
The Faculty of the
School of Engineering and Applied Science
University of Virginia
In Partial Fulfillment of the Requirements for the Degree
Bachelor of Science in Biomedical Engineering

By

Benedict Albergo

November 1, 2021

Technical Team Members: Curtis Creech, Aparna Kola, and Caroline Roden

On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

ADVISORS

Sean Ferguson, Department of Engineering and Society

Steven Caliari, Department of Biomedical/Chemical Engineering

Introduction

Volumetric muscle loss is the traumatic or surgical loss of skeletal muscle resulting in decreased myogenic function. Current traditional and biomaterial approaches to volumetric muscle loss have only been capable of treating simple injuries isolated to skeletal muscle damage. In order to address volumetric muscle loss injuries affecting multiple tissues, Dr. Steven Caliari and his lab have developed a collagen based scaffold for guiding complete skeletal muscle regeneration. Seeded muscle derived cells and neuronal stem cells will be promoted by the environment of the scaffold to produce innervated muscle tissue that can attach to connective tissue and sustain long term function.

Previous literature has shown the benefits of stimulating myogenic cells with electrical or mechanical stimulation to further encourage cell maturation and proliferation. Thus, my Capstone team is developing a novel bioreactor capable of providing electromechanical stimulation to the seeded collagen scaffold. The bioreactor will consist of a single device that provides uniaxial tension along with pulses of electricity to the scaffold simultaneously under the direction of an Arduino based program. Bioreactor conditioning will advance the scaffold towards a model suited for *in vivo* experimentation.

In the spirit of targeted scientific advancement, my STS research paper will focus on the use of race based categories in precision medicine. Raced based categories have recently been called into question due to their use in personalized genomics research and treatment. It is my intent to explore the origin of using this social construct in the name of increasing biomedical diversity imagined by researchers, clinicians, and governmental health agencies. I will then inquire about the increasing use of race as a genomic descriptor as a result of these imaginaries. Finally, I will analyze the effect of race based treatment calculations and strategies on patient health outcomes, specifically for minoritized groups. It is my goal to shed light on the influence of racial stratification in precision medicine and how the social construct's use can be reevaluated going forward.

Technical Topic

Volumetric muscle loss (VML) is a broad term used to describe permanent, large-scale damage to muscle tissue that results in some form of decreased function (Grogan, Hsu, & Consortium, 2011). It is extremely common in military settings, but also affects many civilian populations (Corona, Rivera, Owens, Wenke, & Rathbone, 2015). Traditional approaches to treat VML have had limited success, as muscle grafts require large volumes of tissue which can lead to donor site morbidity (Sarrafian, Bodine, Murphy, Grayson, & Stover, 2018). While biomaterial strategies have been effective at treating simple injuries, most have neglected to consider damage to nervous and connective tissue common in VML injuries. This has resulted in a subpar innervation of muscle and a lack of connection at the musculotendinous junction (MTJ) critical to long term function (Gilbert-Honick & Grayson, 2020).

To address these needs, our advisor Dr. Steven Caliari and his lab have developed a new scaffold for regenerating skeletal muscle. The collagen-glycosaminoglycan polypyrrole (CG-PPy) scaffold is 3D, anisotropically aligned, and electrically conductive (Basurto, Mora, Gardner, Christ, & Caliari, 2021). The scaffold is specifically designed to encourage myogenic and neural cells to mature and proliferate according to these spatial and environmental cues to regenerate VML/MTJ injuries.

Many past studies have indicated the beneficial effects of mechanical or electrical stimulation on myogenic tissue maturation and proliferation (Goldspink, 1999; Maleiner et al., 2018). Thus, my Capstone team is designing and fabricating a bioreactor capable of electromechanically stimulating myogenic cells seeded within the CG-PPy scaffold. Specifically, our aim is to design a bioreactor system that can apply cyclic, uniaxial tension through a custom clamping mechanism that is immediately preceded by electrical stimulation via a network of electrodes. We are focused on creating a tunable stimulation protocol whereby the pulse (electrical) and strain (mechanical) frequency and duration can be modified to optimize cell viability.

Our team has already taken several steps to begin this design process. We have conducted a thorough literature review from which we have established both an electrical and mechanical stimulation protocol (Donnelly et al., 2010; Moon, Christ, Stitzel, Atala, & Yoo, 2008). From the same exploration, we have constructed a circuit design sufficient to control the electrodes within the system. A review of the mechanical properties of the CG-PPy scaffold found by the Caliari as well as a physical evaluation of the cylindrical construct has allowed us to begin designing a custom tensile clamp for the bioreactor (Basurto, Passipieri, et al., 2021). To further inform bioreactor design, we have consulted with the Christ Lab who have previously developed mechanical systems for skeletal muscle conditioning (World Intellectual Property Organization Patent No. WO2006113382A2, 2006). A primary takeaway from these discussions was the importance of creating a sealed off, sterile internal stimulation chamber to ensure cell viability.

Going forward, we intend to begin the process of developing a physical prototype of the bioreactor using additive and subtractive manufacturing techniques. This is being done under the consideration that the bioreactor must fit and function inside an incubator necessary for the maintenance of cells. A sound tensile straining system including a stepper motor as well as a properly aligned electrode network must be further designed and implemented to achieve this goal. We also plan to further source materials that are non-corrosive, easily sterilizable, and non-toxic that will ensure an uncontaminated environment within the bioreactor chamber. My personal responsibilities include designing and simulating the mechanical actuation system as well as developing structural components of the bioreactor. If a successful model is produced, we will begin experimenting on the effect of electromechanical stimulation on seeded myogenic and neuronal cell metabolic activity. This will be compared to fold increases in metabolic activity without bioreactor preconditioning already studied by the Caliari Lab (Basurto, Mora, et al., 2021).

If a tunable bioreactor is produced, the mature tissue construct may guide the repair of clinically relevant VML/MTJ injuries. Overall, this novel biomaterials approach could be significant to restoring muscle function and improving the lifestyle of thousands of civilians and military personnel suffering from VML.

STS Topic

Since the completion of the Human Genome Project, clinicians and researchers have predicted a future of “precision medicine” (PM) in which data intensive biological methodologies and predictive analytics could provide personalized treatment approaches to various diseases. PM relies on large databases that curate a relationship between genetic/molecular features and diseases. However, most early genome-wide association studies from which these databases are established have been dominated by caucasions and lacked genetic diversity. In a push for inclusion, clinicians and government agencies have adopted racial and ethnic categories to guide research and treatment efforts. However, the use of these social constructs in genomics research and clinical treatment is widely questioned due to its legitimacy as a genomic descriptor. Thus, this research paper will explore how the imaginary of objective medicine through genomics differs from its intent by introducing a counter imaginary of negative health outcomes among minoritized groups.

Sociotechnical Imaginaries and the Attempt to Objectify Precision Medicine

Popularized by Jasanoff and Kim, sociotechnical imaginaries emphasize how national actors legitimize scientific and technological development and implementation by combining policy action with imagined forms of social life (Jasanoff & Kim, 2009). Jasanoff and Kim’s framework around sociotechnical imaginaries is demonstrated in this paper through the introduction of race and ethnicity in PM research and clinical use.

To realize the vision of race categories in genomics research, the initial push for inclusion at the advent of PM must be understood. It is well established that minoritized racial

groups within the US, including African Americans and Hispanics, have been notably underrepresented in genomics research. This has mainly stemmed from a limited engagement in clinical trials due to biomedical mistrust, stigma, and competing demands (Cuccaro, Manrique, Quintero, Martinez, & McCauley, 2020). Emphasized by a 2009 study, over 95% of participants in completed worldwide genome wide association studies were of European descent (Anna Need & David Goldstein, 2009). In the era of PM, this has prevented the identification of gene variants more common to different ancestral populations, limiting the knowledge of biological disease pathways that manifest differently between different genetic lineages.

In order to imagine a new course for PM, several governmental agencies have encouraged the use of diversity in genomics research. Guidelines released by the National Institutes of Health (NIH) in 2003 titled “NIH Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research” declared that members of minoritized groups must be included in all NIH-funded clinical research without an exceptional rationale (Callier, 2019). In a similar fashion the Food and Drug Administration (FDA) released guidelines in 2005, recently updated in 2016, that recommend “a standardized approach for collecting and reporting race and ethnicity data in submissions for clinical trials for FDA-regulated medical products” (Lynch & Cohen, 2015). This research paper will further attempt to understand the intent and agenda of these diversity policies and clarify their vision for guiding PM research.

Raced Based Categories in Precision Medicine: A Counterimaginary

While intended to do social good, diversity programs for genomics research, in combination with racial correlations with genetic variants, have also led researchers to use race as a major descriptor to genetic variation and therefore disease. This has been done primarily without the use of other potential classifications, including socioeconomic status and regionality as determinants of disease. Overall, it has had the effect of clinicians considering race based corrections factors in therapeutic approaches frequently resulting in health disparities among

minoritized groups. This counterimaginary is evident when considering the use of race as a variable in the calculation of Estimated Glomerular Filtration Rate (eGFR), a measure of overall kidney function used to determine the need of transplantation. A fixed race correction value recognized by most clinicians was added as a factor to eGFR after a 1999 clinical study determined that African Americans had significantly higher natural levels of creatinine, a waste product that indicates kidney malfunction (Levey et al., 1999). However, a recent investigation has shown that including race has led to a median overestimation in eGFR of 3.2 mL/min/1.73 m^2 for Black patients in comparison to their non-Black counterparts (Inker et al., 2021). Since higher eGFR values indicate better kidney function, an overestimation has likely resulted in fewer African American patients receiving a kidney transplant in a timely manner.

Race based treatment has also been directly reinforced by government health actors attempting to provide clarity in response to research initiatives. For instance, the FDA has issued race based guidelines on epileptic seizure drugs carbamazepine and oxcarbazepine stating that these medications are of heightened risk for individuals across “broad areas of Asia” (Goodman & Brett, 2021). Providing overly generalized guidelines for treatment could lead to confusion in clinical recommendations and prevent essential drugs from being taken by groups within broad broad racial categories that are not in reality at a high risk for significant side effects. In a similar way to eGFR, this results in racial inequities in seizure outcomes among Asian populations.

Under the framework of sociotechnical imaginaries, this research paper will further explore how researchers, clinicians, and nation state actors perpetuate the use of raced based categories in PM research on the foundation of increasing diversity and legitimizing race as a genetic descriptor. Further, it will attempt to determine the impact of race based treatment on health outcomes of minoritized populations.

Next Steps

To complete the Capstone technical project and STS research project by May 2022, the anticipated timetable of work is described below:

Date	*Capstone Objective(s)*	*STS Objective(s)*
November 2021	● Finalize design of electrical and mechanical stimulation system ● Simulate static stress and fatigue of scaffold using known mechanical properties to refine a strain protocol ● Submit Capstone proposal	● Submit Prospectus to STS professor and Capstone advisor ● Receive Prospectus sign-off from STS professor and Capstone advisor ● Prepare Prospectus presentation
December 2021	● Begin prototyping of bioreactor specifically by generating components of the mechanical system and outer framework in CAD ● Start to use additive and subtractive manufacturing techniques to create and assemble the physical components of the bioreactor ● Submit fall progress update	● Submit post Prospectus evaluation ● Continue literature review of raced based categories in precision medicine to further understand the intention of actors perpetuating this system
January - February 2022	● Aid in the formulation of an Arduino script that provides specific instructions to the bioreactor for cyclic electromechanical stimulation. ● Complete experimentation of bioreactor preconditioning on myogenic cell metabolic activity	● Consult with members of medical fields at UVA to hear new perspectives of race in research and clinical treatment ● Complete draft of research paper
March - May 2022	● Finalize conditioning protocol to optimize cellular activity ● Submit technical report	● Submit thesis portfolio

References

Anna Need & David Goldstein. (2009). Next generation disparities in human genomics: Concerns and remedies—ScienceDirect. Retrieved October 17, 2021, from https://www.sciencedirect.com/science/article/pii/S0168952509001851

Basurto, I. M., Mora, M. T., Gardner, G. M., Christ, G. J., & Caliari, S. R. (2021). Aligned and electrically conductive 3D collagen scaffolds for skeletal muscle tissue engineering. *Biomaterials Science*, *9*(11), 4040–4053. https://doi.org/10.1039/d1bm00147g

Basurto, I. M., Passipieri, J. A., Gardner, G. M., Smith, K. K., Amacher, A. R., Hansrisuk, A. I., … Caliari, S. R. (2021). Photoreactive hydrogel stiffness influences volumetric muscle loss repair. *Tissue Engineering. Part A*. https://doi.org/10.1089/ten.TEA.2021.0137

Callier, S. L. (2019). The Use of Racial Categories in Precision Medicine Research. *Ethnicity & Disease*, *29*(Suppl 3), 651–658. https://doi.org/10.18865/ed.29.S3.651

Corona, B. T., Rivera, J. C., Owens, J. G., Wenke, J. C., & Rathbone, C. R. (2015). Volumetric muscle loss leads to permanent disability following extremity trauma. *Journal of Rehabilitation Research and Development*, *52*(7), 785–792. https://doi.org/10.1682/JRRD.2014.07.0165

Cuccaro, M. L., Manrique, C. P., Quintero, M. A., Martinez, R., & McCauley, J. L. (2020). Understanding Participation in Genetic Research Among Patients With Multiple Sclerosis: The Influences of Ethnicity, Gender, Education, and Age. *Frontiers in Genetics*, *11*, 120. https://doi.org/10.3389/fgene.2020.00120

Donnelly, K., Khodabukus, A., Philp, A., Deldicque, L., Dennis, R. G., & Baar, K. (2010). A Novel Bioreactor for Stimulating Skeletal Muscle In Vitro. *Tissue Engineering Part C: Methods*, *16*(4), 711–718. https://doi.org/10.1089/ten.tec.2009.0125

Gilbert-Honick, J., & Grayson, W. (2020). Vascularized and Innervated Skeletal Muscle Tissue Engineering. *Advanced Healthcare Materials*, *9*(1), e1900626. https://doi.org/10.1002/adhm.201900626

Goldspink, G. (1999). Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. *Journal of Anatomy*, *194 (Pt 3)*, 323–334. https://doi.org/10.1046/j.1469-7580.1999.19430323.x

Grogan, B. F., Hsu, J. R., & Consortium, S. T. R. (2011). Volumetric Muscle Loss. *JAAOS - Journal of the American Academy of Orthopaedic Surgeons*, *19*, S35.

Inker, L. A., Eneanya, N. D., Coresh, J., Tighiouart, H., Wang, D., Sang, Y., … Levey, A. S. (2021). New Creatinine- and Cystatin C–Based Equations to Estimate GFR without Race. *New England Journal of Medicine*, *0*(0), null. https://doi.org/10.1056/NEJMoa2102953

Jasanoff, S., & Kim, S.-H. (2009). Containing the Atom: Sociotechnical Imaginaries and Nuclear Power in the United States and South Korea. *Minerva*, *47*(2), 119–146. https://doi.org/10.1007/s11024-009-9124-4

Levey, A. S., Bosch, J. P., Lewis, J. B., Greene, T., Rogers, N., & Roth, D. (1999). A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Modification of Diet in Renal Disease Study Group. *Annals of Internal Medicine*, *130*(6), 461–470. https://doi.org/10.7326/0003-4819-130-6-199903160-00002

Lynch, H. F., & Cohen, I. G. (Eds.). (2015). *FDA in the Twenty-First Century: The Challenges of Regulating Drugs and New Technologies* (p. 568 Pages). Columbia University Press.

Maleiner, B., Tomasch, J., Heher, P., Spadiut, O., Rünzler, D., & Fuchs, C. (2018). The Importance of Biophysical and Biochemical Stimuli in Dynamic Skeletal Muscle Models. *Frontiers in Physiology*, *9*, 1130. https://doi.org/10.3389/fphys.2018.01130

Moon, D. G., Christ, G., Stitzel, J. D., Atala, A., & Yoo, J. J. (2008). Cyclic mechanical preconditioning improves engineered muscle contraction. *Tissue Engineering. Part A*, *14*(4), 473–482. https://doi.org/10.1089/tea.2007.0104

Sarrafian, T. L., Bodine, S. C., Murphy, B., Grayson, J. K., & Stover, S. M. (2018). Extracellular

matrix scaffolds for treatment of large volume muscle injuries: A review. *Veterinary Surgery: VS*, *47*(4), 524–535. https://doi.org/10.1111/vsu.12787

Yoo, J., Stitzel, J., Atala, A., & Christ, G. (2006). *World Intellectual Property Organization Patent No. WO2006113382A2*. Retrieved from https://patents.google.com/patent/WO2006113382A2/en?q=Bioreactor+system+and+method+of+enhancing+functionality+of+muscle+cultured+in+vitro&inventor=george+christ