Bioreactor Design and 3D Bioprinting for Muscle Repair
(Technical)

When Ethical Controversies Should not Slow the Progress of Using CRISPR/Cas9 for Regenerative Medicine
(STS)

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
Emily M Jackson

November 3, 2023

Technical Team Members:
Eirian Crocker and Abigail Boyette

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

Prof. Pedro Augusto P. Francisco, Department of Engineering and Society

Dr. George Christ PhD., Department of Biomedical Engineering

Megan Lobdell, Department of Biomedical Engineering

Poonam Sharma PhD., Department of Biomedical Engineering

Introduction

Volumetric muscle loss (VML) occurs due to a traumatic experience, commonly a war-related injury. VML is the loss of skeletal muscle with resultant functional impairment (Grogan et al., 2011). Normally, skeletal muscle possesses a remarkable capacity to regenerate when injured, but when confronted with major traumatic injury, the regenerative process consistently fails (Aguilar, et al., 2018). Without the regeneration of new muscle, these patients suffer from loss of function. As a possible treatment for VML injuries, the Christ Lab at UVA has developed a Tissue-Engineered Muscle Repair construct (TEMR), which seeds muscle progenitor cells (MPCs) onto a bladder acellular matrix (BAM) before incubation in a bioreactor to prepare the graft for surgical implantation (Machingal et al., 2011).

In my research, I am looking into how ethical concerns slow the use of the clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 technology on human models. CRISPR/Cas9 is a gene-editing technology that makes it possible to correct errors in the genome and turn on or off genes in cells and organisms quickly, cheaply, and with relative ease (Redman et al., 2016). This technology is a hot controversial topic since it can change human genomes. According to the authors of a paper reviewing the ethical concerns of this technology, ‘the most contentious issues concerning human germline modifications are the challenges to human safety and morality such as the risk of unforeseen, undesirable effects in clinical applications particularly to correct or prevent genetic diseases, matter of informed consent and the risk of exploitation for eugenics’ (Shinwari et al., 2018). However, to implement this technology and use its talent on targeted diseases in humans, the ethical concerns have to be overlooked to some degree. One of the biggest future uses is in regenerative medicine.

Regenerative medicine seeks to replace tissue or organs that have been damaged by age, disease, trauma, or congenital issues, versus the current clinical strategy that focuses primarily on treating the symptoms (University of Pittsburgh, 2019). The use of CRISPR/Cas9 for regenerative medicine could be extremely impactful, especially for VML patients.

Currently, ethical concerns are slowing the progress of using this powerful technology on humans. A lab-research group that wanted to see the effect of CRISPR/Cas9 on muscle atrophy did a study on live mice that presented, "with a view to establishing a novel therapeutic modality toward muscle-wasting syndrome, we used CRISPR/Cas9 to directly target Mstn (a target gene that instructs myostatin which activates skeletal muscle movement) in vivo in skeletal muscle cells to prevent loss of muscle mass" (Wei et al., 2016). There are many experiments and trials that have promising results using CRISPR/Cas9 technology for regenerative medicine. The use of CRISPR/Cas9 technology as a potential solution to the VML problem would be the first real regenerative medicine treatment besides functional free muscle transfer and the use of advanced bracing because research into regenerative medicine and powered bracing is ongoing (Grogan et al., 2011).

Bioreactor Design and 3D Bioprinting for Muscle Repair

My team and I hope to solve the problem of volumetric muscle loss with the following bioreactor design. The current model of the bioreactor holds three cassettes that are designed in parallel to hold the TEMR membranes. In order to let the muscle fibers know which orientation they need to grow in, a stepper motor is hooked up to one end to rhythmically pull on the three cassettes. However, the bioreactor is labor-intensive to produce due to the large amount of post-printing processing that needs to be done. The material warps from autoclaving, potentially causing leaks,

and media changes require the opening of the bioreactor, potentially introducing contamination. To address this, we propose the following aims:

Aim 1: ***Update Current 'Solidworks' files to decrease manufacturing time, expand the number of scaffolds held, and allow for recirculation of fresh media in bioreactor***

A. Create a pump system allowing 0.5-5 mL/min flow of media in a fully enclosed environment. Currently, the media is manually changed every 24-48 hours.
B. Improve the fastening of membrane-holding cassettes by using stainless steel nuts and bolts and creating 'through' holes to increase durability and decrease printing and processing time.
C. Add a gasket between the lid and tank to prevent leaks when the material warps.
D. Increase the volume of the bioreactor to hold more than three scaffolds for more efficient TEMR production.

Aim 2: ***Fabricate prototype using a 3D Printer***

A. Print pieces of modified bioreactor using Formlabs BioMed Clear resin.
B. Process printed pieces via washing in isopropanol and filing, then assemble into a full bioreactor with screws, magnets, and a motor.

Aim 3: ***Assess the effect of bioreactor change on graft quality***

A. Seed muscle progenitor cells onto BAM and incubate.
B. Analyze cell metabolic activity via the alamarBlue assay compared to the TEMRs produced by the previous bioreactor.
C. Analyze muscle fiber alignment at multiple time points and cell viability count at the time of seeding by fluorescently staining and imaging cytoplasm with DiD, dead cells' nuclei

with EthD-1, and collagen fibers via autofluorescence at 405 nm analyzed via ImageJ compared to TEMR produced by previous bioreactor (Christensen et al., 2022).

The efficacy of TEMR grafts incubated in a bioreactor has been demonstrated (Machingal et al., 2011). We will improve the ease of manufacturing of the bioreactor by reducing the post-printing processing necessary and introducing a mechanism to recirculate fresh media while ensuring the quality of the TEMR grafts does not decline in cell viability count or fiber alignment. This will make the production of TEMR constructs more efficient and allow for new experiments with perfusion to be done to improve the TEMR graft further. This will increase the efficiency of the production of grafts for further progress in the development of the TEMR graft, and eventually for the production of grafts for patients to help them live more normal and functional lives (Kiran et al., 2021).

When Ethical Controversies Should not Slow the Progress of Using CRISPR/Cas9 for Regenerative Medicine

The research question I am analyzing for my STS portion is “How can we use CRISPR/Cas-9 technology today despite the human ethical controversies?” This question is significant due to the outstanding arguments against using it on humans even though it has been proven as a valuable tool. One of the biggest moral issues is the ability it has to change our genomes. I will be examining this view by giving examples of the fears and the triumphs of this technology.

As stated previously, the biggest fear we have is CRISPR/Cas9’s ability to perform unwanted gene editing. This therapy is used to correct, introduce or delete almost any DNA sequence in many different types of cells and organisms (National Institutes of Health, 2019). After thinking about this possibility, it may be unnerving to hear that humans can manipulate the genome, but we also have to understand what it has already done in that aspect.

In an article, Rob Stein reviews Victoria Gray's astounding experience. "Almost four years ago, Gray became one of the first patients with a genetic disorder — and the first patient with sickle cell disease — to get an experimental treatment that uses the revolutionary gene-editing technique known as CRISPR" Stein stated. "Today, all of Gray's symptoms are gone, and she was in London to describe her landmark experience" (Stein, 2023). This patient would be continuing to fight for her life without this amazing technology. In this case, CRISPR/Cas9 uses gene-edited therapy. To do this. stem cells from the patient are extracted, edited with the technology *in vitro*, and placed back into the patient. The human body does the rest. This amazing technology should be introduced into every situation feasible.

The well-known and loved American businessman, investor, philanthropist, and writer–Bill Gates–has also considered this technology, and his input is well thought out. In his article, "Gene editing for good: how crispr could transform global development" Gates examines more than just the human health benefits of CRISPR/Cas9. He also examines the benefits for agriculture and the environment. New technologies are often met with skepticism. But if the world is to continue the remarkable progress of the past few decades, it is vital that scientists, subject to safety and ethics guidelines, be encouraged to continue taking advantage of such promising tools as CRISPR (Gates, 2018). The world, as he points out, has to continue and evolve to our surroundings. In order for that to happen, new technologies must be implemented.

Ethical issues with CRISPR/Cas9 used on humans must be considered. In order to find new solutions to diseases and medical ailments, it requires us to have an open mind.

These articles cited and many more views are turning the science world inside out with this technology. I will be reviewing the most recent and upcoming articles, interviews, and

presentations I can to confirm my question. I will also consider conducting in-person interviews to get the views of UVA's best scientists and researchers.

Conclusion

Consider the controversies. Should we use CRISPR/Cas9 on humans today? We must be cognizant of the issues that could arise, but no treatment is perfect. Sickle cell disease was corrected in a patient almost four years ago and ongoing human trials are using CRISPR/Cas9 to treat cancer that started in 2019. Many are moving forward cautiously with this technology despite the ethical concerns. So why not give them the green light?

The project with the bioreactor is attempting to reverse VML in patients with bioprinted TEMRs but CRISPR/Cas9's ability to alter genes to rejuvenate muscle could be another possibility.

I will continue to focus on how ethical controversies could be overlooked in order for CRISPR/Cas9 to be used throughout research and how it can help VML patients live like their traumatic injury never happened.

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