Thesis Project Portfolio

Peristaltic Pump to Automate Media Flow for Tissue-Engineered Muscle Repair (TEMR) Construct

(Technical Report)

How do Ethical Concerns Regarding CRISPR/Cas9 and Stem Cell Therapy Affect the Research for Volumetric Muscle Loss (VML)?

(STS Research Paper)

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Executive Summary

In pursuing enhancing medical interventions for Volumetric Muscle Loss (VML) in humans, the convergence of genetic modifications and stem cell therapy has emerged as a pivotal frontier, characterized by substantial ethical considerations. My STS research examines ethical dilemmas in genetic engineering and stem cell therapy. Despite their promise for medical research, these technologies face significant ethical debates due to involvement with human embryos or genome editing, hindering progress toward viable medical solutions. However, their potential to treat conditions like VML is considerable. The cellular composition employed in the technical endeavor comprises a lineage of muscle stem cells derived from murine origins. However, the ultimate aim necessitates transitioning to human stem cells for addressing VML. The ethical considerations inherent in the utilization and provenance of stem cells pose potential impediments to this research endeavor, thereby stalling its progress.

We're developing peristaltic pumps for our Capstone project to perfuse media into a bioreactor with tissue-engineered muscle repair (TEMR) constructs to address VML. These pumps will replace the current manual media change method, reducing contamination risk by establishing a closed system. This aspect is crucial to my research because, without a closed system, there exists a significant risk of contamination upon opening the bioreactor. In such instances, the process of creating and incubating the grafts resets to day one, further delaying this treatment option due to human error. To advance towards finding a cure for this ailment, numerous factors must be carefully considered, including the origin of cells used in the grafts and even the potential for simple mistakes leading to contamination.

With the incorporation of these pumps, researchers can introduce an automated system to facilitate the production of Tissue-Engineered Muscle Repair (TEMR) constructs for patients with Volumetric Muscle Loss (VML). Instead of manually opening the lid of this delicate system to aspirate the used media and add fresh media, these pumps ensure the absence of potential sources of contamination and eliminate the need for manual labor in media replacement. The development of these pumps involved sourcing an open-source article and obtaining files for each component, which were subsequently 3-D printed, assembled, and subjected to testing. Achieving this automated, closed system required sourcing biocompatible tubing compatible with the pump and determining the optimal flow rate to remove used media and introduce fresh media into the bioreactor without compromising the media level below the grafts or impeding media diffusion before disposal. Upon establishing a viable flow rate, experiments were conducted using food coloring dye to confirm proper media diffusion throughout the bioreactor, ensuring uniform exposure to all grafts containing cells intended for eventual human treatment.

Once the pumps were tested to be calibrated to have a flow rate that matched each other, we found a system to experiment with these pumps that worked well and gave promising results. Our experimentation consisted of eight trials that had three independent variables that changed throughout. These variables included flow rate, the volume of liquid pumped in and out of the bioreactor, and a pause time initially thought to give the fresh media enough time to diffuse before it was pumped out. Through our tests, we found that the pump taking out the used media had a faster flow rate than the pump pushing fresh media into the bioreactor which was different from when we calibrated them. Therefore we had to change the location of the tubing placement in the bioreactor so that it would slow the flow rate. We moved the tubing to the top of the bioreactor instead of the bottom side where it should be placed. This fixed our issue throughout

testing. Over the eight different runs using food coloring dye to imitate media, we found that one flow rate and volume showed the best results. To quantify what made the best test, we measured the concentration of dye that ended up in the waste media container. We did this by using a spectrophotometer machine that will measure the concentration by the known wavelength of the color of the dye. By using this information, we found that a flow rate of 1.5 mL/min and 3 mL of volume moved was best. We also concluded that because of the new tube placement of the waste media pump, the pause time was insignificant to this experiment.

The question I am attempting to answer in my research is "How do Ethical Concerns Regarding CRISPR/Cas9 and Stem Cell Therapy Affect the Research for Volumetric Muscle Loss (VML)?" This is significant due to the ongoing debates surrounding the use of genetic modifications and stem cell therapies being proposed and used in laboratories in an attempt to cure ailments and diseases. These debates have the potential to stop important clinical research in its tracks which is hurting the population who is suffering without proper treatments. I will be studying primary sources that are conducting experiments using these techniques and their outcomes as well as secondary sources that mainly consist of reviews of why people fear these two technological advancements and how we may be able to move forward with proper legislation and regulation.

Based on my research findings, several factors contribute to the deceleration of clinical research due to ethical controversies. These encompass debates concerning the provenance and application of stem cells, such as the ethical dilemmas surrounding the utilization of embryonic stem cells and their implications for human rights. Furthermore, there are concerns regarding the potential unethical use of genetic engineering to pursue human enhancement and unauthorized

genome editing. Additionally, the impact of these controversies on funding allocation poses another significant impediment to research progress. If we wish to further proceed with these technologies, proper legislation needs to be put in place so there are ethical and clean studies.