**Improving Tissue-Engineered Muscle Repair construct (TEMR) to Streamline Production of Bioreactors for Tissue Regeneration for Volumetric Muscle Loss**
(Technical Paper)

**Challenges Healthcare Personnel Face in Tissue Implantation and Regeneration**
(STS Paper)

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By
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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.

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**Introduction**

Volumetric muscle loss (VML) is the massive wasting away of skeletal muscle and civilians and military personnel both pay the cost in traumatic events such as car accidents or combat wounds respectively (Testa, Stefano, et al, 2021). Extremity injuries from combat have been estimated to require the most resources for initial treatment and are the leading cause for disability in veterans, resulting in large disability benefit costs. (Masini et al., 2009). VML is an important aspect of extremity injury where trauma-based or surgical removal of muscle tissue results in loss of functionality (Grogan et al., 2011). Because of the substantial muscle loss, it is beyond the inherent regenerative capacity of the body since mammalian skeletal muscles are not capable of extensive muscle fiber regeneration after VML injury (Shayan & Huang, 2020).

One of the main areas of research for VML is regarding acellular biological scaffolds, also known as decellularized extracellular matrices (dECM) (Greising et. al, 2017). A possible treatment called Tissue-Engineered Muscle Repair construct (TEMR) for VML is being developed in the Christ Lab at UVA. TEMR 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). There are numerous applications for this technology, and the greatest interest is how to improve the bioreactor so that production of tissue grafts can be scaled up and mass produced for surgical implementation. The second area of research and development in VML has to do with 3D printing muscle constructs consisting of decellularized Extracellular Matrix (dECM). This research provides a blueprint for future research for clinically engineered human-scaled muscle tissues for “VML treatment, drug development, and toxicity assessment studies” (Yeong-Jin et al., 2019).

The socio-technical barriers that are faced by the healthcare industry are of great interest to better understand how to fix the underlying problems that prevent VML patients from receiving tissue implantation and life-changing surgery. My technical topic is to produce a new and improved third generation model of the bioreactor that meets a number of criteria to improve its effectiveness. To complement my research to improve the bioreactor for tissue engineering, my STS topic is to explore the discrepancies that healthcare personnel face when attempting to treat victims of VML. By understanding the problems that prevent excellent treatment, this knowledge will lead to exposure of what areas need solutions in order to streamline the quality of care for individuals.

**Technical Topic Section**

The bioreactor design includes cassettes to hold the TEMR membranes and repeatedly stretch them to stimulate muscle fiber alignment. 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 opening of the bioreactor, potentially introducing contamination. To address this, we propose the following aims:

1 of 3 cassettes for tissue scaffolding.

**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 changed out every 24-48 hours.

B. Improve fastening of membrane holders by using stainless steel nuts and bolts and creating through holes to increase durability and decrease printing and processing time.

C. Add gasket between lid and tank to prevent leaks when 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 3D Printer***

A. Print pieces of modified bioreactor using Formlabs BioMed Clear resin.

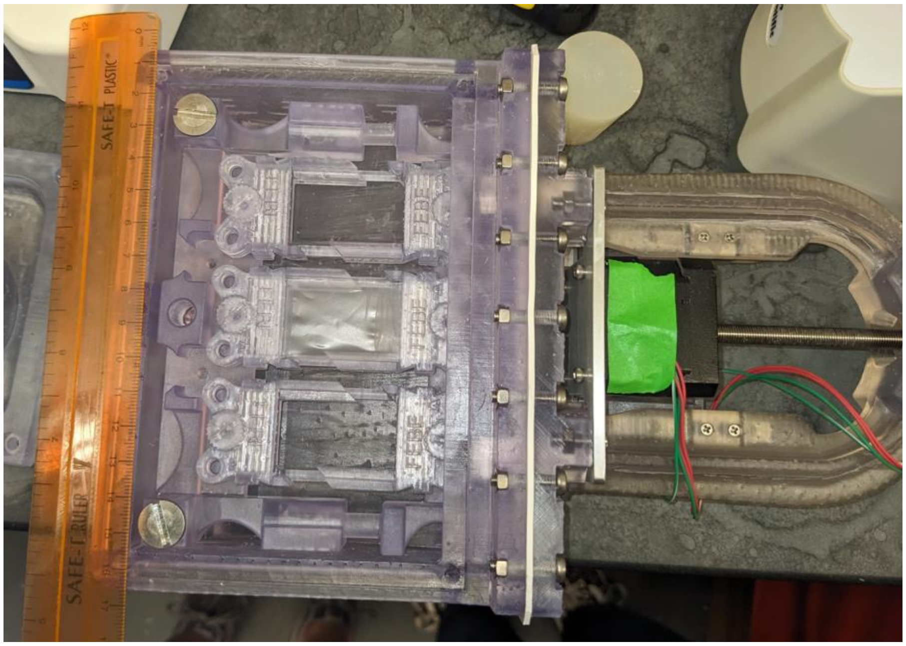
B. Process printed pieces via washing in isopropanol and filing, then assemble into a full bioreactor with screws, magnets, and motor.

**Aim 3:** ***Assess 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 TEMR produced by previous bioreactor.

C. Analyze muscle fiber alignment at multiple timepoints and cell viability at 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).



Bioreactor for tissue regeneration. 3 cassettes inside that are rhythmically pulled by stepper motor to induce instruction on the correct orientation for the cells to grow in.

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 introduce a mechanism to recirculate fresh media while ensuring the quality of the TEMR grafts does not decline in cell viability or fiber alignment. This will make the production of TEMR constructs more efficient and allow for new experiments with perfusion to be done to further improve the TEMR graft. This will increase the efficiency of production of grafts for further progress in development of the TEMR graft, and eventually for production of grafts for patients to help them live more normal and functional lives (Kiran et al., 2021).

**STS Topic**

For my STS research, I want to explore the challenges that hospital personnel face in treating victims of VML and how they go about deciding upon a treatment plan. Additionally, I would like to see how the current state of affairs of treatment plays a role in victims of VML receiving life-changing treatment plans and how improving upon that could bring better quality of life to VML patients. This research question is important in order to identify the challenges that hospital personnel face in treating VML patients so that improvements can be made to provide better care and treatment for victims of VML.

Surgeons and healthcare personnel face many challenges in the world of healthcare and in particular, the challenges they face when trying to treat VML patients prevents them from providing the best possible treatment plan and an improved quality of life. The current clinical standard of care for limb salvage patients involves both physical and/or occupational therapy and intends to restore range of motion (ROM) while increasing muscle strength (Greising et. al., 2016). Current clinical treatment for VML is autologous tissue transfer which is using skin grafted from a different section of the victim’s body. However, due to the failure rates of grafting and the result of scar tissue formation, patients are left with limited functional recovery (Carnes and Pins, 2020).

Primarily, the US Military Health System has pioneered surgical and rehabilitative care for patients with extensive extremity injury over the past decade. By using the team approach for care, “orthopedic surgical care combined with comprehensive physical rehabilitation programs and state-of-the-art technologies within these institutions have produced salient advancements in returning injured Servicemembers and Veterans to the highest level of function and quality of life." (Greising, 2016). This means that the integration of multiple specialties in healthcare is what has produced the best results of caring for victims of VML.

The current technologies and treatments that will be improved upon are those of production of bioreactors that will grow muscle tissue which in turn can be mass produced for use in implantation of volumetric muscle. Currently, there is no standard treatment protocol for VML "to replace the lost tissue with contractile muscle or restore muscle strength" (Shayan & Huang, 2020). Current surgical procedures include methods such as "autologous free flap grafting, scar tissue debridement or minced skeletal tissue transfer, are utilized to reconstruct the tissue defects." However, these methods are limited by the availability of tissue for surgery and by the need for skilled surgeons with expertise in this area to perform the surgery (Shayan & Huang, 2020).

The problem of the challenges faced in the healthcare world that bridge the gap between patients and the technical world, will be analyzed by evaluating which challenges play the most significant role in impairing stellar patient treatment for VML. The best way to analyze the data is to look into the healthcare system through the eyes of the healthcare personnel and to glean from them what challenges they face when treating VML patients. The evidence that will be collected is in regards to what healthcare professionals deem to be challenges in the treatments of VML. Evidence will be interpreted by taking the healthcare professionals perspective and cross-examining it against what are common issues in other disease treatment plans in the healthcare system that serve as a hindrance to treating VML patients.

**Research Questions and Methods**

For my STS research, I aim to answer what issues need to be addressed for healthcare professionals to provide better treatment to patients who are victims of VML. My research will include the current standard of care methods as well as the discrepancies that need to be addressed to better treat VML victims. The methods that will be used to analyze and interpret the data are as follows. First, testimonies from healthcare personnel will be obtained and scrutinized to differentiate what the main issues are in facing tissue regeneration and implantation. Secondly, research will be scrutinized regarding the obstacles that prevent the implantation of regenerative muscle tissue. Through this process, scrutiny of the disparities in VML patients getting the best treatment and return of function to their injured limbs will be evaluated and used to improve upon the current standard of care.

**Conclusion**

By exploring the challenges that hospital personnel face when trying to treat victims of VML, a better understanding will be gained for the problems that need to be addressed and solved to improve the quality of care that is available for these individuals. This research will prove extremely impactful to society by allowing victims of VML to lead more functional and normal lives by receiving life-changing treatment. This research will also be impactful on future research by providing important and necessary insight on the roadblocks healthcare workers face when trying to treat VML patients.

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