Bioreactor Design and 3D Printing for Muscle Repair

Mapping the Networks of Stem Cell Therapy to Reduce the Impact of For-Profit Stem Cell Clinics

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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

Combat veterans sustain life-altering injuries that can affect how they reintegrate with the civilian world and their quality of life. According to a study done by Veterans Affairs, 8% of a cohort of 450 medically retired servicemen were retired due to disability due to volumetric muscle loss injuries (Corona et al., 2015).Volumetric muscle loss (VML) injuries result when large portions of the muscle are removed due to trauma or surgery, overwhelming the body's regenerative capacity (Grogan et al., 2011). The loss of muscle function can cause a permanent disability, impacting the ability of veterans to work or live independently (Masini et al., 2009). However, there are engineers and scientists working to help increase the ability to regenerate the lost tissue via hydrogels, grafts, and bioprinting (Kulwatno et al., 2023). My technical project is to improve the design of a bioreactor for the production of a graft for VML injuries.

This research into grafts is a subset of tissue engineering, a field often dependent upon stem cell engineering technology. However, there is a disparity in the success of bringing products to market in tissue engineering vs stem cell therapies. In part due to regulation allowing for stem cells that are used with "minimal manipulation" and for "homologous use" (Food and Drug Administration, 2020) to be marketed and sold without FDA approval, the quantity of for-profit stem cell clinics have skyrocketed in the past decade (Knoepfler & Turner, 2018). In contrast, there are few tissue engineering products on the market (Mao & Mooney, 2015) due to having to go through rigorous FDA approval and clinical trials because the cells are manipulated (including being expanded in cell culture). Here, I will use Actor Network Theory to compare the networks for stem cell clinics and tissue engineering products to find reasons for this disparity in outcomes.

First, I will explain my technical project and the specific aims my team will achieve in improving the bioreactor design. Then, I will look at the need for mapping the networks around for-profit stem cell clinics and the academic/biotechnology company research system. I will be building upon work done by Paul Knoepfler in mapping the stem cell ecosystem (Knoepfler, 2018). Finally, I will explain the steps I will take to build these network models using Actor Network Theory.

Technical Project

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). One aspect of these extremity injuries is Volumetric Muscle Loss (VML) injuries, where trauma-based or surgical removal of muscle tissue – in civilians or military personnel – 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 (Shayan & Huang, 2020).

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). 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:

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.
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. Currently, the media is changed out every 24-48 hours.
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).

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 Project

Research Question

One of the key tenets of STS is that all of technoscience is socially constructed, so effects and causes of technoscience phenomena are not simply technical (Sismondo, 2010). While figuring out how to manipulate stem cells in engineered biomaterials to create a functional tissue is a more difficult technical problem than simply taking stem cells from a source and inserting them into a patient, I want to see if there is a social or systematic reason for why there are so many more of unproven stem cell therapies sold at for-profit clinics compared to FDA-approved treatments made through academic institutions and biotechnology companies. Paul Knoepfler and Leigh Turner have tracked a drastic increase in for-profit stem cell clinics, often involved in "stem cell tourism", offering experimental, non-FDA approved, and often ineffective stem cell treatments to desperate patients (Knoepfler & Turner, 2018). These are not only ineffective but may be harmful to patients. There are numerous reports of treatments at these clinics resulting in harm to patients, including developing septic arthritis after injection with umbilical cord blood-derived cellular products (Taliaferro et al., 2019) and blindness after stem cell treatment for macular degeneration (Leask, 2019). On the other side, in a 2015 review by Angelo Mao and David Mooney, they found only 10 FDA-approved regenerative medicine (a field that encompasses both stem cell therapies and tissue engineered constructs) therapies (Mao & Mooney, 2015). While that number may be slightly higher now, it still is nowhere near the 700+ for-profit stem cell clinics that are in the US alone (Knoepfler & Turner, 2018).

One reason for this disparity may be the FDA loophole allowing stem cell clinics to operate. While any tissue engineered therapy would be subject to an arduous process of FDA approval involving clinical trials to prove safety and efficacy, stem cell clinics operate in a gray zone. Because the products that they use are "minimally manipulated" and used for the same function they normally have in the body, they are permitted to be marketed and used without FDA approval (Food and Drug Administration, 2020).

Relevant social groups

In a 2018 paper, Knoepfler analyzed the groups and interactions involved in the stem cell product ecosystem, finding that the complexity of the network has drastically increased in recent years compared to the early 2000s. He found that now, "besides patients and their advocates, other key players in this system include academic labs, attorneys, biotech companies, funders including public and private funding agencies, investors, journals, physicians, politicians, regulators such as the US FDA in the USA and equivalents in other countries as well as additional governmental bodies, societies and foundations, and unfortunately, the mushrooming group of unproven, for-profit stem cell clinics" (Knoepfler, 2018). This is an enormous network, and I intend to break it down into two (albeit overlapping) sub-networks: the for-profit clinic network and the academic/biotechnology company network. As Knoepfler notes in his paper, much of the analysis will be focused on the United States, but is applicable to and incorporates the global stem cell ecosystem (Knoepfler, 2018). This paper will not be looking at the impact of groups opposed to stem cell research, as there has been much written on that subject . It has more bearing on historical analysis rather than the current networks of stem cell therapies, as it has

become less of a hot-button, widely debated issue due to time and the introduction of induced pluripotent stem cells providing a way around the ethical issues of embryonic stem cell research (Thompson, 2013).

I will also attempt to incorporate insurance companies, a group that Knoepfler neglected in his paper. Because insurance coverage of a treatment greatly influences who can access that treatment, the willingness of insurance companies to pay for stem cell therapies will drive who has access. Wealthier individuals have greater access to these treatments because they have the disposable income to be able to pay for expensive therapies out of pocket, a luxury that poorer patients do not have. Knoepfler does note that often for-profit clinics will direct patients to raise money online for the treatment (Knoepfler, 2018). This crowdfunding model of treatment targets the desperate, dying patients. Knoepfler notes in his 2018 paper that

> "one meritorious but challenging goal of these kinds of efforts is to shrink the unproven stem cell constellation of the ecosystem. If successful even on a small scale, this effort will free up resources, clarify the state of the clinical science for the public and media, aid public support for stem cells and regenerative medicine and most importantly, protect patients. Admittedly, it is formally possible that there could be unpredicted or even negative consequences to attempting to alter the stem cell ecosystem by reducing the unproven clinic component, but at present the hypothetical risk of trying to change the ecosystem is very much worth it" (Knoepfler, 2018).

By mapping out the sub-networks of the for-profit stem cell clinics and the academic/biotechnology company system using Actor Network Theory (Sismondo, 2010), it may be possible to identify ways to reduce the influence of the stem cell clinic network without harming the other parts of the stem cell ecosystem.

To create these networks, I will research the specific laws and regulations set by the FDA for stem cell therapies and tissue engineering products. I will also look at Medicare and insurance company policies to determine what, if any, stem cell or tissue engineering products insurance companies cover. I can then use these regulations and funding data to make the sub-networks for for-profit clinics and the academic/biotechnology company route. A comparison of these networks may show what factors influence the funneling of patients to unproven treatments at predatory for-profit clinics rather than FDA approved tissue engineering constructs from biotechnology companies. From this, it may be possible to identify a route to cut down on the power of these for-profit stem cell clinics without negatively impacting the other side of the network.

Key Texts

Mapping and driving the stem cell ecosystem, Paul Knoepfler, 2018

Paul Knoepfler outlines the network of actors involved in stem cell research and medicine. He tracks how it has changed as the field has evolved, particularly comparing the early 2000s to 2018. He lays out each of the groups in that network and goes into detail about what motivates them and how they tend to interact with the other groups. He focuses a great deal on for-profit clinics, which he argues are largely unethical and hinder trust in stem cell medicine. I will be building upon his network by adding in insurance companies and separating it out into two separate sub-networks: for-profit stem cell clinics and FDA approved treatment research.

Actor Network Theory in *An Introduction to Science and Technology Studies*, Sergio Sismondo, 2010

Sismondo outlines the key tenants of Actor Network Theory, a framework used for visualizing and analyzing how groups in a network interact to produce technoscience. It involves identifying the actors, or groups, involved in the network, then elucidating the interests of each of the actors. These interests may line up with other actors, and so the groups form associations and alliances with each other to achieve their mutual goals. This facilitates the analysis of the interactions of the groups in the network to determine possible paths to solve a technoscientific problem. This is a useful framework for the analysis of the stem cell ecosystem since it allows for visualization and mapping of how all of the groups work together or against each other.

Regenerative Medicine: current therapies and future directions, Angleo Mao and David Mooney, 2015

This review paper looks over therapies derived from tissue engineering and discusses the technological advancements involved. It lists 10 FDA-approved therapies, which is in stark contrast to the 700+ for-profit stem cell clinics. I plan to use this review as the basis for the claim that there are very few FDA-approved therapies on the market.

Spotting 'unproven' stem cell therapies in the wild, Freya Leask, 2019

Freya Leask looks at typical signs that a stem cell therapy is unproven, including insufficient data, using "tokens of scientific legitimacy", and dramatic claims. She compares and contrasts a successful, legitimate tissue engineering treatment for macular degeneration done by Masayo Takahashi with stem cell treatments for the same disease that resulted in patient blindness. In this comparison, Leask implicates the FDA loophole of allowing products with 'minimal manipulation' for 'homologous use' to be marketed and sold without FDA approval. This key difference is one that I will be working into my model.

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