**Identifying Nuclear Membrane Proteins that Facilitate Chromosomal Mechanotransduction**

(Technical Paper)

**The Effect of Ethical Restrictions on Stem Cell Research and Innovation Since 1998**

(STS Paper)

**A Thesis Prospectus Submitted to the**

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Allison Horenberg
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Technical Project Team Members
Victoria Hinchberger

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

## Introduction

Understanding the pathogenesis of a disease is crucial to the development of effective and curative treatments. This understanding is often derived from the research performed at the university and institutional level through early stage discovery research. While scientists in the field are supportive of their own research techniques, the general public and the government may raise ethical concerns that halt or diminish the progress of research innovation. One field that often experiences ethical pushback is the field of tissue engineering. Tissue engineering utilizes the understanding of the natural *in vivo* environment to develop biomaterial constructs and processes that can help regenerate tissue post injury (Rogers, n.d.). Within tissue engineering, regenerative medicine seeks to utilize potent cell lines that can differentiate into any cell type in the body (Stocum, n.d.). Both tissue engineering and regenerative medicine aim to development effective treatments for disease and injury, thus, restrictions on these fields could lead to a lack of adequate health care innovation and curative treatments.

Throughout the past twenty years, there has been significant innovation in stem cell biology, a field within regenerative medicine, to understand specific differentiation of stem cells and their effect on wound healing and tissue regeneration. This progress, while beneficial to the development of autologous treatment options, has not been widely accepted for research development due to ethical restrictions, and thus, safe and effective treatments may have been rejected or undiscovered through these techniques. Through the proposed STS research paper, the effect of ethical reactions on stem cell research will be explored. Furthermore, through my technical project, I will utilize tissue engineering laboratory research techniques to identify a novel protein signaling pathway involved in force mechanotransduction, which could potentially serve a target for curative therapies for Idiopathic Pulmonary Fibrosis (IPF). These two projects will

contribute to the field of biomedical engineering in two distinct, yet related ways. The technical project has potential to identify previously unknown targets for IPF treatment through laboratory research and the research paper will aim to identify how laboratory research on stem cells has been affected by ethical responses.

**Technical Topic: Identifying Nuclear Membrane Proteins that Facilitate Chromosomal Mechanotransduction**

*Introduction*

Upon injury, tissues begin the wound healing process. Rather than regaining functionality, the tissue is often remodeled as a scar resulting in tissue fibrosis. In the case of Idiopathic Pulmonary Fibrosis (IPF), the interstitial region between the vasculature and alveoli is progressively damaged and scarred. With the loss of healthy pulmonary tissue, the functionality of the lungs is compromised. IPF is the most severe among a family of interstitial lung diseases. There is only a median 3.8-year survival rate for IPF, highlighting the need for better treatments and therapies (Olson, Gifford, Inase, Pérez, & Suda, 2018).

The current state-of-the art treatment fails to effectively treat IPF. While double-lung transplants are a viable treatment option, IPF patients have the highest mortality rates on the waiting list among patients with indications for lung transplantation (George, Arnaoutakis, & Shah, 2011). Additionally, lung transplants have the lowest survival rate, 54.4% after five years, of all solid organ transplants (George et al., 2011). These issues with lung transplants have given momentum to pharmaceutical treatments for IPF. There are two Food and Drug Administration approved treatments, Nintedanib and Pirfendione. Nintedanib inhibits tyrosine kinases which are proteins involved in expression of profibrotic mediators including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF)

(Rivera-Ortega, Hayton, Blaikley, Leonard, & Chaudhuri, 2018). Pirfenidone inhibits the small molecules like VEGF, procollagen I, and FGF, blocking the functions of these factors and thereby reducing fibrotic activity (Margaritopoulos, Vasarmidi, & Antoniou, 2016).

While both Nintedanib and Pirfenidone slow down the progressive fibrosis, they are not curative. These treatments are important, however, in that they have given momentum to studying profibrotic mediators as targets for fibrotic development. As mentioned earlier, tissue damage has been shown to initiate the wound healing process (Kotton & Morrisey, 2014). The wound healing process includes hemostasis, inflammation, proliferation, and scar remodeling. Due to the low regenerative capacity of pulmonary tissue, fibrotic remodeling causes mechanical integrity is maintained at the expense of functionality (Kotton & Morrisey, 2014). As pulmonary tissue becomes more fibrotic, the stress experienced as a result of myofibroblast contraction increases (Hinz, 2012). These stresses are of particular interest in fibrotic development, as it is well known that force-induced mechanisms are involved at each step of the wound healing progress, and that these mechanisms lead to fibrotic remodeling.

*Taking Advantage of Mechanotransduction*

The conversion of force to biochemical signals is referred to as mechanotransduction (Duscher et al., 2014). This signal transduction pathway has recently been implicated as a potential target for IPF treatment. The force initiates a response through extracellular (ECM) proteins like fibrinogen and fibronectin that are bound to integrins. Integrins are transmembrane proteins that connect to these ECM proteins on one end and cytoskeletal actin on the other end. The intracellular binding to cytoskeletal proteins is accomplished through cross-linking proteins. This interwoven network creates the mechanical connection that allows for force transduction. Continuing down the pathway, cytoskeletal proteins have recently been shown to connect to the

linker of nucleoskeleton and cytoskeleton (LINC) complex (Belaadi, Millon-Frémillon, Aureille, & Guilluy, 2018; Wang et al., 2018). After this complex, proteins that are still-to-be elucidated reach chromatin and DNA, serving as transcription factors for gene expression of profibrotic activity.

While there has been recent discovery of proteins and complexes like the LINC complex, there are still missing pieces in the protein puzzle of the mechanosignaling pathway (Belaadi et al., 2018; Wang et al., 2018). Utilizing *in situ* and *in vitro* methods, this capstone project aims to both predict and validate proteins that are involved in mechanotransduction and fibrosis.

*Creating the Network*

I will be working with Victoria Hinchberger under the supervision of Dr. Thomas Barker and Dr. Chiuan-Ren Yeh. All experimental and computational work is split jointly between Ms. Hinchberger and myself. Approximately 10 hours per week per group member are dedicated to the in-laboratory experiments discussed below. The approximate timeline for research and writing is in Figure 1.

To visualize predicted novel proteins, an open source software, Cytoscape will create biomolecular reaction networks based off of already known protein interactions (Su, Morris,

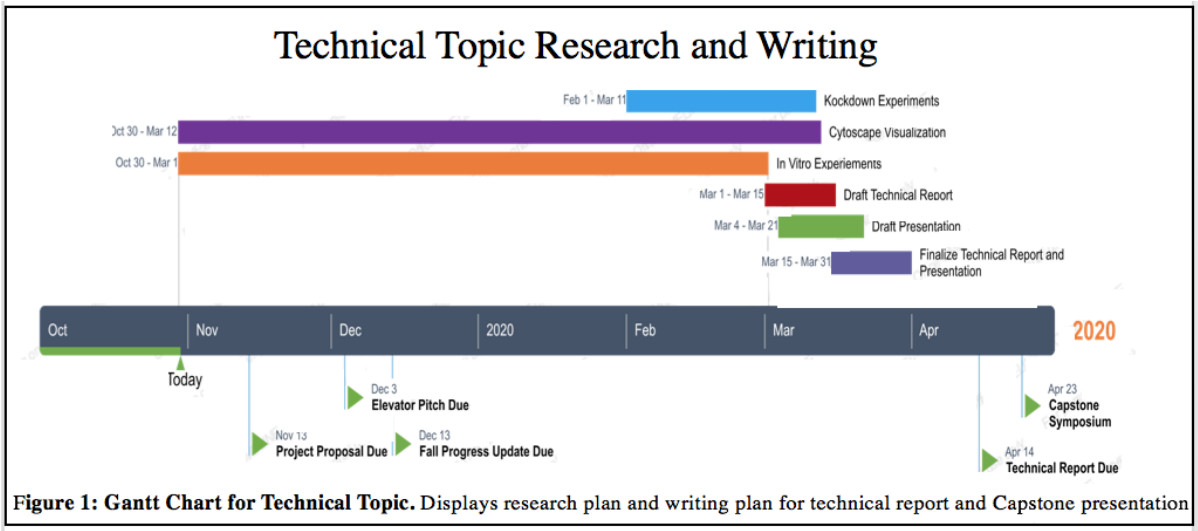


**Figure 1: Gantt Chart for Technical Topic.** Displays research plan and writing plan for technical report and Capstone presentation

Demchak, & Bader, 2014). This program is especially useful in large-scale networks like the mechanosignaling pathway because there is a plethora of potential interactions between proteins. Although the proteins present after force induction can be found through *in vitro* proteomics data, it is difficult to determine how they are spatiotemporally relevant in the pathway without an analysis of gene expression and direct protein interactions. This high-throughput screening of interactions will allow us to more efficiently and accurately predict proteins involved in the network.

In order to determine the proteins specifically involved in the mechanosignaling pathway, a magnetic precipitation technique is used, in which force is induced by a magnetic force on fibronectin coated beads. This application of force induces the mechanosignaling pathway and formation of protein complexes and connections that will ideally mimic the early response caused by force applied to pulmonary tissue *in vivo.* Following cell lysis, only the proteins involved in the mechanosignaling pathway will remain for analysis. Previously, magnetic precipitation has been used to extract protein from egg whites and for experiments in which the proteins of interest are already known (Cao, Zhang, He, Chen, & Zhang, 2014). The current project applies magnetic precipitation towards studying mechanotransduction, and it is being used to extract unknown proteins. Two controls are used in this experiment - force negative and negative controls. No magnetic force is applied for the negative control and the beads are added with the lysis buffer to account for potential background interactions. The force negative control tests for the presence of proteins that may naturally bind in the presence of the magnetic beads. These controls are important to ensure that the correct candidate proteins are focused on for the force-induced, mechanosignaling pathway. The proteins isolated from these experiments have been subject to

high performance liquid chromatography to initiate the search for the novel proteins through a high-throughput method.

Other *in vitro* methods will be used to identify and validate the identity of such proteins. Western blotting will test for the presence of specific proteins by molecular weight. Using a marker well with respective molecular weights serves as comparison for both the expected molecular weights and expression levels of proteins found in the experimental and control groups. Immunofluorescence imaging will confirm the localization of the proteins of interest by visualizing fluorescent immunoglobulin staining through confocal microscopy. If proteins are localized to their respective cellular compartments, further interaction analysis can be performed. A proximity ligation assay (PLA) will be used to confirm the interactions of these proteins within a 40 nanometer range (Bagchi, Fredriksson, & Wallén-Mackenzie, 2015). Successful PLA experiments will drive the future steps toward defining the mechano-signaling pathway and confirming direct and indirect interactions between proteins isolated through force mechanotransduction.

By predicting proteins *in situ* via computational methods and validating through *in vitro* studies, the technical project aims to identify novel proteins involved in the mechanosignaling pathway to elucidate the mechanisms involved in fibrotic development. The identified proteins have potential to support the development of an upstream curative therapeutic that could halt the progression of IPF.

**STS Topic: The Effect of Ethical Restrictions on Stem Cell Research and Innovation Since 1998**

Stem cell research has greatly expanded in the past twenty years with the discovery of both embryonic stem cells and induced pluripotent stem cells, allowing therapies to create any cell lineage in the body from many different cell lines (Wertz, 2002). For example, bone marrow cells

can be differentiated into muscle cells and oligodendrocyte precursors can be differentiated into neurons (Morrison, 2001). While these discoveries have the potential to create autologous therapies to treat a myriad of injuries and diseases, ethical concerns due to the use of human embryos have led to a rejection of these technologies and innovations. There have been several papers that have justified or refuted these ethical reactions (Outka, 2002; Sandel, 2004; Sugarman, 2008; Wainwright, Williams, Michael, Farsides, & Cribb, 2006), however, up to this point, there has yet been an analysis of the effects of the restrictions on the research field as a whole. Through this research paper, I will analyze the effect to which the ethical restrictions promoted or restricted further research in the field.

I will categorize "eras" in stem cell research and development since 1998 that define periods of either research innovation or ethical restriction. I will ultimately conclude the extent to which ethical reactions have affected this research with respect to both positive and negative impacts. This paper focuses on stem cells, which are cells that have two key abilities: differentiation and proliferation. Stem cells can both differentiate into many cell types, depending on their potency, and also proliferate and self-renew (Zomer, Vidane, Gonçalves, & Ambrósio, 2015). There are multiple types of stem cells currently used in research. Arguably the most controversial stem cells are the pluripotent embryonic stem cells (ESCs) that are isolated from the inner cell mass of a blastocyst and can differentiate into any tissue in the body. Mesenchymal stem cells (MSCs) are isolated from adult tissue and are multipotent, thus, they can only differentiate into their respective germ layer. The most innovative stem cell line are the induced pluripotent stem cells (iPSCs) which are MSCs transformed back into pluripotent cells using specific transcriptions factors (Zomer et al., 2015).

All three stem cells have generated ethical concerns, but the main reason for rejections of these methods is the use of the human embryo for isolation of embryonic stem cells (Holden & Vogel, 2008). Both political and religious arguments surround this debate as the embryo is destroyed during the isolation process. Additional ethical concerns associated with stem cell research question cell types used for isolation, procurement processes, *in vivo* research use, and intellectual property (Sugarman, 2008). These ethical reactions have resulted in legislation that has restricted the use to these cells in research. The main legislation that I will focus on is the Bush administrations restrictions and guidelines governing stem cell research, which put research on hold throughout his term as president (Levine, 2011). These policies were critical to the development of other stem cell research methods, however, they also halted progress of ESC research that was already in progress.

The stakeholders that are relevant to my research paper are the patients with stem cell treatable injuries or diseases. Furthermore, patients with diseases that are moderated by innate stem cells could also be affected by restrictions on research due to the potential for development of a mechanistic understanding of the disease progression that could be discovered through research methods. Scientists and other researchers hold a significant stake in this work as public discourse and political response controls funding and motivation for their work. Therefore, politicians and the general public are also major stakeholders in this problem as they ultimately contribute to the acceptance of the new research technologies. The physical artifacts involved in this work are the research technologies, stem cells, and human embryos used to understand disease, injury, and tissue regeneration. The non-physical artifacts include the public ethical sentiments and the political legislation used to control research progress.

One STS theory that will frame this problem is Langdon Winner's *Do Artifacts Have Politics?* (Winner, 1980). This theory suggests that artifacts can have politics in two distinct ways: inherently or developmentally. For example, Winner argues that cotton is inherently political due to its necessity for authority to accomplish the diverse set of tasks to create the finished thread, and thus, the creation the cotton system requires the adoption of specific authority, regulations, and guidelines. In another example, Winner suggests that low-hanging overpasses are developmentally political, due to the impossibility of bus travel under these highways, resulting in less minorities and low-income populations in suburban areas. Determining whether an artifact is inherently political can help to justify the response of the general public to its creation and adoption. Through this lens, I will explore the connection between the legislative policy established to control stem cell research. Through the discussion of whether or not this research is inherently political, I will determine if the restrictions were inevitable or if they solely existed due to the effects of stem cell therapies. One critique of this theory discussed in a master's thesis by Richard W. Donnelly in is that Winner has an inconsistent explanation and understanding of "politics" and "technology," which leads to a conceptual misunderstanding and a loss of historical context (Donnelly, 1990). He argues that incorporating a focus on human choice and control for technological decision making will clarify who is making the political decisions and reconcile ambiguities in his argument. To incorporate this opinion into my research paper, I will ensure to perform policy analysis to determine where the policies and political responses stem from, adding historical context to my research.

Another STS theory that will frame this problem is Thomas P. Hughes's explanation of *Technological Momentum*. Hughes suggests that technological momentum is "A more complex concept than determinism and social construction, technological momentum infers that social

development shapes and is shaped by technology" (Hughes, Smith, & Marx, 1994). Therefore, momentum is time-dependent and will shift within a spectrum of social constructivism and technological determinism. Within the eras of stem cell research defined in my paper, I will determine which side of the determinism-constructivism spectrum the eras fall towards. Using these ideas, I will identify which factors, either social or technical, are defining the progress of research and innovation. The theory of technological momentum is widely accepted today, thus, current critics focus on the expansion of the theory, rather than exposing the holes in the analysis. Mika Panzer discusses the potential for technological momentum to be expanded and intertwined with actor network theory, where artifacts are "not only…embedded within systems which constrain human behavior, but…artifacts accomplish an agency" (Pantzar, 1997). To incorporate this addition into my analysis, I will be sure to acknowledge both human and non-human agents when considering the ethical effects on the field.

Overall, this research is important and worthy of attention because it will provide prospective on the impact of public sentiment and political restriction on research and development of a novel technology. Specifically, within the focus of stem cells, I will argue whether ethical restrictions were detrimental or stimulating to the research field. Furthermore, this research will also conclude with the current state of stem cell research providing insight into the potential and

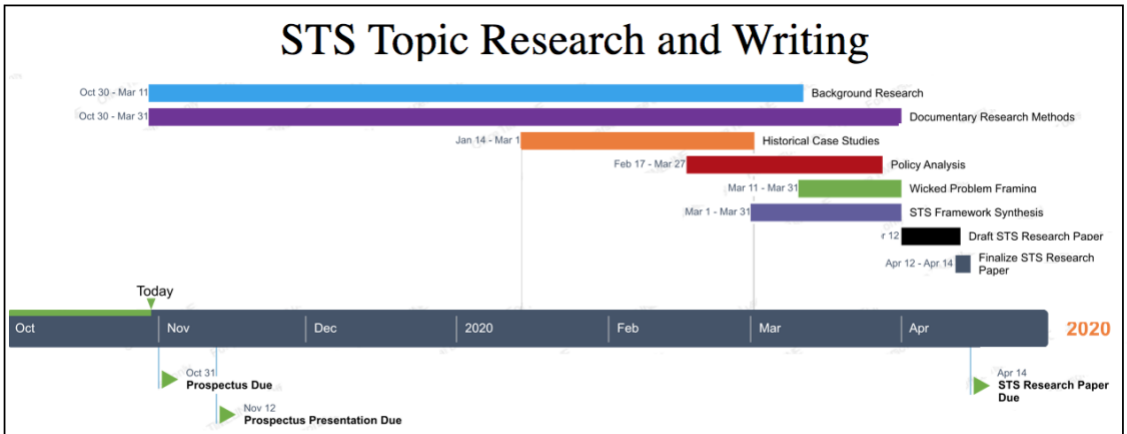


**Figure 2: Gantt Chart for STS Topic.** Displays research plan and writing plan for the STS research paper

future for the field. To effectively communicate these frameworks, I will organize my research paper into stem cell research "eras" that contextualize the trends in research during that time period by a period of innovation or a period of restriction. Within each era, I will incorporate technological momentum as described above. I will conclude the paper will an argument using technological politics to determine the extent to which ethical and political restrictions affected the development of this research. A timeline for the research and writing is seen in Figure 2.

**Research Question and Methods**

To investigate my STS research topic, my research question is "how has ethical rejection of stem cell research affected the progress of the field from 1998 to present day?" To pursue this research question, I will use documentary research methods, historical case studies, policy analysis, and wicked problem framing. Documentary research methods will be utilized to organize and synthesize data gathered from primary research articles and review papers. Sources will be organized into the research, ethical restrictions, and public reactions of their respective time period to shape the ideas of that time. Careful documentation will help to organize the research into subsections that will ultimately for the intended "eras" of stem cell innovation and restriction that will be discussed in the paper. Historical case studies, such as the development of the induced pluripotent stem cell and the discovery of the embryonic stem cell, will play a key role in the development of the historical analysis of my research paper. These studies will allow me to qualitatively understand how the ethical reactions to stem cell research have shifted throughout history, as well as characterize general public sentiments within the specific eras. Policy analysis of the Bush administration restrictions will not only give additional evidence for the public sentiment of the respective times, but it will also contribute to the connection of the STS framework to the scope of this research paper. The policies will directly show how research

innovation has effected policy decisions and vice versa. Finally, wicked problem framing will organize the focus of the paper to understand how the historical analysis has ultimately effected stem cell research as a whole. I will connect the ideas of ethics, research innovation, and policy to frame the root ideas of my STS research paper.

These methods align well with my research question through the organization of relevant evidence and effective problem framing. By utilizing policy analysis and historical case studies, I will gather and analyze the evidence necessary to justify the ethical concerns and rejections of innovation in stem cell research. Documentary research methods and wicked problem framing will ultimately be the key methods to the overall conclusions made in my research paper as I will ultimately only focus on the major effects that truly effected the stem cell field. Through these methods I will develop a strong argument to justify the effect of ethical rejection on research innovation.

**Conclusion**

Through the Technical Topic and the STS Research Paper, I will elucidate key mechanisms to understand current biomedical research and technologies. The technical project will identify key proteins involved in force mechanotransduction that could play a role in the development of idiopathic pulmonary fibrosis. Attention will be paid to nuclear membrane proteins that may have direct interactions with gene expression and cell signaling. The technical report will detail the development of this research and the future potential for this work. The STS paper will make conclusions on the effects of ethics on stem cell research innovation throughout history by categorizing time periods of either restriction or innovation. Through the described methods, I will effectively analyze primary and secondary research to develop the STS frameworks of Technological Momentum and Political Technologies. Overall, I anticipate a correlation between

strong ethical concerns and political restrictions, which has ultimately discouraged innovation in stem cell research.

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