Investigating the Role of Canonical Wnt signaling in Dental Regeneration as Aided by FGF-2 (With Some Applications to Cancer Treatment)

Grant Proposal

Lilian Amer

Massachusetts Academy of Math and Science at the Worcester Polytechnic Institute

Worcester, Massachusetts

Abstract (RQ) or Executive Summary (Eng)

Regeneration, the process of restoring damaged tissues, is a fast growing solution to various diseases in the medical field. Human regenerative capabilities are limited in organs such as teeth, where pulp necrosis poses significant health challenges, affecting an estimated 2.44 billion people globally. Current treatments, including dental implants, carry risks such as peri-implantitis and jawbone resorption. Research into molecular pathways like Wnt and fibroblast growth factor-2 (FGF-2) signaling offers a promising avenue for dental pulp regeneration. This study investigates how modulation of the canonical Wnt signaling pathway, using a combination of lithium chloride and FGF-2, influences stem cell proliferation and differentiation in a Schmidtea mediterranea model. Results demonstrated that combining lithium chloride and FGF-2 enhanced regeneration rates and odontoblast-like differentiation, offering a minimally invasive solution for dental pulp regeneration. These findings provide a foundation for advancing regenerative therapies in dentistry and highlight broader applications in tissue engineering and cancer treatment.

Keywords: Wnt signaling pathway, dental pulp, stem cells, lithium chloride, basic fibroblast growth factor (bFGF)

Investigating the Role of Canonical Wnt Signaling in Dental Regeneration (With Some Applications to Cancer Treatment)

Regeneration, the process by which organisms restore lost or damaged tissues, has become a central focus of medical research due to its vast potential in treating various diseases and injuries. While the ability to regenerate tissues is more commonly associated with animals like salamanders, which are able to regrow entire limbs, humans demonstrate similar activity on a smaller scale (Joven et al., 2019). In humans, natural regenerative abilities are limited in tissues such as the heart, brain, and teeth, which do not regenerate as efficiently as other organs, such as the liver or even the skin (Mowbray, 2023). However, the potential for inducing regeneration in these tissues through molecular signaling pathways has led to promising research in regenerative medicine, particularly cellular regeneration caused by stem cells. Stem cells are undifferentiated cells with the potential to develop into various cell types, and they play a significant role in tissue regeneration because of their ability to proliferate, differentiate, and replace damaged cells (National Institutes of Health [NIH], 2016). The regenerative potential of stem cells has shown to be useful in dental research, particularly in efforts to restore dental pulp tissue lost to pulp necrosis (Kwack et al., 2022). Pulp necrosis — a serious condition that affects approximately 2.44 billion people worldwide — is when cells that make up the pulp begin to die (World Health Organization, 2022). It is commonly caused by cavities that progress beyond the dentin layer and reach the pulp, exposing it to bacterial infection that leads to pulp necrosis (Ricucci & Siqueira, 2010). The dental pulp makes up the center of the tooth, and it contains all the nerves, blood vessels, connective tissues, and specialized cells, essentially keeping the tooth alive (Morotomi et al., 2019). Thus, it is imperative to find a treatment, as pulp necrosis can also eventually lead to periodontitis, or the infection of gums, which can affect other teeth surrounding the dead tooth (NIH, 2024). Current treatments for pulp necrosis include dental implants; however, these have been found to increase the risk of peri-implantitis, which is an irreversible condition in which the hard and soft tissue surrounding the osseointegrated dental implant become infected and begin to break down (Barootchi & Wang, 2021). Dental implants lack periodontal ligaments, which normally protects against the mastication forces of chewing. The risk of jawbone resorption increases without periodontal ligaments, making implants unsustainable for long-term oral health (Angelova Volponi et al., 2018). Thus, researchers have looked

into cellular regeneration mediated by the Wnt signaling pathway as a viable solution to reversing the effects of pulp necrosis. A signaling pathway is a network of biochemical reactions that enables intercellular communication, allowing cells to carry out their specific functions (Nesterova et al., 2020). The Wnt signaling pathway is especially promising in tissue regeneration, as its proteins regulate both proliferation and differentiation of dental pulp stem cells (DPSCs), offering potential for reversing pulp necrosis (Angelova Volponi et al., 2018).

The Wnt signaling pathway regulates various cellular processes, such as differentiation, proliferation, and migration. However, its role in determining the fate of dental pulp stem cells (DPSCs) — particularly whether they differentiate into odontoblast-like cells, which are responsible for dentin formation, or maintain a stem-like phenotype — requires further research. Therefore, we aim to study the activation or inhibition of the Wnt signaling pathway by usage of fibroblast growth factors (FGF), such as bFGF (or FGF-2), and how it influences the behavior of DPSCs in the context of dental regeneration. Basic Fibroblast Growth Factor (bFGF) is a single-chain polypeptide that primarily binds to heparan sulfate proteoglycans (HSPGs) on the cell surface, which act as a co-receptor to facilitate its interaction with its primary receptor, the Fibroblast Growth Factor Receptor (FGFR) (Mundhenke et al., 2002). Wnt and FGF signaling pathways have been shown to work together to aid in cell proliferation, and separately to determine cell lineage specification (ten Berge et al., 2008). In dental pulp stem cells (DPSCs), fibroblast growth factor 2 (FGF-2) signaling significantly influences both proliferation and differentiation into odontoblast-like cells, which are essential for dental tissue regeneration. Studies have demonstrated that early delivery of exogenous FGF-2 to exposed pulp leads to the proliferative expansion of αSMA-positive progenitor cells and their accelerated differentiation into odontoblasts (Vidovic-Zdrilic et al., 2018). Additionally, FGF-2 has been shown to enhance the osteo/odontogenic differentiation ability of stem cells from the apical papilla (SCAP) by inhibiting the PI3K/AKT pathway (Wang et al., 2024). However, the precise mechanisms by which FGF-2 regulates DPSC self-renewal and differentiation remain under investigation. By further exploring how FGF-2 signaling impacts DPSC behavior, this study aims to uncover its potential for optimizing dental tissue regeneration. It is hypothesized that modulating Wnt signaling might direct DPSCs toward optimal regeneration outcomes.

The effects of FGF-2 on cell proliferation, differentiation into odontoblast-like cells, and the formation of a functional pulp-like tissue in vitro, will be assessed using the model Schmidtea mediterranea (planaria). Schmidtea mediterranea has been used most commonly in cancer research for its remarkable regenerative abilities, including the regeneration of complex tissues like the brain, gut, and reproductive organs. This regenerative capacity is driven by their population of pluripotent stem cells, known as neoblasts, which respond to various signaling pathways, including Wnt. Several different activators that have been proven to aid in dental regeneration, such as lithium chloride, will be tested to measure the rate of regeneration of the Wnt signaling pathway with and without the activator (Ishimoto et al., 2015). The combination of lithium chloride with bFGF will be tested to determine whether it could enhance the rate and quality of tissue regeneration. Then, the two will be used to develop a preliminary topical application that could be applied directly to the tooth after pulp extirpation, offering a minimally invasive solution that avoids the complications associated with more traditional treatments like implants or root canals.

Section II: Specific Aims

Research Problem

Dental pulp necrosis is a serious global health issue with limited sustainable treatment options, as current methods like dental implants and root canals often result in complications such as peri-implantitis and jawbone resorption (Angelova Volponi et al., 2018). The canonical Wnt signaling pathway has been proven to promote dental pulp regeneration by modulating the proliferation and differentiation of human dental pulp stem cells (hDPSCs). However, the mechanisms through which the pathway regulates hDPSC fate — balancing proliferation, differentiation into odontoblast-like cells, and maintenance of a stem-like state — are currently unclear. This proposal aims to investigate the role of basic fibroblast growth factors (bFGF), as well as Wnt signaling activator lithium chloride, in enhancing dental regeneration.

Long-Term Goal

The long-term goal of this project is to develop a minimally invasive, biologically-driven therapy for dental pulp regeneration that can replace current treatments like implants and root canals with more sustainable solutions.

Hypothesis

By better understanding how bFGF signaling and its regulation impact Schmidtea mediterranea stem cell behavior, we hypothesize that targeted modulation of this pathway, potentially enhanced by lithium chloride-induced Wnt signaling, can optimize stem cell-driven regeneration and offer a biologically-based alternative to current dental treatments.

Specific Aim 1: Investigate the effects of Canonical Wnt-signaling pathway modulation on *Schmidtea mediterranea* behavior using FGF-2.

Specific Aim 2: Evaluate the combined effects of bFGF and lithium chloride on regenerative outcomes in *Schmidtea mediterranea*.

Specific Aim 3: Develop a topical agent (bioactive gel) for promoting dental pulp regeneration based on findings from *Schmidtea mediterranea*.

This work is expected to reveal how bFGF and lithium chloride modulate hDPSC behavior to promote dental pulp regeneration. The findings will guide the development of a bioactive gel as a minimally invasive, sustainable treatment for pulp necrosis.

Section III: Project Goals and Methodology

Relevance/Significance

Dental pulp necrosis is a pervasive condition affecting billions of people worldwide. Current treatments, such as implants and root canals, increase the risk of other dangerous conditions like jawbone resorption. Thus, the regenerative potential of the Wnt signaling pathway as induced by FGF-2 in promoting dental pulp regeneration is a promising alternative to these existing solutions. This research aims to explore the role of FGF-2 in modulating dental pulp stem cells (DPSCs) and its potential for treating pulp necrosis by enabling tissue regeneration. This project is significant because it could lead to a minimally invasive, biologically-driven therapy that addresses a major gap in dental care.

Innovation

This project is innovative because it studies the combined use of FGF-2 and LiCl for enhancing dental pulp regeneration, an area of research that has not been fully explored. While FGF-2 is known to enhance osteo/odontoblast differentiation, it also inhibits mineralization, a major reason for its current lack of usage (Osathanon et al., 2011). However, LiCl has been shown to aid in mineralization, so the usage of the two together might prove to be beneficial in treating pulp necrosis. Developing a bioactive gel for topical application could promote pulp tissue regeneration without the need for invasive procedures such as implants or root canals, which could also be considered a novel approach in dental medicine. Additionally, the use of *Schmidtea mediterranea*, a non-traditional and highly regenerative model organism, helps apply the research not only in the context of dental regeneration, but also in fields such as cancer research, where regenerative mechanisms are similarly important.

Methodology

Specific Aim #1: Investigate the effects of Canonical Wnt-signaling pathway modulation on Schmidtea mediterranea behavior using FGF-2.

The objective is to investigate how FGF-2 influences stem cell behavior in Schmidtea mediterranea. Our approach will expose the Schmidtea mediterranea neoblasts (pluripotent stem cells) to varying concentrations of FGF-2. Then, we will monitor the ALP production, which will indicate cellular energy levels and proliferation. This method will help evaluate the impact of FGF-2 on stem cell proliferation and differentiation by assessing the blastema regeneration rate. This will be compared against the control group (figure 1 and 2) to draw conclusions regarding the effect of bFGF on blastema regeneration rate.

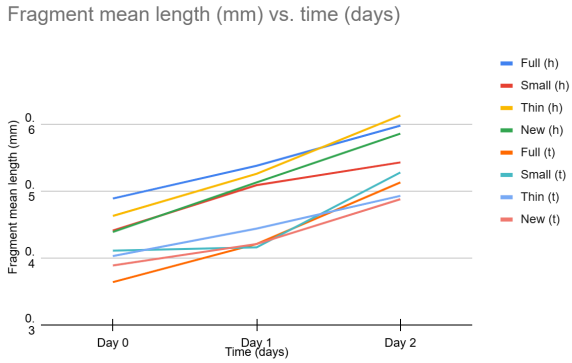

Figure 1. Mean length of planaria (in millimeters) vs. time (for three days). See appendix 1 for exact measurements.

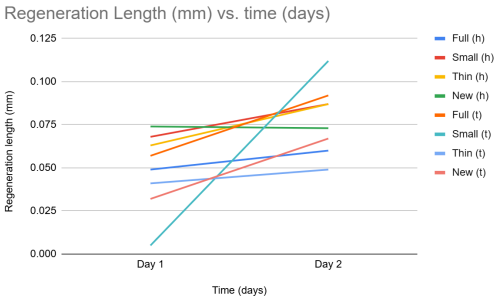

Figure 2. Regeneration length of planaria (in millimeters) vs. time (for three days). See appendix 2 for exact measurements.

Justification and Feasibility. It has been shown that FGF-2 aids in promoting the proliferation and differentiation of dental pulp stem cells (DPSCs) (Wang et al., 2024). It does so by increasing the mRNA and protein expressions of DSPP, DMP1, and BGLAP — osteo/odontogenic-related genes — which supports the feasibility of using this factor in Schmidtea mediterranea to investigate its effects on stem cell regeneration. An ALP staining assay is valuable in this context because it measures enzymatic activity linked to tissue differentiation.

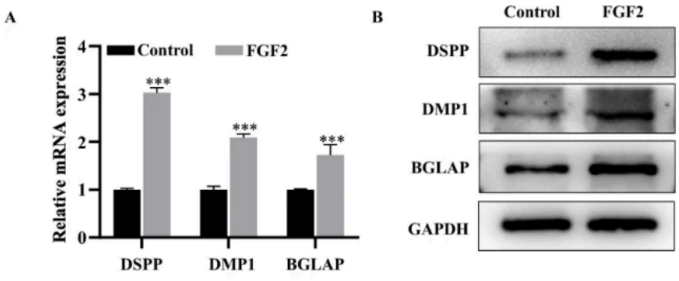

Figure 3. FGF2 increased mRNA and protein expression of DSPP, DMP1, and BGLAP.

This method has been used in previous studies to evaluate stem cell proliferation in response to various factors, such as in the Wang et al. (2024) study that explored the effects of FGF-2 on tissue regeneration.

Expected Outcomes. It is anticipated that FGF-2 treatment will lead to increased stem cell proliferation and differentiation into dentin-like cells in Schmidtea mediterranea. Preliminary data is expected to show elevated ALP activity, which would indicate enhanced cell viability and regenerative potential in treated planaria compared to controls. The expected outcome is to determine the concentration of FGF-2 that optimally promotes stem cell proliferation and differentiation into dentin-like cells in Schmidtea mediterranea, providing insights for dental pulp regeneration strategies.

Potential Pitfalls and Alternative Strategies. One expected pitfall is variability in stem cell response. If any such inconsistencies arise, FGF-2 concentrations will be adjusted to enhance regenerative outcomes.

Specific Aim #2: Evaluate the combined effects of FGF-2 and lithium chloride on stem cell-driven regeneration.

This aim will explore how the combination of FGF-2 and lithium chloride (LiCl) affects neoblast proliferation, differentiation, and tissue regeneration in Schmidtea mediterranea. While FGF-2 is useful for neuronal regeneration, it inhibits the mineralization of the tooth (Osathanon et al., 2011). Lithium Chloride, however, has been shown to increase tooth mineralization (Ali et al., 2019), as induced by canonical Wnt signaling, which may work synergistically with FGF-2 to improve regenerative outcomes. We will treat Schmidtea mediterranea neoblasts with varying concentrations of both FGF-2 and LiCl and assess regenerative responses through ALP assays.

Justification and Feasibility. Previous studies have demonstrated the regenerative potential of lithium chloride in promoting dentinogenesis through the activation of the Wnt pathway (Ali et al., 2019). LiCl has been shown to induce transdifferentiation of pulp cells into odontoblast-like cells, thereby promoting dentin repair and the formation of a dentin bridge. A study by Ishimoto et al. (2015) demonstrated that topical application of LiCl in a 10 mM concentration

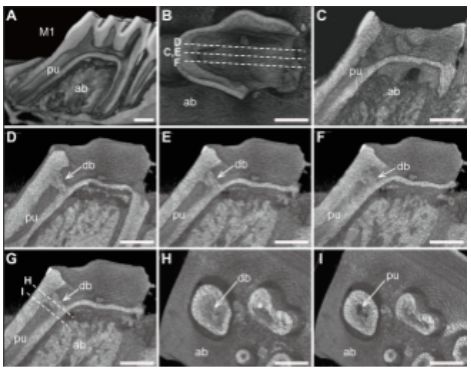

Figure 4. The microCT imaging results of dentin formation in LiCl-treated mouse teeth (Ishimoto et al, 2015).

significantly enhanced dentinogenesis in a mouse model of pulp injury, as seen in figure 4 (Ishimoto et al., 2015).

Expected Outcomes. We expect that the combination of FGF-2 and lithium chloride will significantly enhance stem cell proliferation and differentiation in Schmidtea mediterranea. Preliminary data should show an increase in cell viability and proliferation, measured by ALP activity, with a stronger regenerative response at the site of injury compared to controls. Specifically, we anticipate that lithium chloride will synergistically promote FGF-2-induced differentiation of neoblasts into dentin-like cells, as indicated by higher ALP activity. These results would suggest that the combined modulation of Wnt signaling via lithium chloride and FGF-2 can enhance tissue regeneration and stem cell differentiation, which could provide insights into potential therapeutic strategies for promoting dental pulp regeneration without inhibiting mineralization.

Potential Pitfalls and Alternative Strategies. We expect challenges in achieving consistent modulation of both FGF-2 and lithium chloride in Schmidtea mediterranea. If the combination of FGF-2 and lithium chloride does not yield desired regeneration results, we will test alternative concentrations.

Section IV: Resources/Equipment

All the research will be conducted in the laboratory at the Massachusetts Academy of Math and Science in Worcester, Massachusetts. This lab provides access to standard cell culture equipment, analytical tools, and experienced faculty mentorship, increasing the likelihood of project success. The laboratory provides strong institutional support for this research, as it has previously done with other students. The teacher overseeing this work has guided numerous students in successfully completing similar projects, further ensuring a supportive and experienced environment for achieving the proposed aims.

Section V: Ethical Considerations

This research involves the use of *Schmidtea mediterranea*, a model organism widely studied for its regenerative capabilities. Ethical considerations include ensuring humane treatment of the planaria during experiments, minimizing unnecessary harm, and adhering to established guidelines for their care and use. Efforts will be made to limit the number of organisms used while still achieving statistically significant results.

Section VI: Timeline

Timeline

Task	Duration	Time Frame
Set up and standardise cultures	<1 week	Week 1
ALP assays and FGF-2, LiCl exposure	2.5 weeks	Weeks 1 - 3
Data analysis and validation	<1 week	Week 4
Finalize Report	1 week	Week 4 - 5

Section VII: Appendix

	Full (h)	Small (h)	Thin (h)	New (h)	Full (t)	Small (t)	Thin (t)	New (t)
Day 0	0.489	0.441	0.463	0.439	0.364	0.411	0.403	0.389
Day 1	0.538	0.509	0.526	0.513	0.421	0.416	0.444	0.421
Day 2	0.598	0.543	0.613	0.586	0.513	0.528	0.493	0.488

Appendix 1. Table of recorded fragment mean length in millimeters vs. time in days for eight planarian fragments.

	Full (h)	Small (h)	Thin (h)	New (h)	Full (t)	Small (t)	Thin (t)	New (t)
Day 1	0.049	0.068	0.063	0.074	0.057	0.005	0.041	0.032
Day 2	0.06	0.087	0.087	0.073	0.092	0.112	0.049	0.067

Appendix 2. Table of recorded regeneration length vs. time in days for eight planarian fragments.

Section VIII: References

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