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

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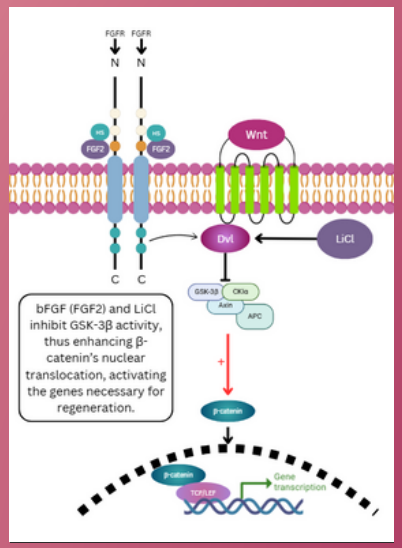

Background:

- **Dental pulp necrosis** affects billions worldwide
  - The pulp contains all the **nerves**, **blood vessels**, and **connective tissues** that keep the tooth alive (Morotomi et al., 2019)
- Current treatments like root canals and implants come with serious drawbacks, including **peri-implantitis** (Ting & Suzuki, 2024)
- Regeneration offers a better alternative by restoring the tooth's natural function rather than replacing it
- The **Wnt signaling pathway** plays a major role in stem cell growth and tissue repair (Angelova Volponi et al., 2018)
  - **Lithium chloride** (LiCl) and **basic fibroblast growth factors** (bFGF) activate Wnt signaling, promoting stem cell **proliferation** and **differentiation** (Ishimoto et al., 2015; ten Berge et al., 2008)
- **Schmidtea mediterranea** (planaria), a highly regenerative flatworm, was used as a model to study how Wnt pathway modulation influences regeneration
- The LiCl and bFGF combination and its effects on dental pulp regeneration were studied

Methodology:

The study used *S. mediterranea* as a model organism to study the effects of 0.4 mM lithium chloride (LiCl) and 10 ng/mL basic fibroblast growth factor (bFGF), prepared following standard dilution procedures (Farooq et al., 2021; Molano, 2024). A compound microscope and a Celestron digital imager were used to examine the regeneration rate. The Celestron S-Viewer Software was used to quantify the area of tissue regrowth over time, ensuring precise measurements. *S. mediterranea* specimens were then transected using a fine scalpel to induce regeneration, then monitored for 7 dpa.

Research Question:

How does the canonical Wnt-signaling pathway, as modulated by lithium chloride and basic fibroblast growth factors, influence the proliferation and differentiation of stem cells to enhance dental pulp regeneration?

Hypothesis:

It was hypothesized that the modulation of canonical Wnt-signaling can increase the rate of proliferation and odontoblast differentiation, particularly when induced by the combination of basic fibroblast growth factors (bFGF) and lithium chloride (LiCl).

Main Takeaway:

The combination of 0.4 mM lithium chloride and 10 ng/mL basic fibroblast growth factors increases the rate of regeneration in Schmidtea mediterranea, reducing the regeneration time by up to four days.

Results:

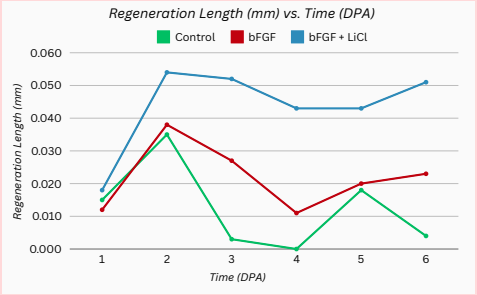

Figure 1. The average fragment length of *S. mediterranea* treated exogenously with basic fibroblast growth factors alone (red line) show a significant increase in the rate of regeneration compared to the average fragment length of the control group (green line). However, the average fragment length of the *S. mediterranea* treated exogenously with the combination of basic fibroblast growth factors and lithium chloride (blue line) show an even more significant increase in the rate of regeneration. This implies that the lithium chloride and basic fibroblast growth factors work synergistically to increase the blastema growth rate, thus inducing regeneration by modulating the Wnt signaling pathway.

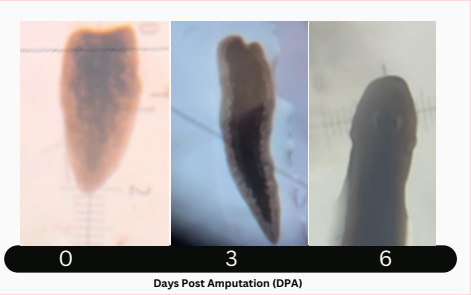

Figure 2. Schmidtea mediterranea treated with basic fibroblast growth factors under the microscope. The *S. mediterranea* were transected anterior to the pharynx using a fine scalpel to induce regeneration. The photo above shows the what one of the planaria looked like 0, 3, and 6 days post amputation (DPA). A compound microscope and a Celestron digital imager were used to examine the regeneration rate. The Celestron S-Viewer Software was used to quantify the area of tissue regrowth over time, ensuring precise measurements. Then, each photo underwent dimensional analysis to accurately measure the rate of regeneration for each worm.

Analysis:

This study demonstrated that the combined application of lithium chloride (LiCl) and basic fibroblast growth factor (bFGF) significantly enhanced regeneration in Schmidtea mediterranea by modulating the Wnt signaling pathway. The findings suggest that these factors work synergistically to accelerate regeneration, with planarians treated with both showing faster blastema growth than those treated with bFGF alone. Given the evolutionary conservation of Wnt signaling, this approach may also support the growth and development of human dental pulp stem cells (hDPSCs), potentially offering a solution for dental pulp necrosis.

A strength of the study was its use of Schmidtea mediterranea, a highly regenerative model organism, to investigate tissue repair mechanisms. However, while there is strong evidence that Wnt pathway modulation plays a role, we have not directly measured Wnt activity in our samples. Future studies should include direct assays of Wnt pathway activation, such as β-catenin localization or expression analysis of downstream targets.

Conlcusion:

The study investigated whether combining lithium chloride (LiCl) and basic fibroblast growth factor (bFGF) could enhance regeneration in Schmidtea mediterranea by modulating the Wnt signaling pathway. Comparing regeneration rates in planarians treated with LiCl alone versus both LiCl and bFGF, it was found that there was a synergistic effect that accelerated regeneration. Given the evolutionary conservation of Wnt signaling, this approach may have broader applications in regenerative medicine, particularly in promoting human dental pulp stem cell (hDPSC) proliferation and differentiation. If effective in human cells, this strategy could advance tissue repair methods in clinical settings, particularly in dental and regenerative medicine.

Future Work:

- Experimenting on human dental pulp cells in vitro to study the effects of bFGF and LiCl
- Adjusting concentrations for maximum proliferation and differentiation
- Developing a bioactive gel for topical application to teeth after pulp extirpation

Citations

* not included in this document - flip-page effect, scroll down to view

Citations:

Angelova Volponi, A., Zaugg, L.K., Neves, V., Liu, Y., & Sharpe, P. T. (2018). Tooth Repair and Regeneration. *Current Oral Health Reports, 5*, 295–303. https://doi.org/10.1007/s40496-018-0196-9

Farooq, M., Khan, A. W., Kim, M. S., & Choi, S. (2021). The role of fibroblast growth factor (FGF) signaling in tissue repair and regeneration. *Cells, 10*(11), 3242. https://doi.org/10.3390/cells10113242

Ishimoto, K., Hayano, S., Yanagita, T., Kurosaka, H., Kawanabe, N., Itoh, S., Ono, M., Kuboki, T., Kamioka, H., & Yamashiro, T. (2015). Topical application of lithium chloride on the pulp induces dentin regeneration. *PLOS ONE, 10*(3). https://doi.org/10.1371/journal.pone.0121938

Citations:

Molano, A. (2024). Developmental Effects of Lithium and Zinc in Planaria. https://doi.org/10.1101/2024.08.30.610554

Morotomi, T., Washio, A., & Kitamura, C. (2019). Current and future options for dental pulp therapy. *Japanese Dental Science Review, 55*(1), 5–11. https://doi.org/10.1016/j.jdsr.2018.09.001

ten Berge, D., Brugmann, S. A., Helms, J. A., & Nusse, R. (2008). Wnt and FGF signals interact to coordinate growth with cell fate specification during limb development. *Development*, *135*(19), 3247–3257. https://doi.org/10.1242/dev.023176

Ting, M., & Suzuki, J. B. (2024). Peri-implantitis. *Dentistry Journal*, *12*(8), 251. https://doi.org/10.3390/dj12080251