Investigating the Role of Canonical Wnt Signalling in Dental Regeneration as Aided by FGF-2 (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 a variety of 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. However, the potential for inducing regeneration in these tissues through molecular signaling pathways has led to promising research in regenerative medicine, particularly cellular regeneration as caused by stem cells. Stem cells are undifferentiated cells with the potential to develop into various cell types, and they are significant 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 proven to be useful in dental research, particularly in efforts to restore dental pulp tissue lost to pulp necrosis. 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). Pulp necrosis is the process in which the cells that make up the pulp begin to die. 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). Pulp necrosis is a serious condition, affecting approximately 2.44 billion people worldwide (“Global Oral Health Status Report towards Universal Health Coverage for Oral Health by 2030,” 2022). Pulp necrosis can also eventually lead to periodontitis, or the infection of gums, which can affect other teeth around the dead tooth (NIH, 2024). Current treatments for pulp necrosis include dental implants, but those 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 a periodontal ligament, which normally helps protect against the mastication forces of chewing. Without it, the risk of jawbone resorption increases, making implants

unsustainable for long-term oral health. Thus, researchers started to look into cellular regeneration mediated by the Wnt signaling pathway as a viable solution to reversing the effects of pulp necrosis. 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).

However, critical knowledge gaps remain regarding how Wnt pathway modulation governs stem cell fate, particularly the balance between odontoblast differentiation and maintaining a stem-like phenotype. The Wnt signalling 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. In the following research, the activation or inhibition of the Wnt Signalling 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 was investigated. 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 was 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, was 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, were tested to measure the rate of regeneration of the Wnt signalling pathway with and without the activator (Ishimoto et al., 2015). The combination of lithium chloride with bFGF was tested to determine whether it could enhance the rate and quality of tissue regeneration. Then, the two were 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.

Problem Statement/Researchable Question/Mathematical Conjecture

How does the canonical Wnt-signalling 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

Hyp. 1a: It is hypothesized that the modulation of canonical Wnt-signalling can increase the rate of proliferation and odontoblast differentiation.

Hyp. 2a: The canonical Wnt-signalling pathway can be modulated using basic fibroblast growth factors (bFGF) and lithium chloride (LiCl).

Hyp. 2b: It is hypothesized that the combination of bFGF and LiCl will increase the rate of regeneration in the *Schmidtea mediterranea* model as induced by the Wnt-signalling pathway.