# Development biology lab grant proposal biology essay

Science, Biology

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## Research Proposal

The primary intention of this research project is to investigate the process of heart tissue regeneration in the zebrafish. It has been demonstrated zebrafish display a remarkable capability to regenerate tissue in a variety of organs. The zebrafish can revitalize cellular tissues in the fin, the spinal cord, and the retina. However, the processes the zebrafish go through in order to accomplish this regenerative feat are not completely understood. The aim of the investigation offered in this experimental proposal is to scrutinize some of those factors which occur when the zebrafish restores cardiac tissue. The hope is the information gleaned through this investigation will promote new knowledge which may be applied to the treatment and rehabilitation of damaged cardiac tissues in human beings. By adding to the scientific knowledge base scientists and applied medical specialists may be able to conduct new investigations into tissue regeneration which can lead to medical applications which promote the repair of human cardiac tissue when

it becomes damaged or in danger of being destroyed by pathological processes.

## Background

Cardio-vascular pathology and heart disease are major causes of severe health problems in human beings around the world and a leading cause of human mortality. Unlike the more simplistic zebrafish, complex human beings do not have the capacity to regenerate cardiac tissue after it has been compromised or destroyed by disease or trauma. All mammals have the restorative ability to regenerate skin cells, except under select traumatic conditions such as severe burns, and some mammals also exhibit the capacity to regenerate liver tissues. Mammals, including human beings, do not display the ability to rejuvenate cardiomyocytes. However previous investigators have demonstrated it is possible for cardiomyocytes to be stimulated to grow through the use of Fibroblast growth factor-1 (Fgf1) and P38 Mitogen Activated Protein (MAP) kinase in culture. Mammal hearts are composed of four chambers. After a mammal experiences a myocardial infarction fibrin deposition occurs at the site of injury. This fibrin is then replaced by scar tissue which remains as a permanent part of the damage sustained. The zebrafish is a teleost fish with a two-chamber heart including one atrium and one ventricle. In zebrafish a fibrin clot forms at the injury site and is then subsequently replaced with new cardiac muscle. The replacement of damaged tissue by healthy tissue in the zebrafish takes approximately 1 to 2 months. This phenomenon can be observed over time. However, the reason why and how this transformational process occurs

essentially baffles investigators. By the time tissue regeneration becomes completed there are no recognizable differences between the newly regenerated cardiac tissues from those tissues which formerly made up the heart muscle. Along with this cardiomyocyte proliferation in the zebrafish, injury to the myocardial tissue is believed to activate the surrounding epicardium. The epicardium is the outer non-muscle layer of the heart. It is speculated the role of the epicardium in zebrafish cardiac regeneration is to contribute endothelial and smooth muscle tissue to the coronary vasculature. This process is assisted by two genes known to be expressed within the embryonic epicardium. These genes are found to be present at the wound site, indicating an organ-wide response to the cardiac injury. These molecular responses occurring after injury are of great interest and could shed light on how the process of heart tissue regeneration occurs in the zebrafish. When the processes of gene expression and proliferation are complete within the epicardial layer, these newly generated cells surround the wound and penetrate several layers into the wound and regenerate the muscle. These epicardial cells seem to play similar roles in the embryonic heart when the heart muscle is developing inside the developing fetus.

## Previous Research

Prior research has demonstrated Fgf receptors 2 and 4 are expressed near the site of cardiac injury in the zebrafish. Signaling by these Fgfs appears to be necessary for epicardial cell activity during the regeneration process in the zebrafish. Previous inquiries using rodent hearts established Fgf supplementation along with the P38 MAP kinase did stimulate

neovascularization and decreased the size of the myocardial infarction and the size of scarring. Researchers have indicated mammalian hearts are responsive to Fgfs after myocardial infarction. This intriguing observation provides incentive for creative research investigation into the multiple factors which result from the effects of Fgfs. Additional variables involved in the response to Fgfs are worth exploring in order to increase our knowledge of the effects Fgfs have on the repair of heart tissue after it has been damaged. Fgf signaling has been shown to decrease the size of injury in rodent hearts and is thought to be a key factor in the regeneration process in zebrafish. Amphibians also have shown the ability to regenerate tissue. Although amphibians can regenerate tissue in the spinal cord, brain, limbs, and retina, they only show modest levels of tissue replacement accompanied by scarring. Research has shown that at least partial regeneration of the newt heart is possible following a mechanical injury.

## Experimental Design

Impaired mural cell coverage in endothelial-specific Tβ4-knockdown vasculature is investigated utilizing a mouse model. Mutant Tie2-Cre–Tβ4shRNA embryos at E10. 5, revealed a deficit in NG2-positive mural cells in comparison to control littermates expressing Cre alone in a recent case. Significant knockdown of Tβ4 was confirmed by qRT-PCR on dissected aortas from E10. 5 control and mutant embryos in the same study. The defect in mural cell coverage in the knockdown vasculature was both quantifiable and highly significant. Immunostaining for VE-cadherin confirmed the

endothelium was intact in the mutant dorsal aorta after endothelial-specific knockdown of Tβ4.

## Expected Result

This study should reveal Tβ4 is required for systemic vascular development. Additionally, this study should demonstrate an endothelial source of Tβ4 functions is required to maintain adequate recruitment and differentiation of mural cells/pericytes. Positive experimental findings will encourage the future investigation of what is required to maintain the stability of the developing aorta, cranial, and trunk vessels. Tβ4 functions with the TGFβ pathway to regulate mural cell development and vascular wall stability. Thus, the implication and application of modern techniques could provide the basis for further research utilizing a different model, notably the zebrafish. Zebrafish exhibit a vigorous regenerative capacity in a variety of tissues including the fin, spinal cord, retina, and heart, making it the sole regenerative vertebrate organism currently amenable to genetic manipulation. Genetic research investigations using the zebrafish have revealed a large number of mutants affecting embryonic development. Most of these mutants exhibit embryonic or larval lethality, making it impossible to assess roles for specific genes in adult tissue regeneration, unless such mutations have effects in heterozygous animals. Investigators have circumvented this issue in a small number of studies by searching for conditional (temperature-sensitive) or hypomorphic alleles of genes that may be important for regeneration. Unfortunately, the idea of a direct screen for heart regeneration mutants comes with many challenges. Transgenic fish

allowing inducible ectopic expression of a wild-type gene or a dominant-negative construct have been used recently and give similar benefits as conditional mutants. Future studies are also likely to take advantage of lineage tracing tools that have been utilized in mice for progenitor cell studies, to define progenitor/progeny relationships during heart regeneration.

## Materials and supplies

$4, 995. 00 - Fully stocked RT-PCR.$300. 00 - Chemical reagent kit.$2, 000. 00 - Mice and upkeep.$427. 14 - Mini centrifuge.$300. 00 - General Lab equipment: pipettes, pipette tips, microcentrifuge tubes.