BIODOME PROJECT

MOUSE TAIL REGENERATION WITH BIODOME

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Lastly, I am grateful to all of those who supported me in any respect during completion of this project

GLOSSARY

A

Anode - an electrode in which the electric current flows into a polarized electric device. Typically positive.

Apical Epithelial Cap – a layer of signal cells that induce development of epimorphic regeneration.

B

Blastema – a mass of undifferentiated cells capable of growth and regeneration**.**

C

Cathode – an electrode in which the electric current flows out of a polarized electric device. Typically negative.

Current (electrical) - electrical current is the flow of electric charge; denoted in amperes [A]

D

Dedifferentiation – a cellular process in which a partially or terminally differentiated cell reverts to an earlier developmental stage.

Differentiation – a process by which a less specialized cell type becomes a more specialized cell type.

Dragonskin® - a platinum-cured silicone elastomer used in molds.

E

Elastin – a protein in the connective tissue that allows for many tissues in the body to resume their original shape after deformation

Epithelium- layer of tissues composed of cells that line the cavities and surface of structures

through out the body

F

FGF-18- Part of FGF family; it regulates proliferation and differentiation of midline cerebellar structure

I

Inflammation (wound healing) – First phase in wound healing; it is a biological response of vascularized tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammatory phase involves clotting cascade, vasoconstriction, and migration of neutrophils and macrophages.

O

Osteoblast- mononucleate cell responsible for bone formation

Osteoclast- multinucleate cell that resorbs bone tissue by mineralized matrix and breaking up the bone

P

Proliferation (wound healing) – the second phase in wound healing that involve angiogenesis, fibroplasia, epitheliazation and contraction.

R

Regeneration- Healing process in which complex, three dimensional organic structures can be completely restored to its original shape and function by processes such as cell renewal, cell dedifferentiation, or stem cell differentiation

W

Wound healing- an intricate process in which the orga repairs itself after injury. It separates into three main phases: (1) inflammation, (2) proliferative, and (3) maturation.

ABSTRACT

Every organism in nature regenerates to certain extent, yet the process of regeneration is still not fully understood. While prosthetics are able to provide a limited solution for limb amputations, they are not able to fully restore the lost function and structure of the original limb. Regeneration requires complex orchestration of cell migration, proliferation, and outgrowth, controlled by the bioelectrical and biochemical pathways. The Biodome project aims to induce regenerative pathways in an amputated murine specie tail by altering membrane potential and providing external electrical stimulations. Biodome is an apparatus designed to deliver chemical solutions, while enabling electrical stimulations. By altering the bioelectrical signals at the wound, the Biodome project was able to produce preliminary regenerative responses by preventing formation of stratum corneum and promoting bone remodeling.

INTRODUCTION

Traditional treatments for limb loss involve implantation of prosthetics. However, they are susceptible to material degradation, immune system rejection, and inflammation (Zhang, 2009). Furthermore, prosthetics cannot fully replace the original function of the limb. Therefore regenerative medicine holds much promise to replace the prosthetics with fully functioning limbs.

The biggest difference between regenerating organisms and non-regenerating organism is the presence of injury current at the site of the wound. Regenerating organisms maintain a steady current density over the period of healing and beyond. However, the injury current in a non-regenerating organisms diminishes slowly as the limb heals (Borgen, 1979). Previous researches have been able to artificially replace the injury current with an electrical current, inducing limited regenerative responses in non-regenerating organisms.

Recently, studies have shown that bioelectrical signals play a crucial role in regeneration. Bioelectric signals are responsible for cell migration, positioning, proliferation and differentiation. More specifically, the depolarization of cell membrane by sodium ion gradient promotes the process of regeneration. Several studies have shown that long term depolarization of membrane potential can manipulate adult somatic cells to re-enter the cell cycle, a crucial process in regeneration (Levin, 2009).

Recent findings have increased our understanding of bioelectricity and the regeneration process. As a result, increasing number of researches is conducted to induce regeneration in non-regenerating organism by manipulating bioelectric signals. If we can fully understand the regenerative

process, these understandings will lead to significant advances in regenerative medicine. The objective of this project is to stimulate the regeneration at amputated murine tail by chemical depolarization and artificial replacement of injury currents. We hope that by inducing regenerative pathways in primitive mammals such as mice, we will be able to induce limited regenerative responses on more complex mammals such as humans in the future.

BACKGROUND/SIGNIFICANCE

There are 1.7 million amputees living in the United States. While prosthetics are able to provide limited functions, the amputees never recover the original structures and function of the lost limb. In addition, prosthetics are susceptible to material degradation, immune system rejection, and inflammation (Zhang, 2009). However, if a patient is able to regenerate the severed limb, the original structure and the function can be fully restored. The process of regeneration is still not fully understood, yet it holds much promise for treating limb amputations in the future.

All organisms are able to regenerate to certain extent. Studies suggest that regenerative ability declines with increased creature complexity (Alvarado, 2000). For example, simple amphibians are known to regenerate, yet the regenerative ability of complex mammals is non-existent.

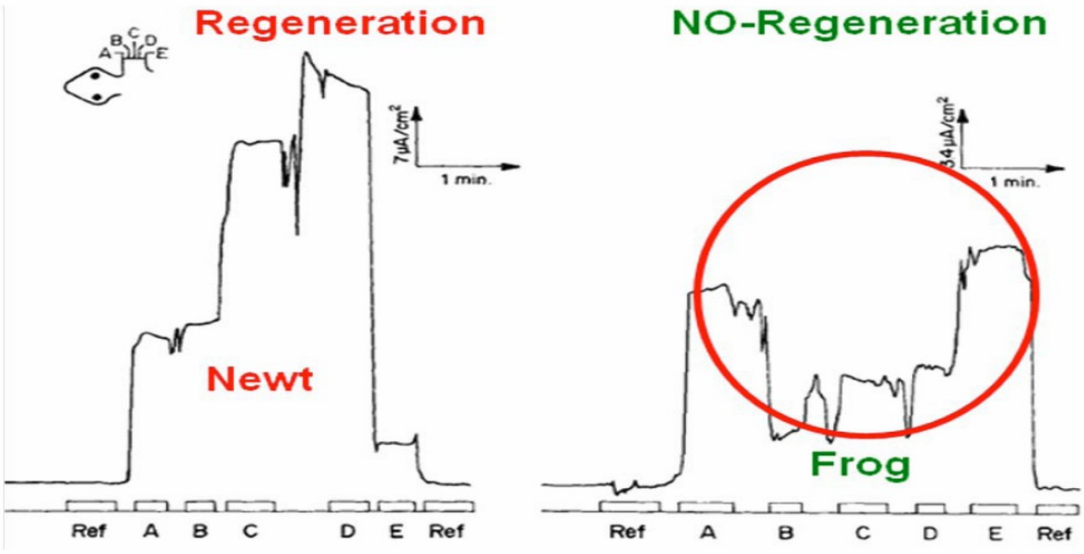
Organisms respond to a traumatic injury in two ways: wound repair or regeneration. The main purpose of the wound healing is to close the wound quickly to minimize blood loss, infection susceptibility, and energy expenditure. Even though this process closes and heals the wound, it fails to restore the original structures and functions. In contrast, the regeneration process is able to restore the original structure and function through cell renewal and differentiation (Clark, 1998).

Regeneration and Bioelectricity

The biggest difference between the regenerative pathway and the wound healing process is the

presence of injury current at the wound site. Injury current is a steady DC current produced by ion gradient between extracellular and intracellular spaces (McCaig, 2005). It flows out and around the injured limb. In a study by Borgens in 1979, he demonstrated that while regenerating animals maintain a steady current density over the period of healing and beyond, the current density in non-regenerating organisms diminishes slowly as the limb heals. Borgen measured the current densities from the stump surface of a newt, regenerating organism, and a frog, non-regenerating organism. The newt stump showed current densities of approximately 20-40 μamp/cm^2 while the frog stump only showed corresponding density of 7amp/cm^2. He suggested that the lower current density of the frog stump is directly related to its inability to regenerate (Borgen, 1979). A recent study by Levine further supports this idea, explaining that bioelectrical signals carry specific morphogenetic information used to position, pattern, differentiate, and proliferate cells.

Since the discovery of the injury current, many studies have been aimed to find its source. While all organisms carry bioelectricity, researchers were curious to know how the steady injury current formed. Recent studies have found that ion transporters, more specifically sodium channels, are responsible for producing the injury current. Ion transporters produce ion current and generate voltage gradient across the cell membrane. This leads to alteration of cellular pH, voltage gradient, and ion fluxes. This coordinated ion transport provide cues for migration and positioning of the cells (Levine, 2007).



Bioelectricity controls three critical components of regeneration: cell migration, positioning and proliferation. Interestingly, the electric guidance is also found during embryonic development. In embryos, patterns of voltage gradients form coordinates guiding cell movement during complex morphogenetic processes. Also known as galvanotaxis, cells utilize field lines and voltage gradients as migratory cues, moving towards the anode or cathode, depending on the cell type. During regeneration, the injury current produced at the wound site is able to guide migratory cells to the right position (Levine, 2009).

In addition to cell positioning, regeneration requires differentiation of cells at the site of the wound. Previous studies have shown that extracellular ion concentrations are directly related to the process of differentiation (Barth, 1974). Barth has demonstrated that ventral ectoderm explants can be differentiated into different cell types by manipulating the extracellular ion concentration (Levine, 2007). Recently, studies have shown that membrane voltage controls the differentiation of human mesenchymal stem cells. More importantly, depolarization promotes de-differentiation of cells. Studies have shown that even mature neurons can be manipulated to re-enter the cell cycle by long term depolarization. This raises possibility that depolarization of the membrane potential can produce stem

cell-like plasticity even in terminally differentiated adult somatic cells (Levin, 2009).

Another important aspect of regeneration is the proliferation of cells at the wound site. While some cells migrate to the wound, cells at the wound must proliferate in order to produce outgrowth of a limb. Bioelectric signals also controls mitosis. The mitotically active cells tend to be more depolarized than terminally differentiated somatic cells. Studies have shown that cell proliferation is controlled by the membrane potential, and depolarized cells are more mitotically active (Levine, 2009).

Recent findings have shown that sodium gradient is responsible for producing injury currents. Increased sodium concentration is an indicator of proliferative activity, and many growth factors are aimed at increasing sodium entry into cells in order to upregulate proliferation. Regenerating organisms are able to pump sodium ions inward producing an ionic gradient that leads to injury current. So in order to induce regeneration, activation of the sodium channels is critical (Meiri, 1981).

In this study, we looked to apply electrical stimulations and chemical depolarization concurrently. We believe that by combining both artificial stimuli, more extensive regeneration responses could be induced.

Inducing Regeneration

As stated above, non-regenerative organisms lack bioelectrical signal at the wound site. Several studies have been performed to provide artificial electrical currents at the wound site. Most notably, Borgen have been able to induce extensive nerve growth by applying a minute current to the forelimb stump of an adult frog. When adult *Xenopus* and *Rana* forelimbs have been treated with 0.1mA DC current, bifurcated structures have formed. The produced stump contained nerve trunks with thin cartilage cone and mature epidermal papillae (Levine, 2007).

Same methods have been applied to non-regenerating organisms such as mammals. When

weak DC current have been applied to the amputated rat hind paw, nerve cell regeneration have been achieved. While the nerve cell formations have not been as extensive as with the frogs, limited regenerative responses have been achieved.

Depolarization is also critical to regeneration. Studies by Levine showed that long term depolarization induced stem cell plasticity in adult stem cells. Since cell proliferation and differentiations are critical to regenerations, depolarization could aid this process.

HYPOTHESIS AND AIMS

The overall objective of the Biodome project is to induce regenerative responses on amputated murine tail through artificial electrical and biochemical stimulations. Biodome project combines both electrical and biochemical alterations. The missing injury current is replaced by artificial electrical stimulations. Also by keeping the wound site depolarized, we are hoping to hyper-activate mitotically active cells at the wound site. Lastly, monensin is added to the solution to activate sodium channel, in order to mimic the natural biochemical state of the naturally regenerating organism.

Through out this process, a new Biodome has to be designed to decrease necrosis, while providing protection to the wound.

We hypothesize that electrical stimulation along with depolarizing solution and monensin can produce appropriate signaling for amputated mouse tail regrowth.

EXPERIEMENTAL DESIGN/ METHODS

Project Background

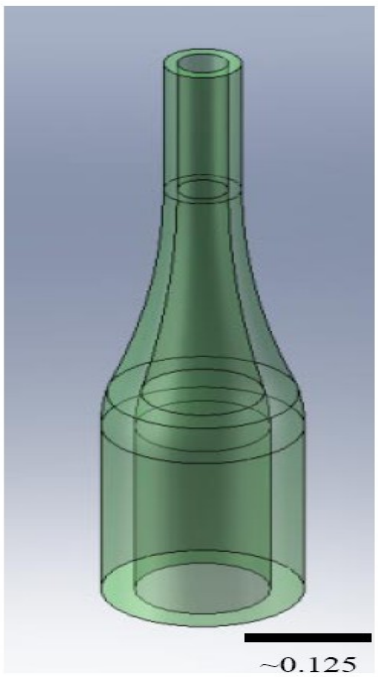
Many studies were successful at replacing the injury current by electric current or electric field. As many of these studies produced promising results, the specific parameters of the electrical stimulation were tested to produce the most optimal results. In this 1987 study, McDevitt *et al.* have varied the amounts of current delivered to an amputated rat limb. In nature, minute amount of current

is delivered and it has been known to vary constantly. They have found that 10μA of direct current for 30 minutes per day expressed unparalleled nerve regeneration compared to control animal and those receiving higher or lower amounts of currents. Also he has demonstrated that intermittent stimulation improved the nerve regeneration compared to continuous stimulation (McDevitt, 1987). Other studies by Becker have supported these results. He also concluded that prolonged electrical stimulation over 30 minutes could have deleterious effects to the wound.

In order to activate regenerative pathways, the wound site must contain mitotically active cells, and must be free of signals that could cue wound healing process. Factors such as dry external environmental and bacterial infection could inhibit the regenerative process and lead to the wound healing pathway. The depolarizing solution can provide the cues necessary to promote cell proliferation while keeping the wound site moist and antibacterial (Zhang, 2009).

The depolarizing solution was prepared by Dr. Levin of Tufts University. It contains higher concentration of sodium [Na^+] (150mM) and potassium [K^+] (170nM) than the normal extracellular ion concentrations. In addition, the solution contains lower concentration of chloride ions [Cl^-] (50nM) to further promote depolarization. Veratridine was also added to the solution. Veratridine has been known to open sodium ion channels by preferentially binding to Na. Lastly, insulin and serotonin were also added to increase sodium conductance and prevent excess bleeding (Zhang, 2009).

Another method to induce depolarization is through monensin. As an Na^+/H^+ antiporter, monensin is able to transport Na^+ across the lipid membrane. Addition of monensin causes rapid influx of Na, producing a transient increase intracellular pH. Increase in intracellular Na stimulates Na, K ATPase, which increases electrogenic pump activity. The influx of sodium leads to 20~30mV increase in electrical potential across the plasma membrane. This potential produced across the membrane could be used as cues for cell proliferation, migration and positioning (Lichtshtein, 1979). Along with these



properties, monensin also works as an antibiotic.

Biodome Project

Biodome Project is a continuation of the Biodome Digit Project (Zhang,2009). The Biodome was originally design to investigate the effects of electrical stimulation and different growth cocktails in 2009. The primary purpose of the current Biodome Project is to promote regenerative pathways by applying electrical and biochemical stimulations concurrently.

Biodome is an apparatus that provides specific stimuli to enclosed tissue in vivo systems. It provides a protected, sealed, and humid environment to promote regeneration. It can be connected to an electrical circuit to provide specific electrical stimuli. Biodome is an apparatus specifically designed for this study. It is able to protect the wound site and keep the depolarizing solution in contact. It isolates the amputation site from the external environments, preventing infection. By maintaining the depolarization solution in contact with the amputation site, the cells at the wound site can be maintained depolarized. Figure 2 provides a schematic diagram of a Biodome. The Biodome in Figure 2 has been specifically designed for a mouse tail regeneration research.

Please note: the Biodome Project was approved by the IACUC for all animal handling and surgical procedures.

Specific Project Goals

Specific Aim #1: Finding the optimal Biodome design to minimize necrosis.

Previous studies with Biodome have faced issues with severe necrosis of the tail. This was due

to the limitations of the previous Biodome designs. Previous Biodome molds had several issues with formation of air bubbles, leading to holes in Biodome. In order to overcome this shortcoming less flexible and breathable Dragon Skin 20 was used. While this addressed the problem, inflexibility of the Dragon Skin 20 applied too much pressure on the tail. We believe that the severe tightness and glue led to necrosis. In the first part of the study, we look to modify the mold, Biodome and the protective sleeve design in order to minimize necrosis

Study #1: Optimizing Biodome design

Experimental Design

In order to improve upon the Biodome design, a new mold was designed using computer aided design software. After new mold was printed using a 3D printer, the new mold was filled with Dragon Skin10. The new Biodomes were then tested for 14 days on the amputated tail. Vetbond instead of Urobond- IV was used in order to adhere Biodome to the tail. Previous studies used Urobond-IV. After 14 days, the site of attachment was inspected for signs of necrosis.

Specific procedures: Redesigning Biodome

Several mold designs were tested. The original design was kept as a control. A new mold design increased the diameter of the neck and the reservoir of the Biodome. Instead of open and shut mold design, the new mold design utilized injection molding.

The protective sleeves were also redesigned. In order to reduce the stress on the tail, shorter and wider protective sleeves were designed. Also the protective sleeves were designed with clip on systems in order to easily attach and remove them.

Pre-amputation

Prior to surgical procedures, the surgical workplace is disinfected with sodium hypochlorite

solution. Isoflorane was used as the method of anesthesia. Mice were sedated prior to amputation and installation of Biodome.

Amputation

After mice are sedated, the length of the tail is measured from tip of the tail to the base of the tail. The 1/4 length of the tail is marked from the end and is cleaned with 70% ethanol to prevent skin infections. The tail is amputated at the marking using a sterilized razor blade. The amputated tail tip is properly discarded

Biodome installation

Depending on the length of the tail, Biodome is selected and trimmed to proper length. The Biodome was not installed until 24 hours after amputation. After 24 hours, the amputated tail is inserted into the bottleneck cuff of Biodome, until apical tip is halfway in the Biodome reservoir. After placing the Biodome, Vetbond is injected with polypropylene dispensing needle, between tail surface and Biodome cuff. To ensure through adhesion between the tail and Biodome, pressure is applied over the area where the adhesive is injected. Once Biodome is held tightly in place, the depolarizing fluid is inserted using a pair of hypodermic needle. One syringe delivers the fluid, while the other syringe removed the air enclosed in Biodome. The Biodome was maintained for 14 days after installation.

Changing Depolarizing solution

Everyday, the depolarizing solution in the Biodome is replaced with fresh solution. This is repeated until the Biodome is removed.

Inspecting the wound site

After the 14 days period, the Biodome is removed and the wound site is inspected. First the site is inspected visually for any signs of necrosis. The wound site is amputated and fixed with

formaldehyde. It is sent for histology.

Euthanasia

Carbon Dioxide asphyxiation is performed on mice as the preferred method for euthanasia. Death is verified by observing animal's lack of breathing and palpitations before they are disposed. Cervical dislocation is also performed after CO_2 inhalation to verify death.

Control

As a control, a mouse tail will be amputated without the Biodome attached. After 14 days, it will be fixed with formaldehyde and be sent to histology.

Specific Aim #2: Induction of regeneration by chemical and electrical stimulations.

The most significant factor of regeneration is the electrical current present at the site of the amputation. There are two ways to replace this injury current, through direct replacement of the electrical current or chemically by altering the sodium gradients across the membrane. The objective is to induce regenerative pathway at the site of amputated mouse tail. Our central hypothesis is that electrical stimulation will produce extensive nerve cell formations.

Study #1 Depolarization Solutions with Monensin

Experimental Design

Our experiment applied electric current to the site of the amputation along with the depolarization solution. In order to apply electrical current to the amputated mouse tail, Biodome is used to cover and protect the site of the wound. The depolarizing solution and monensin were added to the Biodome, and in contact with the wound to promote depolarization of the wound site. The current of 10μA is applied for 30 minutes daily for 14 days. After specified days, mice are euthanized following proper animal handing procedures and the site of amputation is analyzed with histology.

Specific methods

Pre-amputation

Prior to surgical procedures, the surgical workplace is disinfected with sodium hypochlorite solution. Isoflorane was used as the method of anesthesia. Mice were sedated prior to amputation and installation of Biodome.

Amputation

After mice are sedated, the length of the tail is measured from tip of the tail to the base of the tail. The 1/4 length of the tail is marked from the end and is cleaned with 70% ethanol to prevent skin infections. The tail is amputated at the marking using a sterilized razor blade. The amputated tail tip is properly discarded

Biodome installation

Depending on the length of the tail, Biodome is selected and trimmed to proper length. Immediately after amputation, the amputated tail is inserted into the bottleneck cuff of Biodome, until apical tip is halfway in the Biodome reservoir. After placing the Biodome, VetBond is injected with polypropylene dispensing needle, between tail surface and Biodome cuff. To ensure through adhesion between the tail and Biodome, pressure is applied over the area where the adhesive is injected. Once Biodome is held tightly in place, the depolarizing solution is inserted using a pair of hypodermic needle. One syringe delivers the fluid, while the other syringe allows air enclosed in Biodome to escape.

Electrical stimulation

A custom designed constant current source delivers the electrical stimulation. A steel cathode is carefully inserted into the Biodome reservoir using a syringe. Anode acupuncture need is placed intramuscularly near the rear haunch of the mouse. Once proper current flow is determined with

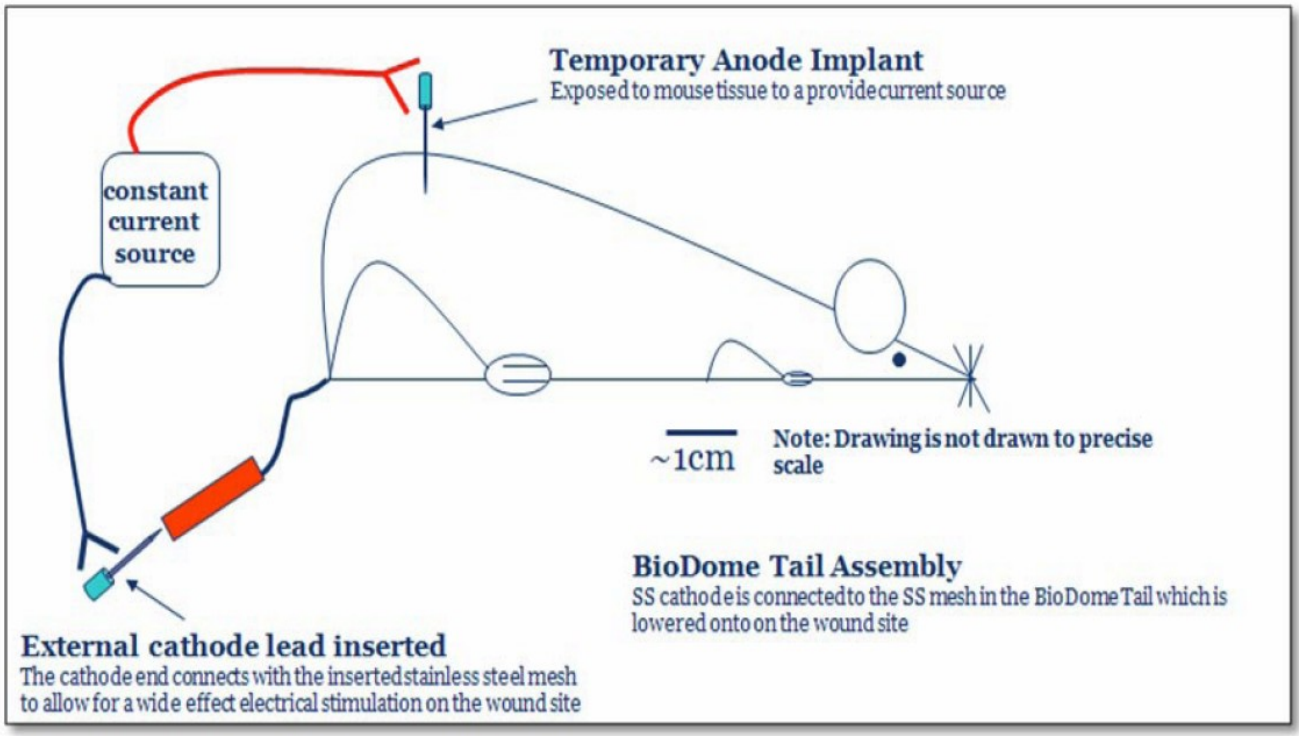
potentiometer, the power source is connected to the electrodes. 10μA of electrical stimulation is applied for 30 minutes for 14 days. Figure 3, provides a schematic drawing of the step up.

Animal Recovery

The mouse is placed on the heating pad until animal shows normal vital signs. Once the mouse is able to move autonomously, the animal is placed in individual cages and given adequate nutrition for duration of the experiment.

Euthanasia

Carbon Dioxide asphyxiation is performed on mice as the preferred method for euthanasia. Death is verified by observing animal's lack of breathing and palpitations before they are disposed. Cervical dislocation is also performed after CO_2 inhalation to verify death.



Histology

The wound site is amputated and fixed with formaldehyde. It is sent for histology..

Control Groups

There will be three groups in this experiment. First group will received Biodome implantation over the amputated tail. The depolarizing fluid, monensin(500µM) and electrical stimulation will be administered. Second group will receive Biodome with depolarizing fluid and electrical stimulations. Third group will received Biodome with HBSS with electrical stimulations. Last group will just have an amputated tail without Biodome or any treatments and served as control.

Study #2 Depolarization Solutions with varying concentrations of Monensin

Experimental Design

In this study, we looked at how varying amount of monensin effect the regenerative processes. Varying concentrations of monensin (100µM, 150µM, 500µM) were added to the depolarizing

solution. The electrical stimulation was provided through the attachment of the Biodome.

After 14 days, mice were euthanized following proper animal handing procedures and the site of amputation is analyzed with histology.

Specific Methods.

All surgical procedure were same as Study1.

Control Groups

There will be three groups in this experiment. First group will received Biodome implantation over the amputated tail. First group was administered the depolarizing fluid with monensin(100μM) and electrical stimulation. Second group was administered the depolarizing solution with monensin (150 μM) and electrical stimulation. Third group was administered the depolarizing solution with monensin (500 μM) and electrical stimulation. Fourth group was administered the HBSS without electrical stimulations. Last group had an amputated tail without Biodome or any treatments and served as a control.

RESULTS

Study #1: Optimizing Biodome design

In order to improve upon the previous Biodome design, an entirely new mold had to be designed. The process of pouring Dragon Skin into the mold produced air bubbles. While this could be overcome with use of Dragon Skin 20, the less flexible and breathable Dragon Skin 20 applied too much pressure on the tail, leading to necrosis.

We made several improvements to overcome this issue. Instead of directly inserting the Dragon Skin into the mold, it was inserted into a vacuum chamber before molding. This process removed most of the air bubbles from the solution, leading to less air bubbles. The mold itself was also

improved. Instead of pouring the Dragon Skin, the new mold was designed for injection molding. With all the components intact, Dragon Skin was injected using a syringe. This reduced amount of the air bubble formation. These two new processes allowed us to use Dragon Skin 10, which is more flexible and breathable than Dragon Skin 20.

The design of the Biodome was also improved. The diameter of the neck of the Biodome was increased. The size of the reservoir was also increased in order to hold more liquid. By increasing the diameter, we were able to reduce the pressure that is put on the tail.

The new protective sleeves were attached along with the Biodomes. The shorter and lighter design was more effective. By decreasing the length of the sleeves, it allows mice to move around more freely. The mice seemed less irritated by the new design. Even with shorter and lighter design the mice were not able to access the Biodome to remove them.

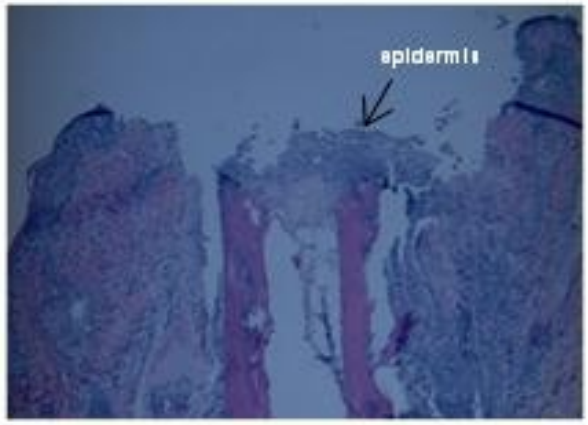
After 14 days, the tail was inspected for signs of necrosis. The new Biodomes showed significant improvement from the previous Biodome. There were no visible signs of the necrosis. No discoloration was found, and the hairs were intact. Overall, the tail looked healthy. This was a significant improvement from the previous Biodome. Older Biodomes showed significant amounts of necrosis that Biodomes were not able to stay attached to the tail. The new Biodome seemed not to disturb the tail at all. The tail remained healthy for 14 days.

Specific Aim #2: Induction of regenerative responses by chemical and electrical stimulations.

Study #1 Depolarization Solution.

Once the new Biodomes were tested and showed satisfactory results, we started applying depolarizing solutions and electrical stimulations. Four different sample groups all showed different results.

The control group without depolarizing solution or electrical stimulation showed typical wound healing pathway. The wound was quickly closed with thick layer of epidermis. Stratum corneum was also noticeable. Two processes that are directly related to the wound healing process. This was not visible in other samples.



The second sample was administered HBSS with electrical stimulations. Compared to the control

group there was much thinner layer of epidermis. We did not see a formation of stratum corneum.

The third sample was administered the depolarizing solution with electrical stimulations. This group did not show any significant signs of regeneration.

Last sample group was administered depolarizing solution with 500 μM of Monensin. This group also did not show any significant signs of regeneration.

Study #2: Depolarization Solutions with varying concentrations of monensin

The last study investigated how different concentrations of monensin affect regenerative process. Five sample groups were studied: Control, HBSS with electrical stimulation, depolarizing solution and100μM monensin with electrical stimulation, depolarizing solution and 150 μM monensin with electrical stimulation, and depolarizing solution and 500 μM monensin with electrical stimulation. After 14 days, when the tails were inspected visually, no signs of necrosis were visible. More importantly, 150 μM monensin sample had a piece of a tissue that was protruding from the tail. It looked like a piece of bone. Visual inspection could not provide adequate information and histology was performed.

The histology results showed different results for all five samples. The control group showed thick layer of epidermis and formation of stratum corneum. This result is consistent with the results from the previous study.

The second group was administered HBSS with electricity. While we notice the significant presence of dermis and hypodermis, no significant signs of regeneration were noticeable.

The third group was administered the depolarizing solution with 100μM of monensin along with electrical stimulation. The histology results showed some epidermis formation. High density of osteocytes was noticeable. This was not visible in previous two sample groups.

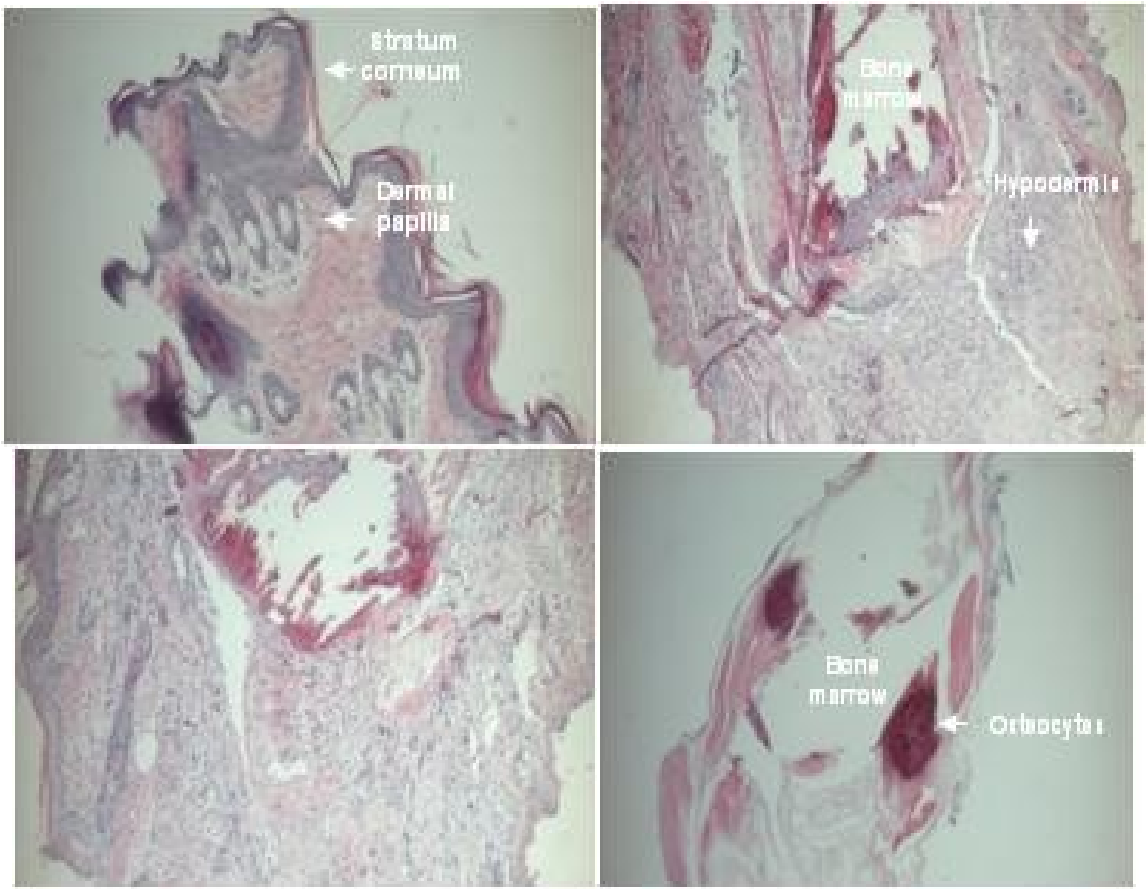
The last two groups contained depolarizing solution with 150 μM of monensin or 500 μM of monensin along with electrical stimulations. Both groups showed some dermis and hypodermis formation. These two samples also had formation dense osteocytes near the wound site. However no significant sign of regeneration was noticed.

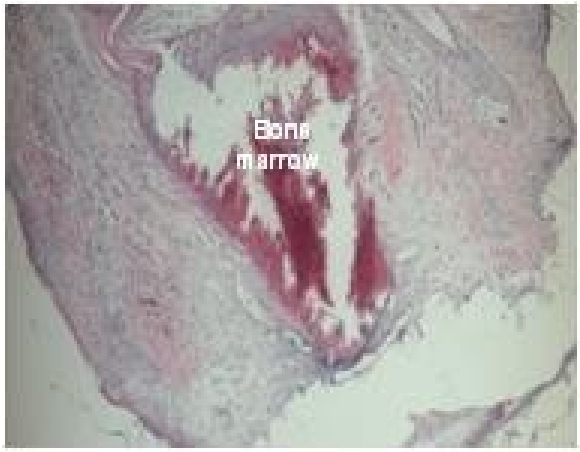
Study #1: Optimizing Biodome design

The new Biodome have improved significantly from the older design. Using Dragon Skin 10 increased the flexibility and breathability of the Biodome. More importantly, the new Biodome is able to stay on the tail for 14 days without causing necrosis. Previous Biodome have caused severe necrosis; almost entire tail was susceptible to necrosis. New Biodome did not cause any necrosis and the tail looked very healthy. There was no bruising on the tail and most of the tail seemed to stay intact.

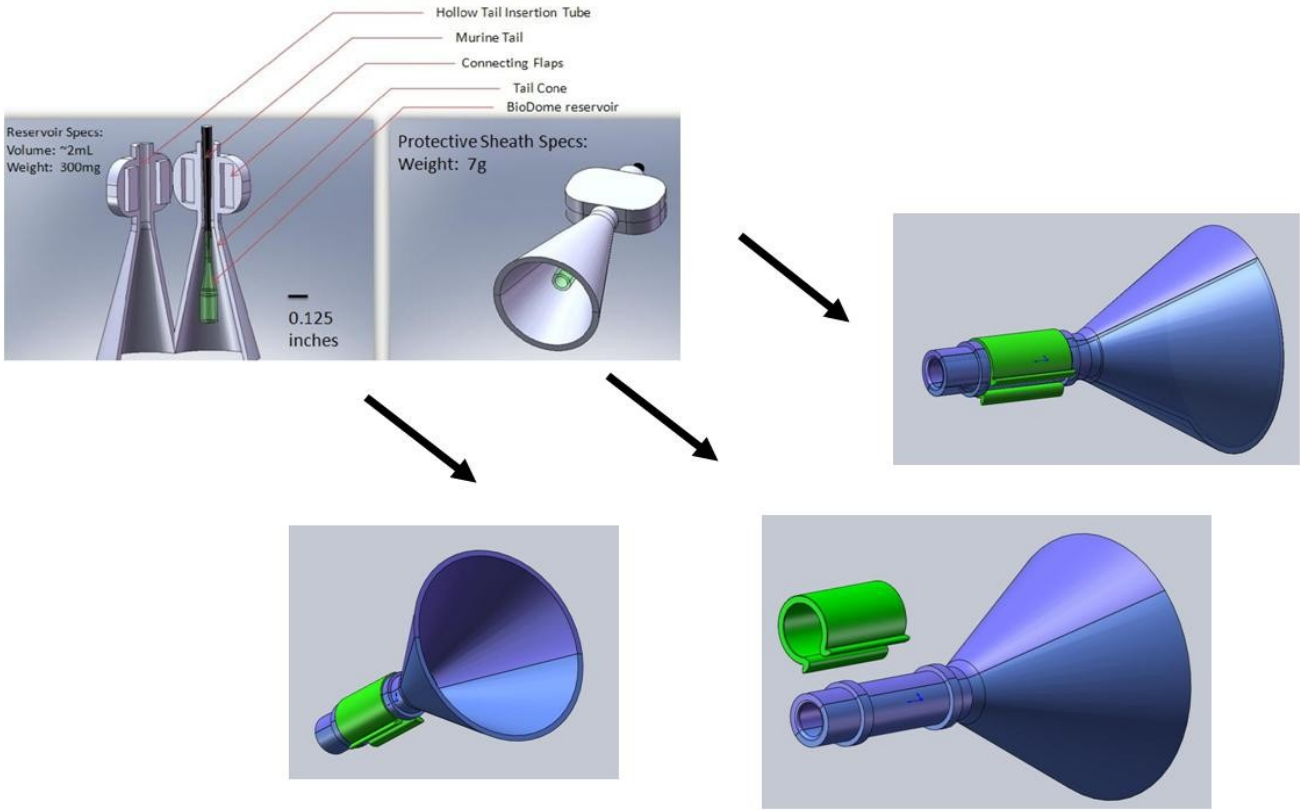
This improvement was able to be achieved by altering several factors. First the design of the Biodome has changed. By increasing the diameter of the Biodome, we were able to make sure that Biodome was not too tight on the tail. The new mold design addressed the air bubble issue that was present in previous design. By utilizing injection molding, air bubble formation had been dramatically reduced, allowing usage of Dragon Skin 10 instead of Dragon Skin 20.

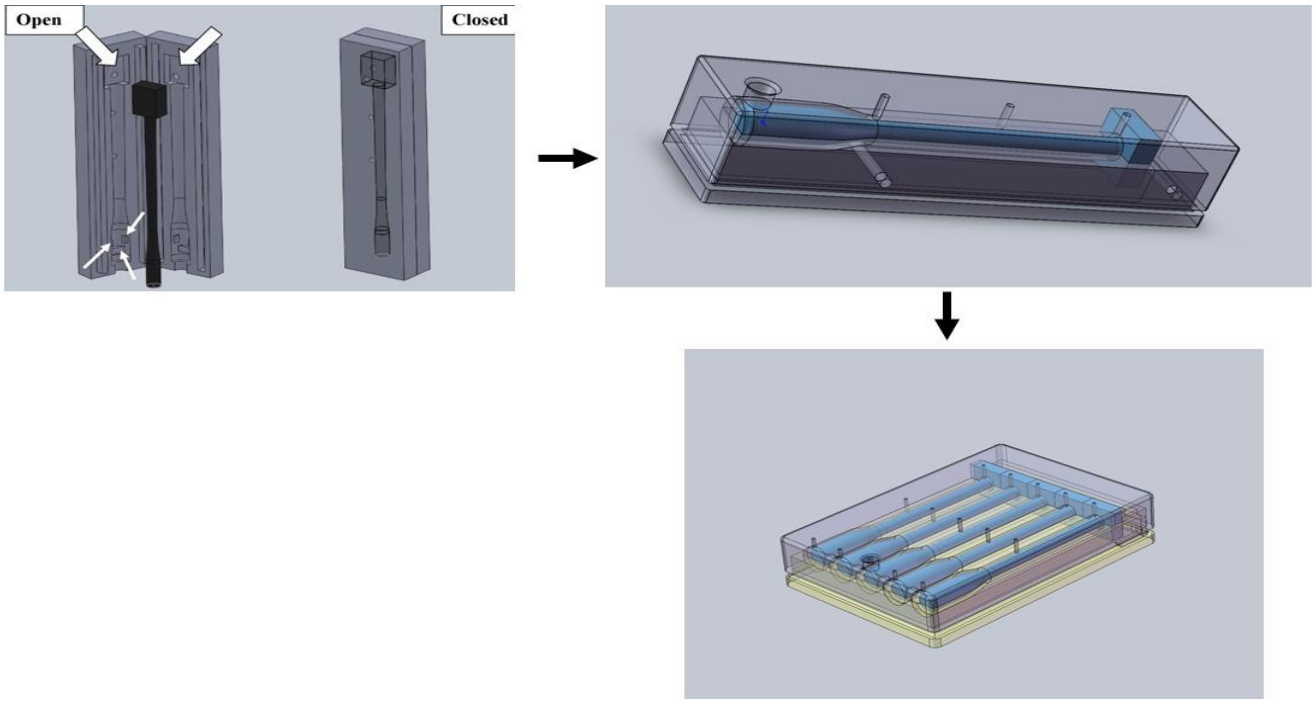
Another minor alteration that produced a significant improvement is the glue. Previous study used Urobond-IV adhesive in order to install the Biodome. Urobond-IV is the adhesive of choice for internal surgical procedures on humans. It has been used previously successfully on animal studies. However, its high viscosity made it very hard to work with. We used M3 Vetbond as a substitute on our study. It is a veterinarian adhesive used for animal surgical procedures. It is easy to apply with a syringe. It is very effective and has no harmful effects on the tails. This has allowed us to install Biodomes more efficiently, without cumbersome process of applying the Urobond-IV.


stratum
corneum
Dermal
papilla
Bone
marrow
Hypodermis
Bone
marrow
Osteocytes


Bone
marrow

DISCUSSION


Hollow Tail Insertion Tube
Murine Tail
Connecting Flaps
Tail Cone
BioDome reservoir
Reservoir Specs:
Volume: ~2mL
Weight: 300mg
0.125
inches
Protective Sheath Specs:
Weight: 7g



Lastly, the improvement of the protective sleeves made it easy install and remove Biodomes. Previous protective sleeves were glued onto the Biodome, making it almost impossible to reuse them. However, our new sleeves used the clip-on designs. This has made it extremely convenient to remove and reinstall them. Also its shorter and lighter design has helped with mice mobility. They are able to move around more freely around the cage.

With these improvements, we have been able to alter the mold design to increase the production of the Biodomes. Since the Biodome is required to be changed every day, large quantities of Biodomes are required before the experiment could start. With previous design, we were only able to produce one Biodome per mold. However, with injection molding, we have altered the mold design to produce five Biodomes in one mold. This has expedited the preparatory process of the experiment.

Overall new design of the Biodome is a success. The new design did not cause any necrosis on the tail. The new mold allowed mass production of the Biodome.

Specific Aim #2: Induction of regenerative responses by chemical and electrical stimulations.

Study #1 Depolarization Solution.

Once the Biodome was ready for experiment, depolarizing solution and electrical stimulations were added. The overall results showed thinner epidermis formation along with absence of stratum corneum. Formation of thick epidermis and stratum corneum is a typical wound healing process, which was shown with the control group. When a limb is lost, the wound site is quickly closed with epidermis in order to prevent blood loss and infections. Formation of stratum corneum is a hall mark of this wound healing process. Stratum corneum has a thick layer of dead cells that serve as protection. On the other hand, regeneration process does not form a thick layer of epidermis or stratum corneum. In order to regenerate, the cells at the wound site need to be actively proliferating. This proliferation along with cell migrations leads to limb growth.

The other three samples showed some signs of regeneration. Compared to the control group, all three groups showed much thinner layer of epidermis at the wound site. Regenerating organisms show thinner layer of epidermis during the regeneration process because the cells at the wound are constantly dividing. We were not able to see any extensive nerve growth or tissue formation at the wound site. While this study produced very primitive regenerative responses, more studies must be conducted in order to conclude if the depolarizing solution along with electrical stimulation produced any significant results.

Study #2 Depolarization Solutions with varying concentrations of Monensin

The second study was aimed at how varying amounts of monensin affect the regeneration process. The control group again showed thick formation of epidermis and formation of stratum corneum. The other four groups showed different results. Thick epidermis formation was not noticeable in all other groups. Most noticeably, other groups showed high concentration of the

osteocytes at the wound site. The group that was administered 100 μM of monensin with depolarizing solution showed highest density of osteocytes. Osteocytes are mitotically inactive cells, and in most cases, they destroy the bone. Even though destroying the bone is not desired for the immediate regenerative process, it might be beneficial in the long term regeneration process. Osteocytes could destroy the damaged bone at the tip of the wound site. Once damaged parts are removed, new bone could grow out from the wound. Further studies should be conducted to see how osteocytes affect the regenerative processes.

Two rounds of experiment is not adequate to provide any significant conclusions. Further studies must be conducted in order to study regenerations. Alternative approaches could be considered in order to improve the regeneration processes, such as adding mesenchym stem cells into the depolarizing solution. Further analysis of the data should be conducted such as RT-PCR or FACS in order to study which genes are activated and which cells are present at the wound site.

Overall, our experiment was able to lay the solid ground work for future studies. Most importantly, we were able to improve upon the Biodome, mold, and protective sleeve designs. New designs do not cause necrosis on the tail. The Biodome project now has stable apparatus and repeatable procedures that can be used for future studies.

FUTURE WORKS

Continuous studies must be conducted in order to produce more informative conclusions. Most importantly, more rounds of experiments must be conducted. New rounds of experiments should look to alter the period of study. For example, instead of sacrificing the mice right after 14 days, future studies should study the mice without stimulations after 14 days to see how this time frame influences the tail regrowth.

More thorough data gathering procedures must be conducted such as RT-PCR and FACS. RT-

PCR should look at the specific genes that are related to cellular proliferation and regeneration such as BMP, FGF, CAP43, Ta1 Tubulin. FACS allows visualization of different cell types at the wounds.

Lastly, novel approaches should be taken to promote cell proliferation and differentiation. One possibility is adding embryonic stem cells into the solution to see how this effects regeneration.

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