Regenerative Mechanisms: Decoding Axolotl Regeneration for Human Healing

This project explores the regenerative abilities of axolotls which can regenerate lost tissues, organs, and entire limbs. This study aims to understand how their unique abilities could be applied to human medicine in the future.

Section 1: Overview of Regeneration

Importance of Regenerative Medicine

- Regenerative Medicine: refers both to the regular and repeated renewal of a particular structure or tissue throughout the life of an organism and holds to answer to treating numerous conditions
    - Injury repair: bone fractures, tissue damage (spinal cord injuries)
    - Degenerative diseases: Alzheimer's, Parkinson's, and other age-related conditions
    - Organ failure: potential for growing replacement/repairing organs (heart, lungs, etc.)
- Improved regeneration can help the body function as it did before aging or other diseases

Challenges of Human Regeneration

- Humans are able to regenerate certain tissues and parts of organs through compensatory hypertrophy mostly in liver and skin but the process is flawed
    - Compensatory hypertrophy: ability for an organ to increase in size after being damaged or removed
    - Have limited regenerative abilities for more complex structures like limbs, heart, spinal cord, and brain
- When humans suffer injuries they often form scar tissue instead of regrowing fully functioning tissue.
- Comparing humans to amphibians
    - Adult stem cells: in humans adult stem cells have a limited ability to become other cells unlike amphibian stem cells
    - Blastema: humans do not form blastemas to compensate for a damaged tissue or limb (will elaborate later)

Stem Cells

- Stem Cells: These are undifferentiated cells capable of turning into various specialized cell types.

    - Adult Stem Cells: found in specific tissues and can produce new cells for repair (ex. skin stem cells, bone marrow stem cells)
    - Embryonic Stem Cells: have the ability to become any type of cell in the body
    - Induced Pluripotent Stem Cells (iPSCs): reprogrammed from adult cells and makes it useful during regeneration
    - Perinatal Stem Cells: found in the mother's amniotic fluid
- Progenitor cells: an undifferentiated cell that can form tissues (like stem cells) that are more specialized and can repair the body

Types of Regeneration

- Physiological Regeneration: happens to maintain regular functional homeostasis with regular replacement of different cells in the body
- Reparative Regeneration: triggered by injury or damage, seen when tissues grow back after being injured
    - Incomplete regeneration: only partial restoration of the former structure which include replacement of finger tips in young children
    - Complete regeneration: mostly observed in amphibians but rarely in mammals, and happens when the new tissues is the same as the old tissue

Differences in Regeneration

- While humans have some regenerative abilities they can't regenerate entire limbs, organs, or other tissue that is exactly identical to to old tissue
- A main limitation in human regeneration is that the cells do not dedifferentiate (become more like stem cells) after injury as easily as they do in amphibians like axolotls

Section 2: Axolotl Regeneration

Axolotl

- Axolotl (*Ambystoma mexicanum*), a species of salamander that belongs to the family Ambystomatidae
    - Known for its ability to keep its juvenile features and never leave the water known as neoteny
        - Neoteny: is the phenomenon in which an organism keeps its juvenile characteristics throughout its life

            - Allows axolotls to retain regenerative abilities that would typically fade in other species after they mature
            - They don't undergo metamorphosis like other amphibians meaning they maintain juvenile stem cells throughout their lives
            - This is due to their lack of thyroid-stimulating hormone: thyroxine
                - An injection of thyroxine or iodine can stimulate their metamorphosis but their regenerative capacities can be affected and their lifespan shortened
- Unlike humans, axolotls are able to regenerate complex body parts and organs (eye, brain, spine, heart, gill, limbs, etc.)
    - Regenerated tissues are identical to former tissue with extreme precision
    - Scar free process
- The regenerative process of other amphibians such as salamanders and newts are very similar and follow the same steps
    - No other amphibian is able to perfect this process as well as the axolotl

Limb Bud Formation

- In every organism their limbs must have been formed at some point in life
- Limbs are developed through limb buds while the organism is still an embryo
    - These limbs buds are full of progenitor cells and stem cells
    - Progenitors differentiate and multiply
    - Nerves grow into the limb bud and newly developed blood vessels fuel the entire process
    - Eventually the limb bud grows into a full infant limb
- Limb generation aims to mimic this process

Limb Regeneration Steps

- **Wound Healing**
    - The wound site is covered rapidly through fibrosis with epidermis cells (skin cells) to form the wound epidermis rather than scar tissue
    - With nerve stimulation, the wound epidermis thickens and forms an apical epithelial cap
- **Dedifferentiation**
    - The cells around the limb stub are signalled by nerves that provide neurotrophic factors to go back to a more primitive state called progenitor cells

        - Wnt signaling regulates cell differentiation while FGF (fibroblast growth factor), BMP (bone morphogenic protein), and TGFβ (transforming growth factor β) promotes the formation of the blastema and regulates the growth of the new limb
- **Blastema Formation**
    - Blastema: a mass of heterogeneous progenitor cells that are activated after injury
        - essential for regeneration because it can differentiate into various tissue types, such as muscle, skin, bone, and nerves, to fully regrow a lost body part
    - This is almost identical to an infant limb bud
        - Made up of recycled and repurposed cells
    - The blastema is essential for regeneration because it can differentiate into various tissue types, such as muscle, skin, bone, and nerves, to fully regrow a lost body part
    - The major nerve signal is the anterior gradient protein (AGP) that supports blastema development and growth
    - Blood vessels bring oxygen fuel this entire process that takes approximately 5 weeks
- **Redevelopment**
    - The limb bud undergoes a period of rapid ontogenetic allometric growth over multiple weeks
        - Ontogenetic allometric growth: rate of growth of a structure
    - The resulting limb is proportionate to the rest of the body
        - Because axolotls may also grow during the period of limb regeneration, we know that there is not a fixed size for a regrown limb but must to regulated throughout the process
        - For the first five weeks after blastema formation progress is rapid (approx. 0.04cm/day) but afterwards, progress slows gradually over 9 weeks (approx. 0.02cm/day) until growth rate is isometric with the other limb
    - Growth regulation
        - Once the regeneration is complete, the proliferative signals are immediately switched off to prevent uncontrolled cell growth or tumor formation
        - Meaning axolotls are basically cancer resistant

Genome

- Axolotl genes are extremely complex genome with 14 chromosome pairs and 32 GB size (humans are 3.4 GB)
    - Around 10 times the length of the human genome

- They have highly repetitive sequences
    - Most of which still have no known function
        - Some are also key genes for regeneration
    - it might not be the genomes themselves but how axolotls regulate them

Section 3: Implications for Human Medicine

Cancer Resistance and Tumor Suppression Mechanisms in Axolotls

- **Role of p53**
    - Axolotls can be used as models for cancer therapy due to their shared genes and signaling pathways with humans.
    - Regeneration is monitored through reprogramming genes of pluripotent adult cells and tumor suppressor genes (p53) which are upregulated during tissue differentiation
        - Pluripotent: a cell able to develop into other cells
        - P53 is a gene that regulates the cell cycle and when it doesn't work properly, cells proliferate and cause tumors that can be cancerous (leading cause of cancer)
    - Axolotl limb tissue extracts has been proven to induce cell cycle arrest of human acute myeloid cells
        - Human acute myeloid leukemia is a type of cancer
        - Further research on this topic can further unlock the anti-cancer potential of axolotl proteins
- **Immune System**
    - Axolotls also have remarkable immune system responses
        - They have an anti inflammatory response when healing wounds
        - Inflammation in humans combats infection and triggers the healing process but can also harm the healthy parts of your body and cause chronic diseases (cancer)
        - Instead, axolotls use macrophages to help carry out cell debris and old tissue
        - Unfortunately, there is not sufficient research on this topic but if we are able to study more about how they heal without an inflammatory response, it could be the solution to many other chronic diseases

Spinal Cord Regeneration in Axolotls

- **Axolotl Spinal Cord Regeneration**

    - After injury, ependymal radial glial (spinal cord lining) cells at the cut site seal the injury and begin to proliferate
        - Leads to the formation of a tube of glial cells, which eventually becomes the new spinal cord tissue
    - The cells in the terminal vesicle (end of the tube) can migrate to extend the tube of new spinal cord tissue
        - They undergo partial EMT (epithelial-mesenchymal transition) and they change their behavior to allow them to move and grow
    - The newly formed tube eventually generates a variety of neurons (nerve cells) and glial (support cells) needed to rebuild the spinal cord
- **Mammalian (Human) Spinal Cord Injury**
    - After injury, the ependymal glial cells primarily generate astrocytes (a type of glial cell)
    - Pre-existing astrocytes and other cells at the injury site form a glial scar, which acts as a barrier to prevent further nerve growth
    - Very little new neurogenesis (generation of new nerve cells) occurs in injured mammalian spinal cords, which limits the ability to repair the damaged tissue
- **The Role of miR-200a**
    - miR-200a: a microRNA that regulates gene expression (tells what stem cells need to become during regeneration)
    - After a spinal cord injury, axolotls need to replace neural cells which help in nerve function
    - miR-200a prevents stem cells from becoming mesodermal (muscle/bone) cells, making sure they stay on track to become neurons or glial cells
    - The study offers insights into stem cell therapies for spinal cord injuries

Blastema

Blastema in Mammals

    - Cellular plasticity is essential for regeneration because it allows cells in the blastema to differentiate into tissues for regrowth
        - Cellular plasticity: ability of adult cells to transform into different types of cells when needed
    - As long as the cells are getting the write signals, regeneration is possible
        - Wnt signaling: Plays a role in the formation of the blastema and the differentiation of cells in the regenerating tissue

        - BMP (Bone Morphogenetic Protein): Regulating tissue differentiation in the blastema
        - FGF (Fibroblast Growth Factor): Important for proliferation and differentiation of the cells within the blastema
        - These signals guide the blastema to form the correct tissue types, ensuring that bone, skin, or muscle cells regenerate where needed
    - Stem cells from various sources (such as the epidermis or fibroblasts) play a key role in forming the blastema
        - The ability to reprogram cells into a regenerative state is essential for successful regeneration
    - By activating stem cells or introducing new ones into the injured tissue, regeneration can be enhanced, making the formation of a blastema more efficient
- **Challenges of Blastema Implants**
    - Scar tissue often forms after injury instead of regenerative tissue, it is non-functional and can hinders proper regeneration
    - There are also limitations in tissue integration
        - Even if new cells are generated, they don't always integrate properly into existing tissue networks (like nerves or blood vessels)
    - Stem cell therapies and other treatments may one day be able to unlock the regenerative capacity of mammals by activating latent regenerative pathways and enhancing the formation of blastemas in humans
- **Applications in Human Medicine**
    - Regenerative therapies could potentially use blastema-like cells to promote the regeneration of tissues lost to injury, aging, or disease
        - Potentially finding solutions to disease like alzheimer's that happens when brain cells die off without being replaced by new, healthy cells
    - Researchers are working on methods to reprogram adult cells or introduce stem cells that can form blastema-like structures
        - Could lead to the regeneration of fingers, limbs, or even internal organs
        - Understanding the formation of the blastema in mammals could lead to breakthroughs in limb prosthetics or biomimetic tissue engineering, where bioengineered tissue is used to replace lost or damaged tissues

Sources

Section 1
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https://www.brookings.edu/articles/regenerative-medicine-a-future-healing-art/
Section 2
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Project Timeline

December 2
- Finalised project idea and topic

December 7
- Written out project aims and objectives
- Completes Basic Project Info form
- Submitted Ethics Due Care 2A form

December 10

- Project is accepted by CYSF

December 23

- Started outlining the format for research
- Splitting the project into many sections, including what topics fit into which parts
- Starting to think about how axolotl regeneration can help human regenerative medicine
  - Helping amputees and cancer patients
  - Can humans replicate their process of regrowing limbs and organs

December 28

- Finding articles relevant to background information on regenerative medicine
  - Basic information about the human limitations

January 1

- Continuing yesterday's research process and going more in depth into how regenerative medicine can be helpful to common diseases
  - Are there any techniques that are currently being used?
- Writing out my hypothesis

January 14

- Learning about how humans heal after injuries
- Researching different types of regeneration in humans and how the process is flawed

January 19

- Studying stem cells and their role in regeneration
- Comparing human stem cells to the stem cells of animals with regenerative abilities

February 5

- Researching the blastema and how they are helpful for other animals when regrowing limbs and organs
  - Is it possible to inject blastema cells into a human amputee's limb stump?

February 7

- Comparing human regeneration to axolotl regeneration
  - How can humans replicate the axolotl regenerative process?

February 9

- Diving into axolotl biology
  - Neoteny and how metamorphosis has an affect on their regeneration
  - Finding the limitations of their regenerative abilities
  - How is the newly grown limb completely identical to the other?

February 11

- Finding the steps that happen after injury
- Learning more about dedifferentiation

- Researching more about the detailed role of the blastema

February 13

- Continuing researching regenerative steps

February 17

- Researching the similarities between a limb regeneration and when the limb is first grown in the mother's womb (limb buds)
  - Since humans are also able to grow limbs in the womb, how do we replicate this process later in life?

February 20

- Studying the axolotl genome and how it regulates the entire process
  - Does a longer genome mean a more regulated system?

February 22

- Researching about other regenerative amphibians

February 23

- Decides to focus on axolotls because the process with other amphibians is extremely similar and axolotls are so far the only ones who have perfected this ability

February 28

- Researching human implications of axolotl studies
- Answering previous question about a potential blastema implant
  - Although there is little experiments about it, there are many factors that we know could allow us to mimic the regeneration process

March 2

- Researching about how axolotls heal spinal cord injuries
- Learning about how inflammation stops the spinal cord from healing itself
  - Why does the body reserve the use of inflammation to heal injuries if it is really flawed and could even cause other diseases?

March 4

- Continuing research about spinal cord injuries and the gene that is crucial to this process
  - How can we use gene editing in the future for regenerative medicine?

March 5

- Finding studies on the connection between axolotl's resistance to cancer
  - Axolotl tissue can stop further growth of cancer tumors in some type of cancer
  - More research and experiments on this topic can potentially cure some types of cancer
- Planning out the research report format

March 8

- Writing out the problem/introduction of my research report

March 9

- Beginning the background research/overview of regenerative medicine

March 10

- Finishing the research section of my project

March 12

- Explaining the human limitations
- Comparing role of macrophages in humans and axolotls (researched in more depth on the spot)

March 13

- Writing report on spinal cord regeneration and the potential of blastema related therapies in the future

March 14

- Finishing the cancer related data with axolotl tissues
- Writing out my conclusion

March 18

- Adding diagrams and visuals as well as explanations to the report
- Putting my citations into proper formatting and putting in text citations

March19

- Writing out my method and acknowledgements
- Final round of editing and finishing touches