

Stem Cells: Are They Mechanosensitive?

Thesis statement

The goal of this research is to determine how mechanical forces can influence stem cell differentiation and to quantify this relationship.

Background

Stem cell research is a subject of great fascination and intrigue. It is also the subject of immense scientific, ethical, and political debate. Stem cells are precursor cells that can give rise to multiple tissue types, and they have potential in many different areas of health and medical research [1]. Studying stem cells will help us to understand how they transform into the vast array of specialized cells that make us what we are. Some of the most serious medical conditions, such as cancer and birth defects, are due to problems that occur somewhere in this process. Stem cell research has a history of more than twenty years, but the field has gained considerable attention recently due to the successful achievement in culturing and manipulating human stem cells *in vitro*.

Stem cell fates are determined during the development of the organism. Many research groups around the world are attempting to demystify this differentiation process. Their approaches are generally genetic or chemical in nature [2] [3]. For example, cellular signals, such as growth factors, have been shown to influence stem cell fate. However, the influence of mechanical forces, such as tension or compression, on stem cell specialization has not been studied presumably due to a lack of proper tools.

We intend to build a tool to study the effects of mechanical forces on stem cells. We will use the stem cells from the hydra. Hydra is a simple organism that is composed of a head with tentacles and a sticky foot region (Figure 1). It is roughly 1 cm tall and generally lives in water (both freshwater and salt water environments). The central body column is composed of stem cells. These stem cells have one of two fates: they either become part of the head or part of the foot [4]. If the animal is cut in half, the stem cells in the body column will facilitate regeneration of the missing part. Thus, a foot can regenerate a head and a head can regenerate a foot.

The hydra provides a simple vehicle for stem cell differentiation studies. By imposing compressive forces on dissected parts from this animal, we intend to determine whether mechanical compression will influence the hydra's regeneration and thus stem cell differentiation.

Objective and significance of the project

The objective of this project is to study how mechanical compression influences stem cell fate. By characterizing the mechanical aspects of stem cell differentiation, we will gain immense new knowledge in the biology of organism development. Although we will initially study the force-specialization relationship in stem cells from hydra, this relationship may be similar to that found in humans and will likely hold remarkable potential for therapies and cures for disease. With this data, new diagnostic assays and therapies can be developed to alter the fate of stem cells. This in turn may contribute to advances in the prevention, diagnosis, and treatment of human diseases and devastating illnesses such as heart disease, diabetes, and cancer which continue to deprive people of health, independence, and well-being.

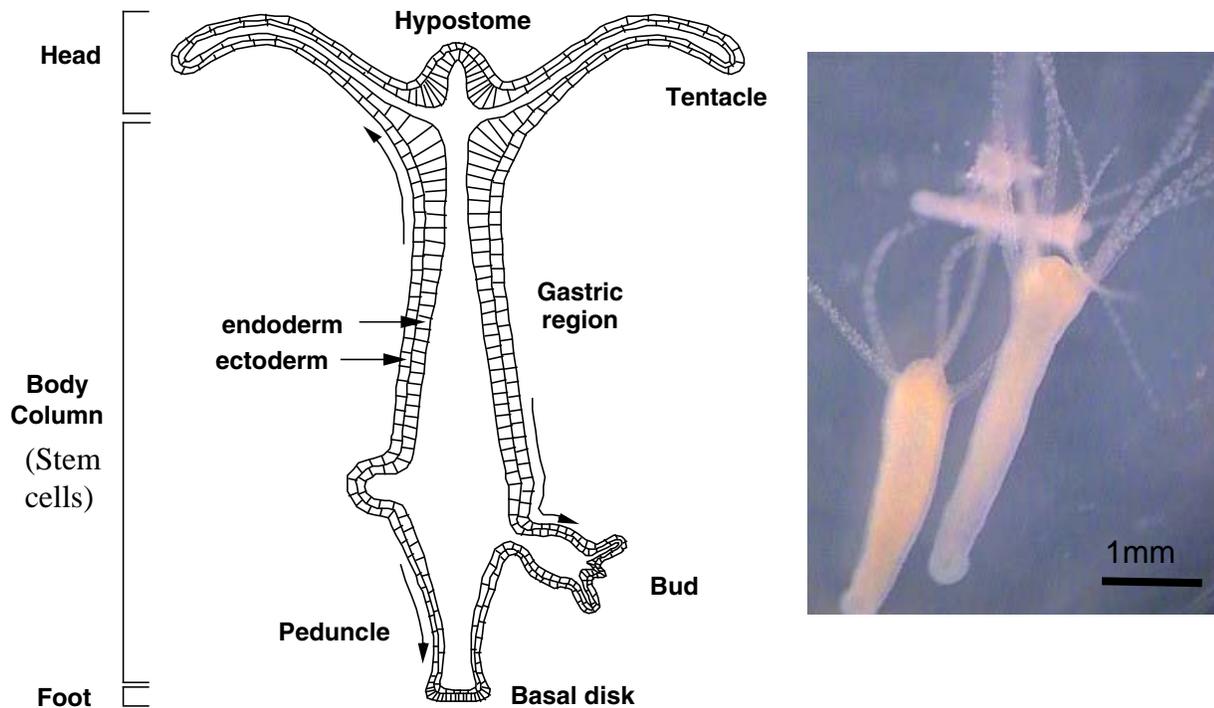


Figure 1. Cross section of hydra. Hydra reproduce asexually by budding. Hydra photo at 40X magnification.

Methods

We intend to impose various amounts of compressive force on dissected tissue containing stem cells from the hydra via a water column. By submerging the parts at various depths, different static fluid pressures will be applied to the tissue and thus different levels of compressive force. The pressure-depth relationship is:

$$P_{\text{total}} = P_{\text{atmosphere}} + P_{\text{fluid}}$$

The unit of pressure is Pascal which is the same as newton/m².

where

$$P_{\text{fluid}} = \rho * g * d$$

- ρ (**rho**) is the density of the hydra media (assumed to be $1.00 \times 10^3 \text{ kg/m}^3$)
- g is the acceleration of gravity (9.8 m/s^2)
- d is depth of the hydra

$$P_{\text{atmosphere}} = 1.01 \times 10^5 \text{ kg/m}^3$$

This relationship is plotted here:

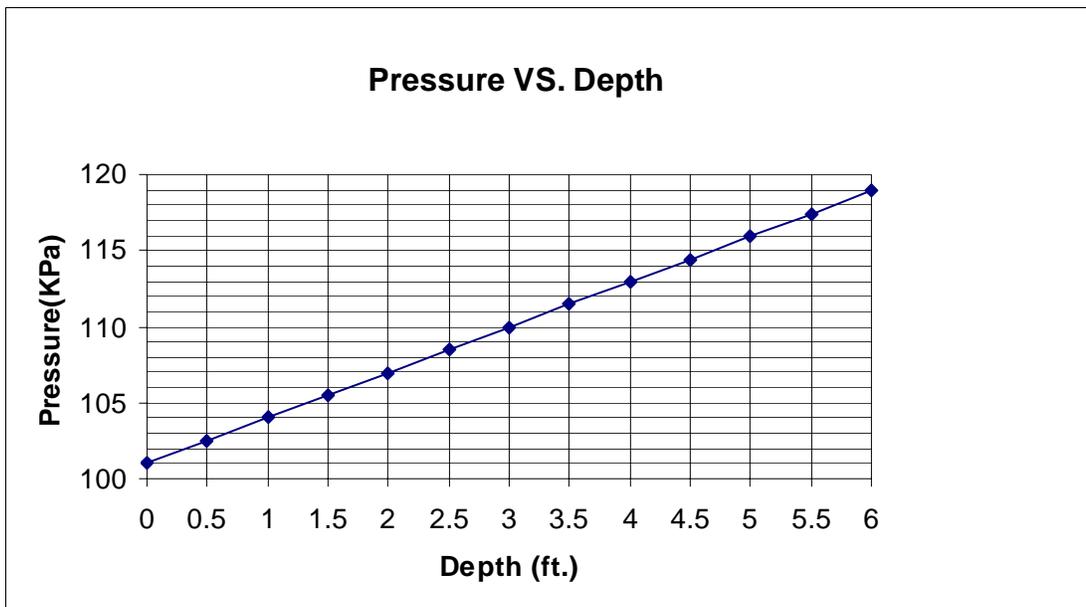


Figure 2. Graph of Pressure VS. Depth in the water column.

The hydra stem cell tissues will be held in microchambers made from polydimethyl-siloxane (PDMS, silicone rubber). The microchambers are designed with 3 channels – one for tissue injection into the chamber, and the other two are used for pressure equalization. The ends of the two equalization channels are capped with gold foil grids with 20 micron holes. Thus, the tissue will not be able to escape but fluid will be able to flow into the chamber. Thus the pressure at the specific depth in the column will be translated to the tissue. A microchamber diagram is shown in Figure 3.

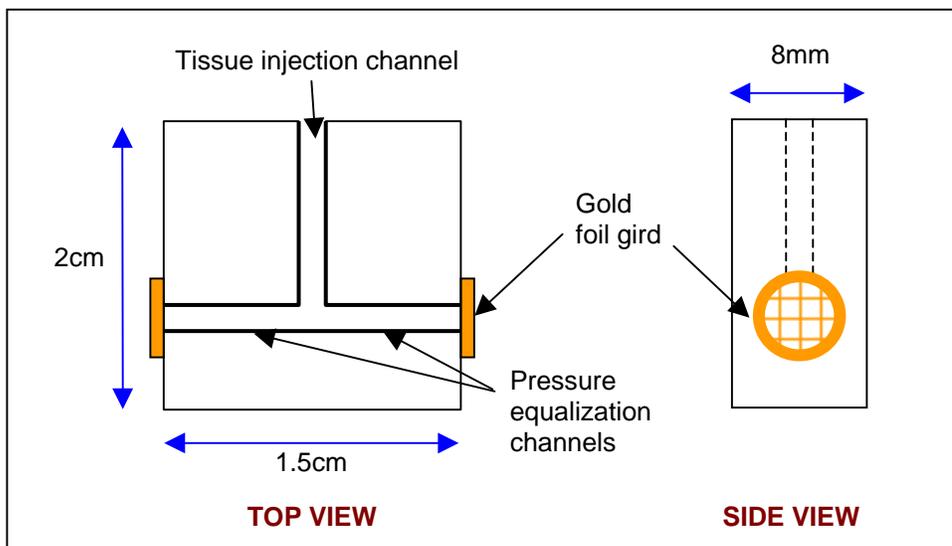


Figure 3. Diagram of microchamber.

To make these microchambers, we will first create a mold using glass capillary tubes (1.5mm in diameter) glued to microscope slides. The mold will be silanized to facilitate easy removal of the PDMS from the mold. Viscous PDMS precursor will then be poured over the mold and cured. After curing the PDMS will be pulled off the mold, leaving open channels. A separate flat slab of PDMS will be prepared to seal off the open channel. The two PDMS parts will be bonded together in an air plasma machine and gold foil

grids glued to each end of the pressure equalization channels, as shown in Figure 4. Flexible plastic tubing will be attached to the injection channel.

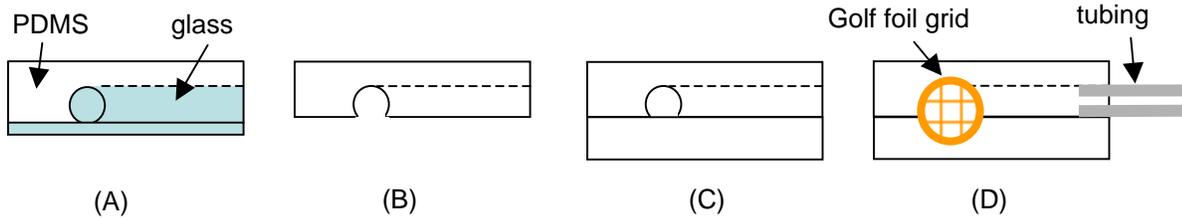


Figure 4. Fabrication process for microchamber. (A) Pour PDMS over silanized glass mold. (B) After curing PDMS, peel it off the mold. (C) Bond another flat piece of PDMS to the first piece to seal the channels. (D) attach tubing and gold grids.

Once the microchambers are prepared, the tissue will be injected into the chamber. The injection channel will then be clamped off, and the microchamber will be lowered into the water column and held at a fixed depth. By having several microchambers positioned at different depths in the water column, different amounts of compression will be experienced by the tissue samples. The duration of the experiment will vary from a few hours, a few days, to a few weeks, after which the tissue samples will be pulled out of the water column and analyzed. The water column will be relatively easy to construct. It will be made from a tube of clear acrylic plastic, sealed at one end. The column will be about 6 feet tall, such that six microchambers can be suspended at each foot using fishing line. The column will be filled with hydra media and each microchamber will be weighted down with a suitable weight. Oxygen will be slowly bubbled through the entire column to maintain consistent gas levels throughout.

Analyzing the tissue consists of looking at it under a high magnification stereoscope to see if regeneration has taken place and determine the extent of regeneration. If animals have fully regenerated, tests can be performed to assess the quality of the animals. For example, the animal's natural instinct to eat may be compromised if in the presence of food it does not eat. Foot formation can be tested by placing a glass rod next to the foot and observing whether it sticks to the rod. Additional tests for light sensitivity and reaction time to disturbances can be performed as well. Other more sophisticated assays can be performed such as staining the tissue to determine whether a proper foot has formed (the stain will only attack specific cells in the foot region if they are present). Ultimately, rigorous genetic tests can be performed on the resulting animals to determine whether they are normal.

Expected results

To date we have studied hydra regeneration under normal conditions (i.e. no mechanical compression). Animals were dissected such that either a head, foot, or both head and foot were removed. We observed that normal hydra regeneration takes roughly 3-4 days. The photos from hydra feet regeneration are shown in Figure 5.

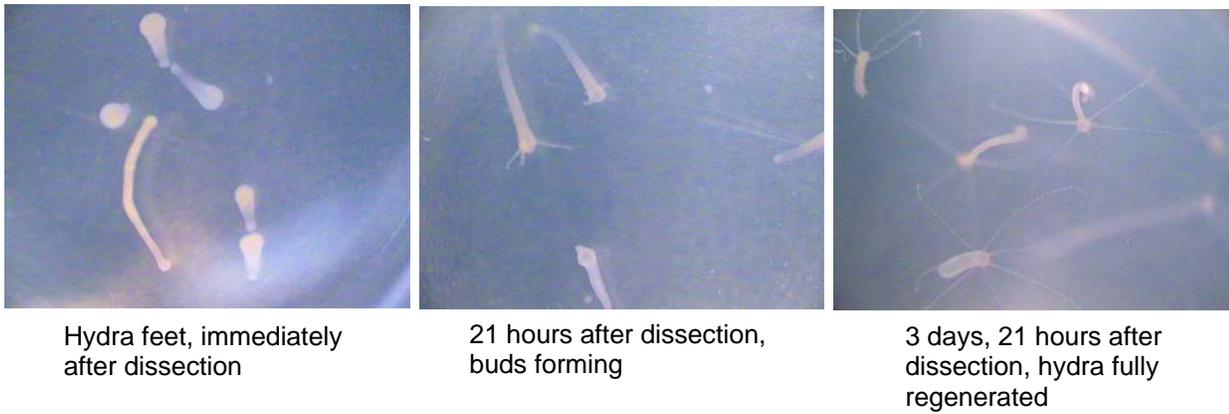


Figure 5. Photos of hydra foot regeneration under normal conditions (all photos 20X magnification).

Under compression we expect that hydra regeneration will be altered. Although it is unclear what the exact alterations will be, we suspect the regeneration process may take longer with greater compression. At the conclusion of a test run, perhaps the animals located at lower depths in the water column will not be fully regenerated compared to animals closer to the surface. If animals have fully formed at all depths in the water column, then we expect the tests listed above to yield interesting differences in animal quality.

Specific responsibilities of the student

I have already begun microchamber construction and testing. In the near future, I will begin water column construction. I will be responsible for microchamber design, water column construction, animal dissection, and acquiring materials under the guidance of Dr. Tang and Dr. Lin. I will also be responsible for collecting and analyzing the data generated from each test run. Through this project, I will learn about the biology behind hydra regeneration and stem cell differentiation. I will also gain first hand experience through observing and analyzing hydra regeneration process under static mechanical force.

Project timeline

The project period is 9 months. List of tasks per quarter:

Fall Quarter:

- (1) Acquire all materials to construct microchambers and water column and perform tests
- (2) Construct microchambers and water column
- (3) Begin testing microchambers to verify proper function

Winter Quarter:

- (1) Complete testing and characterization of microchambers
- (2) Perform short term tests in the water column (duration = hours, few days at most)
- (3) Analyze the dissected tissue samples after each test
- (4) Redesign the microchamber or water column if needed.

Spring Quarter:

- (1) Perform longer term tests in the water column (duration – several days, few weeks at most)
- (2) Analyze the dissected tissue samples after each test.

Budget details & justification

- Materials for microchamber construction, multiple runs (PDMS, glass molds, epoxy, silane, Petri dishes, test tubes, weights, foil grids etc.): \$500
- Materials for water column: \$200
- Materials to make hydra media (various salts, ultrapure water): \$100
- Basic biology and stem cell text books = \$200

TOTAL PROJECT COST = \$1000

NOTE: Hydra will be provided free of charge by the laboratory of Dr. Bode in the Department of Cell and Developmental Biology, U.C. Irvine.

References

- [1] J. Darnell, H. Lodish, D. Baltimore, *Molecular Cell Biology*, 2nd edition, Scientific American Books, New York, 1990.
- [2] H.R. Bode, “The Role of Hox Genes in Axial Patterning in Hydra,” *American Zoology*, vol. 41, pp. 621 – 628, 2001.
- [3] M. Schuldiner, O. Yanuka, J. Itskovitz-Eldor, D.A. Melton, N. Benvenisty, “Effects of Eight Growth Factors on the Differentiation of Cells Derived from Human Embryonic Stem Cells,” *Proceedings of the National Academy of Sciences*, vol. 97, no. 21, pp. 11307 – 11312, October 10, 2000.
- [4] H.R. Bode, “Head Regeneration in Hydra,” *Developmental Dynamics*, Vol. 226, pp. 225 – 236, 2003.