

## **Stem Cells: Are They Mechanosensitive?**

### **Topic or 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 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 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 illnesses such as heart

disease, diabetes, and cancer which continue to deprive people of health, independence, and well-being.

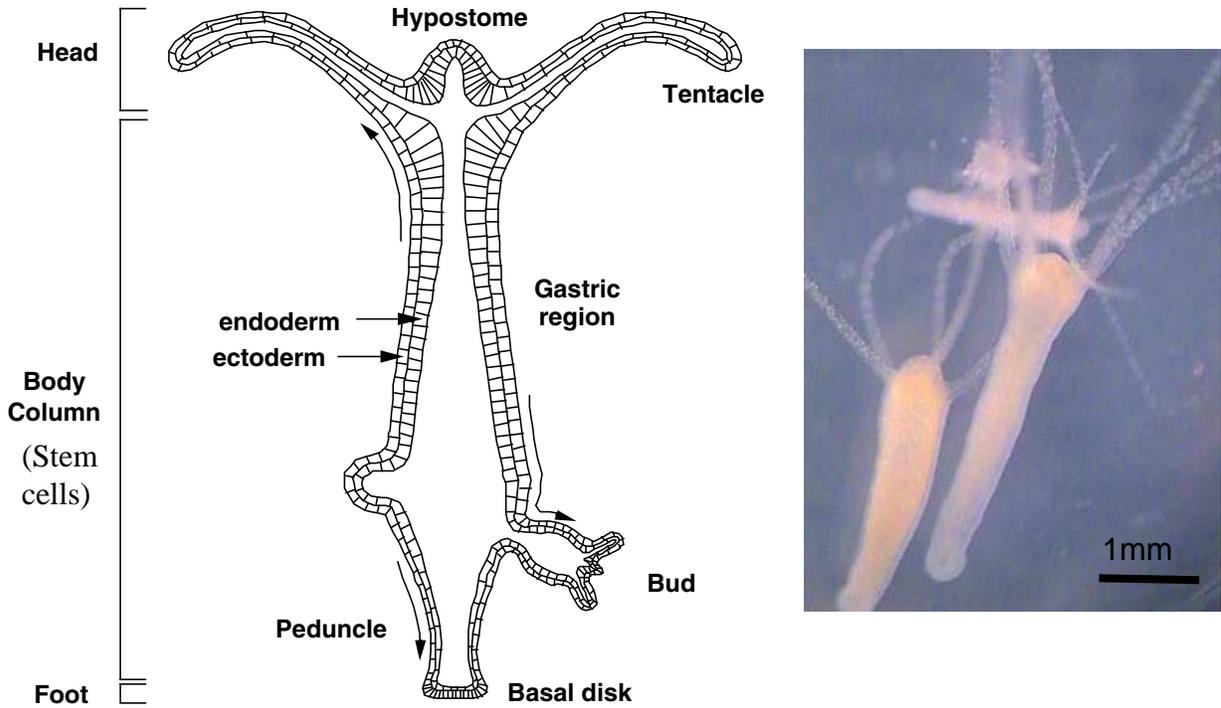


Figure 1. Cross section of hydra. Hydra reproduce asexually by budding. Hydra photo at 40X magnification.

**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 dissected tissue 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:

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

The unit of pressure is Pascal, which is the same as N/m<sup>2</sup>.

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

- $\rho$  (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 ( $9.8 \text{ m/s}^2$ )
- $d$  is depth of the hydra

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

The hydra stem cell tissues will be held in microchambers made from polydimethyl-siloxane (PDMS, silicone rubber) then lowered into the water column at a specific depth. 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 Figure2.

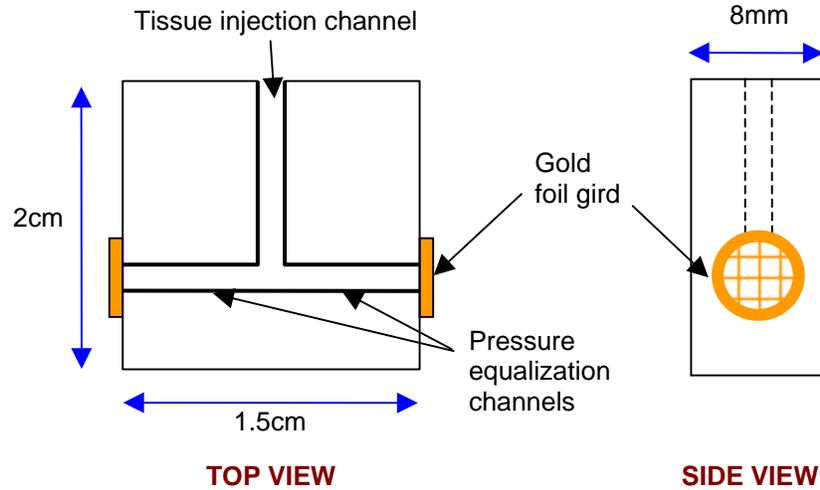


Figure 2. Diagram of microchamber.

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 3. Flexible plastic tubing will be attached to the injection channel.

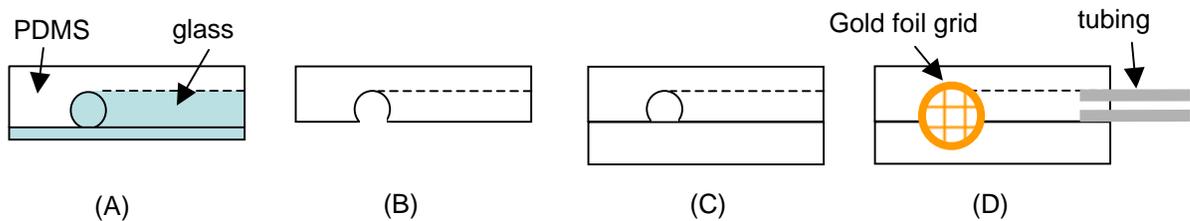
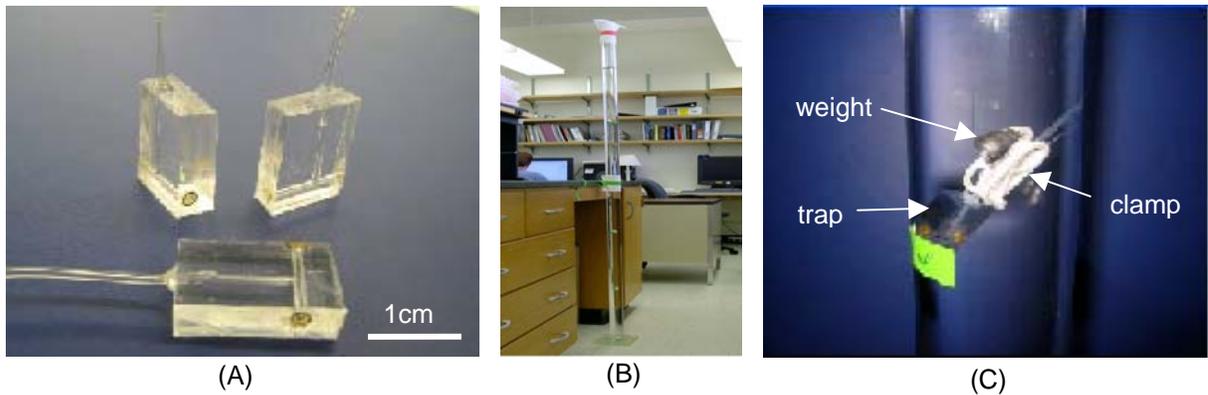


Figure 3. Fabrication process for microchamber. (A) Pour PDMS over silanized glass mold. (B) After curing PDMS, peel it off the mold. (C) Bond another flat piece of PDMS to the first piece to seal the channels. (D) attach tubing and gold grids.

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 labeled water column and held at a fixed depth. Figure 4 shows completed traps, the water column, and a microchamber in the water column at 1-ft depth. The duration of the experiment will vary from 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 was relatively easy to construct. It was made from a tube of clear acrylic plastic, sealed at one end. The column is about 5' 10" tall, such that five microchambers can be suspended at 1-foot intervals using fishing line. The column will be filled with hydra media and each microchamber will be weighted down with a fishing sinker. Each test requires fresh microchambers (the traps cannot be reused). In addition, the water column is cleaned with diluted bleach and rinsed in DI water several times before each new test run to ensure consistent environmental conditions for each test and to prevent bacterial contamination.



*Figure 4. (A) Photo of microchambers (i.e. traps), prior to hydra injection. (B) Photo of water column, secured against the lab bench. (C) Close-up of trap in a water column at 1-ft. Injection tube is clamped off and the whole trap is suspended and weighted using fishing line and sinkers, respectively.*

### **Preliminary Results:**

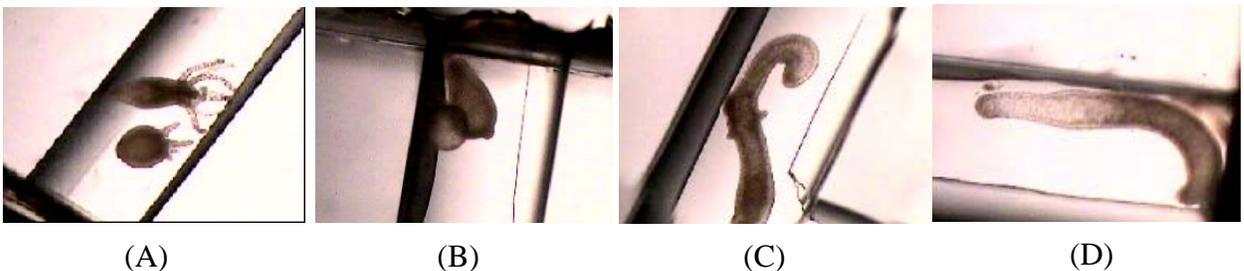
We have studied hydra regeneration under normal conditions (i.e. no mechanical compression). Animals were dissected such that either a head, foot, or both head and foot

were removed. We observed that normal hydra regeneration takes roughly 3 – 4 days. Photos from hydra feet regeneration is shown in Figure 5.



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

Over the past three months, several experiments were performed with the microchambers placed at 1-foot intervals in the water column. After the first test run, we decided to inject only the lower half of the animal (foot region) to facilitate easier observation (i.e. bud formation). Each trap contained two tissue samples taken from the lower half of two separate animals. Our preliminary results suggest a correlation between mechanical compression and stem cell regeneration. With different amounts of pressure, the animals experienced different rates and quality of regeneration. Figure 6 consists of photos of hydra in traps placed at different depths during the most recent test run, and it shows the altered regeneration of hydra in response to mechanical compression. At 1-foot depth, hydra tends to regenerate more tentacles (eight as opposed to a maximum of six under normal conditions, Figure 6(A)). In addition, tentacle buds will normally form around day 2. However, in Figure 6(B), the hydra at 3-foot depth has only regenerated one bud at the end of day 3. Furthermore, Figures 6(C) and (D) show animals at 4- and 5-foot depths, respectively. As shown, the two tissue samples at both depths have fused, but only the tissue at 4-foot depth started to generate buds at the end of day 3, and only three buds total. At the end of the 5-day run, the animals at 3-foot and 4-foot depths had fully regenerated at least six tentacles, with the tissues at 4-foot depth still fused together. At 5-foot depth, the animal seemed to split into two separate animals – one did not survive while the other finally generated buds at one end of the body column.



*Figure 6. (A) Hydra with eight tentacles at 1-foot depth (Day5). (B) Hydra regenerated one bud at 3-foot depth (Day3). (C) Two hydras fused together on the head with bud forming in the middle at 4-foot depth (Day3). (D) Two hydras fused together without buds at 5-foot depth (Day3). This sample eventually separated into two animals, one of which finally generated buds at Day 5.*

Prior runs have produced similar results, such as 8-tentacled hydra at 1-foot depth, as well as dissimilar results (8-tentacled hydra have also appeared at 5-foot depth and animal fusion does not occur every time). However, these preliminary results have led us to hypothesize that there is a relationship between the amount of mechanical compression, available resources (including number of stem cells), and the ability for hydra to regenerate. Under mechanical compression there may be an over-stimulation of the foot portion of hydra to regenerate the missing head. This may explain the appearance of 8-tentacled hydra. Also, under increasing amounts of pressure, more resources may be needed to achieve proper regeneration; hence animals at lower depths (3-foot depth and below) exhibit slowed or incomplete regeneration by the end of the run (day 5). The latest results have led us to suspect that there is a pressure threshold around 3-foot depth that caused animals to join together to recruit sufficient resources (more stem cells, nutrients, etc.) in order to regenerate under higher external mechanical pressure.

During the summer, we intend to verify these hypotheses. We intend to do a sufficient number of additional regeneration runs to verify that the results that we have gathered thus far are repeatable, and thus consistent with the notion that mechanical compression has an effect on stem cell differentiation. By inserting more microchambers into the water column (at 3 – 6 inch intervals) we will be able to pinpoint the pressure threshold for incomplete regeneration. We will also perform longer test runs to allow all animals to achieve full regeneration at all depths. Also, the pH of the hydra media in the water column should be monitored throughout the duration of all future tests to assess whether the media chemical composition is changing during the tests. This may have an adverse effect on the tissue and will affect our results. Once we establish a more consistent baseline for these experiments, further quantitative testing will begin.

To date, 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. In the future we intend to perform more qualitative and quantitative tests to assess the quality of the regenerated animals. For example, the animal's natural instinct to eat may be compromised if in the presence of food it does not eat. Mouth functionality can also be assessed through a mouth opening assay involving glutathione. Foot formation can be tested by placing a glass rod next to the foot and observing whether it sticks to the rod. Additional test 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 in fact normal. However, our preliminary results are encouraging as the regeneration characteristics of the hydra (and thus stem cell differentiation) appear to be influenced by mechanical forces. Now that the testing apparatus and protocol has been established, we intend to spend the summer exploring this possibility further with additional more comprehensive testing.

**Specific responsibilities of the student:**

I will be responsible for microchamber construction, animal dissection, cleansing the water column and acquiring materials under the guidance of my faculty advisor and a post-graduate researcher. I will also be responsible for injecting the animals into each microchamber, performing the test runs, and collecting and analyzing the data generated from each run. Between each test run, I am also responsible for ensuring a consistent environment throughout the water column to prevent inconsistent data by bleaching/cleaning water column prior to each test and pH testing throughout every test. 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 forces as well as developing and confirming hypotheses regarding the response of stem cells to these forces.

**Project timeline:**

The project period is 3 months. List of tasks per month:

**June/July:**

- (1) Perform further regeneration runs (at least 3 runs, 5-days each, one trap per foot in the water column)
- (2) Construct additional microchambers.
- (3) Bleach/clean water column prior to each test and perform pH testing of the hydra media throughout every test.
- (4) Analyze the dissected tissue samples after each test.

**August:**

- (1) Perform regeneration runs (5 – 8 days) with additional data points in the water column (one microchamber at 3 – 6inch intervals in the water column).
- (2) Construct additional microchambers.
- (3) Bleach/clean water column prior to each test and perform pH testing of the hydra media throughout every test.
- (4) Analyze the dissected tissue samples after each test.

**September:**

- (1) Perform longer term tests with additional data points in the water column (duration – 2-3 weeks, two-three traps per foot of hydra media)
- (2) Construct additional microchambers.
- (3) Bleach/clean water column prior to each test and perform pH testing of the hydra media throughout every test.
- (4) Analyze the dissected tissue samples after each test.
- (5) Compare results and hypothesize a relationship between mechanical forces and hydra regeneration.

**References:**

- [1] J. Darnell, H. Lodish, D. Baltimore, *Molecular Cell Biology*, 2<sup>nd</sup> 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.