Sowing the Seeds of Neuroscience

Heads and Tails:

Investigating the Effects of Plant Extracts on Planaria Regeneration

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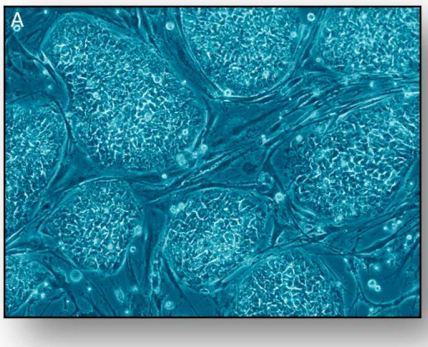
Activity Time: Two class periods, 50 minutes each, to introduce and set-up the experiment, plus additional time (10-15 minutes) in each subsequent class for up to two weeks to assess regeneration.

Lesson Summary:



This lesson is designed to introduce students to the concepts of stem cells and regeneration using planaria as a model organism. Students will learn how neuroscience research promotes health and leads to understanding and therapies. Additionally, students will discuss ways to do such research ethically. In the lab portion, students will explore how the plant extracts they made previously in the *Infusions & Decoctions: Preparing Plant Extracts* lesson affect planaria regeneration.

This lesson should be completed after the *If Worms Drank Coffee: Investigating the Effects of Plant Extracts on Planaria Movement* lesson, which includes introductory material to planaria.

STUDENT UNDERSTANDINGS

Big Idea & Enduring Understanding

- **Regeneration:** Planaria are incredible regenerators and as such, are used as a **model organism** to understand **stem cells** and **regeneration**. Plant extracts may have an impact on the speed of regeneration, among other factors.

Essential Question

- How do plant extracts affect the rate of planaria regeneration?

Neuroscience Core Concepts

- Brain research promotes health and leads to understanding and therapies.
- Neuroscience research must be done in an ethical manner.
- Testing chemicals or plant extracts on animals is a way to determine their likely effects on humans.

- Testing chemicals or plant extracts on animals to determine safety is more ethical than testing on humans before these safety tests have been done.
- Research on humans is an essential final step before new treatments are introduced to prevent or cure disorders.
- The plant world is filled with species that contain chemicals with medical properties, including neuroactive properties.
- There are many people with mental and neurological disorders in our society. Finding cures for these disorders is a social imperative.

Learning Objectives

Students will know…

- Model organisms are non-human organisms that scientists study to better understand something. The hope is that discoveries made on this organism can be used to help understand other organisms, including humans.
- Planaria are used as model organisms to learn about regeneration.
- Planaria have omnipotent stem cells that are flexible; they can become ***any*** other type of cell and can even grow into an entire organism.
- Testing chemicals or plant extracts on animals to determine safety is more ethical than testing on humans before these safety tests have been done.
- Research on humans is an essential final step before new treatments are introduced to prevent or cure disorders.

Students will be able to…

- Demonstrate lab safety procedures.
- Follow the procedures to successfully conduct an investigation of the effect of a plant extract on planaria regeneration.
- Closely observe planaria using a dissecting microscope and draw them at different stages of regeneration.
- Generate and record data of planaria responses to different concentrations of chemical extracts found in plants.
- Evaluate class data to understand and explain the effects of plant extracts on planaria regeneration.

Standards Alignment

Washington State Essential Academic Learning Requirements (EALRs): Science
Science EALR 2: Inquiry • 6-8 INQA—Question • 6-8 INQB—Investigate • 6-8 INQC—Investigate • 6-8 INQD—Investigate • 6-8 INQE—Model • 6-8 INQF—Explain • 6-8 INQI—Consider Ethics Science EALR 4: Life Science • 6-8 LS1A • 6-8 LS1C
Next Generation Science Standards (NGSS)
From Molecules to Organisms: Structures and Processes • MS-LS1-2 • MS-LS1-3
Common Core Standards: English Language Arts (ELA)
Reading Standard for Literacy in Science and Technical Subjects: Key Ideas & Details • CCSS.ELA-Literacy.RST.6-8.1 • CCSS.ELA-Literacy.RST.6-8.3 Writing Standard for Literacy in Science and Technical Subjects: Text Types & Purposes • CCSS.ELA-Literacy.WHST.6-8.2d • CCSS.ELA-Literacy.WHST.6-8.2f Language Standard 4c & 6: Vocabulary Acquisition & Use • CCSS.ELA-Literacy.L.6.4c • CCSS.ELA-Literacy.L.6.6

TEACHER PREPARATION

Materials

Classroom Materials

Item	Quantity
Copies of *Planaria Regeneration Background Reading—Student Handout*	1 per student
Copies of *Ethics of Research with Animals—Student Handout* (located in *Appendix*)	1 per student
Copies of *Planaria Regeneration Planarian Drawings—Student Handout*	1 or more per student, depending on how many days of observation
Copies of *Planaria Regeneration Lab Procedure—Student Handout*	1 per lab group
Copies of *Planaria Regeneration Results & Conclusions—Student Handout*	1 per student
Optional: Copies of *Planaria Regeneration Vocabulary Quiz—Student Handout*	1 per student
Student lab notebook	1 per student
Pen	1 per student
Dissection microscopes	1 per group if possible

Laboratory Materials

Review the materials with your students. It will be helpful to show them each piece of lab equipment and mention how it will be used in this activity.

Most lab materials are provided in the *Sowing the Seeds of Neuroscience* classroom kit. There are enough materials in the classroom kits for ten groups to do this laboratory. Depending on class size, groups should be made of two to three students.

Item	Quantity
Planaria	4 per group
250 ml beaker to dispose of razor blades	1 per teacher
Petri dish (3 extracts, 1 control, and 1 dish for cutting)	5 per group
Paint brush	1 per group
10 ml graduated cylinder	1 per group
Straight razor blade	1 per group
100 ml beaker • One beaker for conditioned water. • One beaker for "recovery"—the planaria will go here after the experiment to rinse off any extracts before they go back to their aquarium.	2 per group
Conditioned water	~150 ml per group

Medicine cup (aka “ketchup cup”)	3 per group
Plant extracts obtained as a result of *Infusions and Decoctions* lab.	1 per group

Safety Materials

Item	Quantity
Safety goggles*	1 per student
Gloves*	1 pair per student

*Safety materials are provided in the classroom kit only if you requested them.

Lab Safety

- Students must not eat or drink anything in the lab.
- Students must never taste any of the plant extracts
- Students will be using razor blades in this investigation. These blades are extremely sharp. Please demonstrate how to safely hold a razor blade and cut a planarian.
- Razor blades must be properly disposed. Set up a labeled sharps container (such as a plastic food container with a hole cut in the lid) in the lab for students to discard their used razor blades. You may be able to dispose of the blades in a medical waste sharps container in your school nurse’s office; alternatively, make sure the blades are in a rigid container with a lid, tape it shut, and label it “sharps.”

Preparation

- This lesson should be completed after the *If Worms Drank Coffee: Investigating the Effects of Plant Extracts on Planaria Movement* lesson, which includes introductory material to planaria. If this lesson is delivered without first completing the planaria movement lesson, then students should be assigned the *Planaria Movement Lab Background Reading—Student Handout* from that lesson.
- Photocopy Student Handouts.
- Assign the *Planaria Regeneration Background Reading—Student Handout* to students as homework; this reading should be completed before students begin the lab. They should answer the questions at the end of the handout, but wait to develop a hypothesis.
- Plan your timing: It will work best to do this lab activity over two full days, with additional time on subsequent days for students to check the stage of regeneration in their planaria.

- On Day One, conduct the *Engage* activities, review with your students how to use dissection microscopes, demonstrate safe use of the razor blade and cutting a planaria in half, and guide students through a review of the background reading, learning goals, and vocabulary. It is also a good idea to talk about which plant extracts might change the rate of regeneration.

- On Day Two, challenge student groups to conduct the investigation as described in the *Explore* section. Also deliver the *Explain*, *Elaborate*, and *Evaluate* activities.

- Determine how you will have students check their regenerating planaria in the next days to weeks. The length of time needed for regeneration will vary depending on the temperature of your classroom. At 70° F, full regeneration takes 4-7 days. At 60° F, it can take three weeks or more. Will you have students check every day or every other day? Will all students need to check and draw what they observe, or can one student per team check and draw what they observe? Will you check every day until something different appears and then prompt students to start checking? How will you score students' drawings and observations?

- Determine how best to set up your classroom for the lab: Most lab materials are provided in the *Sowing the Seeds of Neuroscience* classroom kit. There are enough materials in the classroom kits for ten groups to do this laboratory. Depending on class size, groups should be made of two to three students.

- If you haven't already discussed the ethics of using animals in research—including the use of animals in the science classroom—please do so before delivering this lesson. If students haven't already read the *Ethics of Research with Animals—Student Handout* (located in the *Appendix*), assign this now. In addition, please refer to the *Teacher Background & Resources* section of this lesson plan for a list of helpful teaching resources on this topic.

  Specifically, it is important to discuss that this lab will require students to cut a planarian (flatworm) in half and expose one half to plant extracts. It may be helpful for students to know that planaria can reproduce both sexually and asexually. When they reproduce asexually, the posterior part attaches to a rock and the top pulls away and splits off. When we cut the planarian in half, we do something similar to what occurs naturally in planaria reproduction. No need to feel badly about it. However, like any animals used in the laboratory, we must treat planaria carefully and respectfully.

- Consider an alternate activity that can be provided for students who have an ethical objection to the use of animals in the science laboratory. For example, a student may opt to participate in the lab activities but opt out of being the person who actually cuts the planarian. A list of suggested activities is included in the *Appendix* to this curriculum.

TEACHER PROCEDURE

Day One

Engage

1. Explain the purpose of the lesson and review the Big Idea, Enduring Understanding, and Essential Question.

2. If students did not read the *Planaria Regeneration Background Reading—Student Handout* as homework, they should do so now. They should answer the questions at the end of the handout, but wait to develop a hypothesis.

3. Review with students how identical twins form, as is described on the Student Handout. Ask the students to discuss:

    a. Why can very young embryos split into two parts which grow into two genetically identical humans (identical twins), but older embryos, babies, and adults cannot do this?

    b. What are the differences in regeneration between adult planaria and adult humans? (If you cut a planarian in half, each half will regenerate the other. If you cut a human in half, each half will die.)

    c. What cells, which are present in planaria (and in very young human embryos) but not in human adults, enable this to occur?

4. In this lab investigation, students will use the plant extracts that they made during the *Infusions & Decoctions* lab, exploring how these plant extracts may influence regeneration in planaria after they are cut in half. In order to help students generate their hypotheses, discuss their thoughts on how they think plant extracts might influence regeneration.

5. Ask students to share their ideas about herbs used in their families or cultures to treat injuries or speed wound healing. As listed on the Student Handout, some herbs used to speed wound healing include:

    - *Astragalus*
    - *Centella* (Gotu kola)
    - Comfrey (called “boneknit” or “healing blade”)
    - *Ginkgo biloba*
    - Hawthorne
    - St. John’s wort
    - Arnica (*Arnica montana*)
    - Aloe vera

6. Ask students to share their predictions about how stimulants and depressants might affect regeneration. Stimulants (such as coffee, tea, ginger, or ginseng) speed the metabolism and thus may increase the speed of regeneration. However, these may also slow down regeneration by stimulating other body functions and thus taking nutrients and energy away from regeneration.

7. Allow each student group time to develop their hypothesis, as prompted at the end of the Student Handout. You may want to review and approve each group's hypothesis before they begin the lab investigation.

8. Tell students that though humans have used herbs and plants to help with healing for thousands of years, scientists are only now studying the effects of some of these plants on healing. The investigation they are about to begin might lead to new information that could one day be used in treating neurological disease or helping people recover from accidents.

Explore

9. (**Note:** If students have completed the *If Worms Drank Coffee* lesson, skip this step).

   If students haven't already completed the *If Worms Drank Coffee* lesson and planaria movement investigation, encourage them to conduct a free exploration and observation of the planaria. Take a planarian out of the jar and put it in a petri dish with just a few drops of water. What do students observe? Can they find the anterior and posterior sides? The dorsal and ventral sides? The eyespots?

10. To be successful in this lab investigation, students will need to be skilled at observing their planarian under the dissecting microscope. Allow time for students to practice using the dissecting microscope with a planarian in a petri dish with water. It is much easier to find the planarian and focus the microscope if the planarian is in only a few drops of water. Indicate that they will need to be very comfortable observing their planarian under the dissecting microscope and assist any students who need help.

    Challenge students to draw the planarian, as observed under the dissecting microscope, on the *Planaria Regeneration Drawings & Observations—Student Handout* or in their lab notebooks. Their drawings should be neat, careful, and include a label for the eyespots. Tell students to use the title "Day 1, Uncut Planarian."

11. Review lab safety information with student. Remind your students that the razor blades are extremely sharp. Remind them to be gentle when handling the planaria.

12. Hand out copies of *Planaria Regeneration Lab Procedure—Student Handout* and review the lab procedures.

13. Explain why a "control" is an important part of a scientific investigation. In this case, the conditioned water serves as the ***controlled variable,*** allowing students to observe that it is not the water that affects the speed of regeneration. In this investigation, the ***independent variable*** is the plant extract and the ***dependent variable*** is the speed of the planarian's regeneration. Using the water control ensures that the changes to the speed of regeneration (or death of the planarian) is a result of the bioactive chemicals in the plant extracts, not from the planarian's reaction to water.

14. Some practice in dilution and measurements of solutions will be helpful for the students. Demonstrate how to measure 10 ml using a graduated cylinder and then discuss dilution. Your students will need to carefully follow the directions on the Student Handout to dilute their extracts to 10%, 5%, and 2.5%.

15. Demonstrate how to remove a planarian from the aquarium or beaker using a paintbrush, place it in a few drops of water in a petri dish, and cut it in half.

Day Two

16. Tell students to make a drawing of the cut planaria on the *Planaria Regeneration Drawings & Observations—Student Handout.* They should use the title "Day 1, Cut Planarian." Alternatively, you can ask students to make their drawings in their lab notebooks.

17. Encourage students to work through the procedures on the Student Handout and to record their data in their lab notebooks.

18. When students have all completed the investigation, discuss the plan for checking the planaria, making their drawings, and recording their data. Each time they check their planaria, the students will need to make a drawing and label it with the date.

Explain

19. The length of time needed for regeneration will vary depending on the temperature of your classroom; it could take four days or three weeks for all the "water control" planaria to complete their regeneration (both eye spots visible). When this has occurred, stop the experiment.

20. Distribute copies of *Planaria Regeneration Results & Conclusions—Student Handout*, one per student. Challenge students to work with their group members to answer the questions in the "Results" section of the handout.

21. Bring the class together to discuss the results of their investigation. Ask each lab group to share with the class the results of their investigation.

    - What extract did they use?

    - What similarities and differences between the water control and the different dilutions of the plant extract did they observe? Were there any differences in the speed of regeneration? Did any dilutions cause the death of the planaria? *(If any of the planaria die during the investigation, it is important to discuss with students how this data is helpful in adding to our understanding of how plant extracts effect regeneration; in actual scientific research into treatments for neurological diseases, the death of the model organism provides valuable safety information before the substance in question is tested in humans.)*

    - If more than one group tested the same plant extract, were each group's results similar? In what ways? How did they differ?

    - Did your observations support or refute your hypothesis?

    - Does the class have enough information to determine if any of the plant extracts act as stimulants or depressants on the planaria regeneratioin process? If yes, what is their evidence? If no, what additional evidence would they require?

Elaborate

22. Share with students the following story about the healing qualities of banana plant mash:

When I was your age, I lived on the island of Ponape far out in the Pacific Ocean. It was a tropical, volcanic island covered with jungle and lots of banana plants. One day while playing outdoors with no shoes on (we never wore shoes), I stubbed my big toe on a sharp object. It was bleeding badly! Two gardeners working close by heard my cries. Immediately the younger man came running and taking his long machete, he cut down a small banana plant in our yard. I was confused. I didn't understand why he was chopping it down! The older man filled a tub with water, and started chopping up the stalk of the banana plant in the water to make a mash of banana stalk. All I could do was stare at the mash.

"Put your foot into the tub!" said the older man. Dazed, I followed his directions. To my amazement the blood stopped flowing! I started to pull my foot out. "No, no your foot is not healed yet" he said. "Keep your foot in a little longer." It took about 20 minutes. When I pulled my foot out, my big toe looked as if it were healed! I sat on the ground staring at my toe, thinking, "How can a cut heal so quickly?"

Once in a while I think about this event and I wonder, "What was in those banana plants to create such healing? How can I find out?"

23. Tell students that the plant world is filled with species that contain chemicals with medical properties, including neuroactive properties. Like in the banana story, traditional healers use plants to treat illness and injuries. Current research seeks to understand the potential health benefits of different plants and is exploring whether documented health benefits are due to single chemicals found within the plant or many chemicals within the plant working together.

Evaluate

24. Ask students to return to the *Planaria Regeneration: Results & Conclusions—Student Handout* and to work independently to respond to the prompt in the "Conclusions" section. (A scoring guide has been provided for this learning task).

25. ***Optional:*** Assign a vocabulary quiz using the terms introduced on the Student Handouts. Hand out copies of *Planaria Regeneration Vocabulary Quiz—Student Handout* and administer it like a quiz, with no peeking at lab notebooks or the Background Reading. An answer key for the quiz is provided in the *Scoring Guides* section of this lesson plan.

SCORING GUIDES

Answer Key for Vocabulary Quiz Handout
7 possible points.

1. Conception
2. Dopamine
3. Meniscus
4. Model organism
5. Omnipotent cell
6. Regenerate
7. Stem cells

Scoring Rubric for Drawings & Observations Handout
15 or more possible points. (You may want to increase the total amount of possible points depending on how many drawings/observations are made by students during the regeneration time).

Students are asked to check their planaria on a regular basis (based on teacher's preference). During each of these checks, students should draw a sketch of the planaria on the handout (or in their lab notebooks) and make notes on their observations. In particular, students need to label their sketches with the date and label eyespots if/when they appear.

Dimension	Needs Work (0 Points)	Basic (1 Point)	Proficient (3 Points)	Advanced (5 Points)
Labels	No labels included.	Some items that need to be identified have labels. It is not always clear which label goes with which structure.	Almost all items that need to be identified have labels. It is always clear which label goes with which structure.	Every item that needs to be identified has a label. It is always clear which label goes with which structure.
Drawing	Overall, the quality of the drawings is poor.	Overall, the quality of the drawings is fair.	Overall, the quality of the drawings is good.	Overall, the quality of the drawings is excellent.
Observations	Notes rarely taken or of little use.	Dated notes are taken occasionally, but accuracy of notes might be questionable.	Dated, clear, accurate notes are taken occasionally.	Clear, accurate, dated notes are taken regularly, each time that the planaria is checked.

Rubric created with help from Rubistar.4teachers.org

Scoring Rubric for Results & Conclusions Handout
17 possible points.

Results

Dimension	Needs Work (0 Points)	Basic (1 Point)	Proficient (3 Points)	Advanced (5 Points)
Names the plant extract tested by their group.	Incomplete or incorrect.	Correctly names the plant extract.	N/A	N/A
Provides the hypothesis for the plant extract.	Needed adult assistance to develop hypothesis.	Independently developed a hypothesis somewhat substantiated by previous web research or observation of similar phenomena. Did not use the "If, Then" format.	Independently developed a hypothesis somewhat substantiated by previous web research and observation of similar phenomena. Used the "If, Then" format.	Independently developed a hypothesis well-substantiated by previous web research and observation of similar phenomena. Used the "If, Then" format.
Explains the similarities and differences between the water control and each dilution.	Adult help needed to identify and define the similarities and differences between the control and each dilution.	Independently identified the similarities and differences for the dilutions, but did not make a comparison with the control.	Independently identified the similarities and differences for the dilutions, and made a comparison with the control. If applicable, identifies which dilutions caused the death of the planaria.	Independently identified and clearly defined the similarities and differences for the dilutions and made a comparison with the control. Made references to sketches. If applicable, identifies which dilutions caused the death of the planaria.
Includes a description of the differences in the speed of regeneration	No description provided for the different dilutions.	Somewhat clearly described the changes in the planarian's regeneration	Clearly described the changes in the planarian's regeneration among the	Clearly described the changes in the planarian's regeneration among the

		among the control and the different dilutions.	control and the different dilutions.	control and the different dilutions and supported the description with evidence.
States whether observations supported or refuted hypothesis. Supports this statement with evidence.	No conclusion was apparent or important details were overlooked.	Student provided a conclusion with some reference to the data and the hypothesis statement.	Student provided a somewhat detailed conclusion clearly based on the data and related to the hypothesis statement.	Student provided a detailed conclusion clearly based on the data and related to previous research findings and the hypothesis statement.

Rubric created with help from Rubistar.4teachers.org

Conclusions

15 possible points.

Dimension	Needs Work (0 Points)	Basic (1 Point)	Proficient (3 Points)	Advanced (5 Points)
Includes a Hypothesis	Needed adult assistance to develop hypothesis.	Independently developed a hypothesis somewhat substantiated by previous lab results or observation of similar phenomena. Did not use the "If, Then" format.	Independently developed a hypothesis somewhat substantiated by previous lab results and observation of similar phenomena. Used the "If, Then" format.	Independently developed a hypothesis well-substantiated by previous lab results and observation of similar phenomena. Used the "If, Then" format.
Describes Investigation Set-up		Somewhat clearly described an investigation to test the effect of banana plant extract on planarian's regeneration.	Clearly described an investigation to test the effect of banana plant extract on planarian's regeneration. Included but did not clearly define	Clearly described an investigation to test the effect of banana plant extract on planarian's regeneration. Included and clearly defined a

		Did not include a controlled variable (water).	a controlled variable (water), independent variable (banana plant), and dependent variable (speed of regeneration).	controlled variable (water), independent variable (banana plant), and dependent variable (speed of regeneration).
Explains Methods of Data Collection or Type of Data to be Recorded	No description provided.	Somewhat clearly described the type of data to be collected.	Clearly described the type of data to be collected.	Clearly described the type of data to be collected and explained how the data would be organized or communicated.

EXTENSIONS

- Planaria are ***phototaxic*** and will move to avoid light. However, they can only avoid the light if they have functioning eyespots. Students can use a flashlight to direct a bright light at uncut planaria and observe their behavior. Students can test this every day with their cut planaria (or every day after their planaria develop eyes) and record the number of days that are required after cutting before the planaria actively avoid the light. It is likely that the eyes can be seen before they start functioning. (**Note:** The light avoiding behavior is more obvious if planaria are kept in a dark room, in a drawer, or covered with a cloth.)

- Many plants and herbs are used by acupuncturist, naturopaths, and traditional healers to help people heal from injuries. Challenge students to choose one of the plants listed below, research their healing and/or tissue regenerative properties, and write a story similar to the banana plant mash story provided in the *Elaborate* section of this lesson plan:

    - *Astragalus* (Huan Qi)
    - *Centella* (Gotu kola or ji xue cao)
    - Comfrey (called "boneknit" or "healing blade")
    - *Ginkgo biloba* (bai guo)
    - Hawthorne (Shan zha)
    - St. John's wort
    - Arnica (*Arnica montana*)
    - Aloe vera

- Stem cells and model organisms are currently being used to understand several neurological diseases such as Parkinson's disease, Huntington's disease, cerebral ischemia, and multiple sclerosis. Challenge students to research one of these diseases and to write a report describing

how neuroscience research has formed the basis for significant progress in treating the disorder. Also, encourage students to explore the following tutorials:

Stem Cell-Based Therapies—Click & Learn
HHMI Biointeractive
http://www.hhmi.org/biointeractive/stem-cell-based-therapies

Stem Cells: Cells with Potential
Exploratorium
http://www.exploratorium.edu/imaging-station/research/stem_cells/story_stem_cells1.php

- Share with students one or more of the animations or tutorials listed in the *Resources & Background* section of this lesson plan, including animations showing tissue regeneration in different animals.

- Challenge students to choose one of the careers listed in the Career Connections section of this lesson plan and to consider how a person in that career might use model organisms and plant extracts.

TEACHER BACKGROUND & RESOURCES

Resources

- **The Science and Ethics of Stem Cell Research: Plenty of Planaria**
  A lesson plan for grades 7-12 focusing on stem cell research, using planaria as a model organism. This lesson is part of a larger curriculum, *The Science and Ethics of Stem Cell Research*, from the Northwest Association for Biomedical Research.
  http://www.nwabr.org/education/pdfs/STEM_CELL_PDF/Planaria_Teacher.pdf

- **The Science and Ethics of Animal Research**
  A curriculum for grades 6-12 from the Northwest Association for Biomedical Research about the ethics of using animals in scientific and medical research.
  http://nwabr.org/curriculum/animals-research

- **Speaking Honestly – Animal Research Education (SHARE)**
  A program designed to guide educators in leading a discussion-based classroom activity on the different views on the use of animals in research. The activity requires a single class period of 50-75 minutes.
  http://sharehappens.org/

- **Using Model Organisms**
  Sowing the Seeds of Neuroscience
  http://www.neuroseeds.org/links/model-organisms

- **About Planaria**
  Sowing the Seeds of Neuroscience
  http://www.neuroseeds.org/links/planaria

- **Planarian Regeneration & Stem Cells Video**
  HHMI Biointeractive, 11:47 minutes
  http://www.hhmi.org/biointeractive/planarian-regeneration-and-stem-cells

Animations & Tutorials

- **Newt Limb Regeneration Animation**
  HHMI Biointeractive, 1:21 minutes
  http://www.hhmi.org/biointeractive/newt-limb-regeneration

- **Zebrafish Heart Regeneration Animation**
  HHMI Biointeractive, 2:30 minutes
  http://www.hhmi.org/biointeractive/zebrafish-heart-regeneration

- **Tissue Regeneration in Animals—Click & Learn**
  HHMI Biointeractive
  http://www.hhmi.org/biointeractive/tissue-regeneration-animals

- **Stem Cell-Based Therapies—Click & Learn**
  HHMI Biointeractive
  http://www.hhmi.org/biointeractive/stem-cell-based-therapies

- **Planaria: A Window on Regeneration**
  Exploratorium
  http://www.exploratorium.edu/imaging-station/research/planaria/story_planaria1.php

- **Stem Cells: Cells with Potential**
  Exploratorium
  http://www.exploratorium.edu/imaging-station/research/stem_cells/story_stem_cells1.php

Career Connections

Aquarist: An aquarist is a caretaker for aquatic plants and animals. An aquarist might work for a public aquarium, caring for the fish, coral, and other organisms that are on display. Alternatively, an aquarist might work for a research organization that conducts research with aquatic species.

Biologist: A scientist that studies living organisms and their environments. Biologists may specialize in botany (the study of plants), zoology (the study of animals), or many other specialties.

Chemist: A scientist specializing in chemical science. A chemist studies the properties of matter. Biochemistry, a specialty within the field of chemistry, is focused on the chemical processes that occur among living organisms, such as plants.

Neurologist: A physician who has specialized in neurology, the study of the brain and nervous system. Neurologists are trained to diagnose and treat neurological disorders.

Neuroscientist: A scientist who studies the brain and nervous system. Neuroscientists are primarily concerned with research.

Pharmacologist: A biomedical scientist who studies the interactions between drugs and cells, tissues, organs, or entire organisms. Pharmacologists are primarily concerned with research.

Physician: A medical doctor who provides healthcare for his or her patients. In the United States, a physician must have a M.D. (Doctor of Medicine), O.D. (Doctor of Osteopathic Medicine), or N.D. (Doctor of Naturopathic Medicine) license to practice medicine.

Research scientist: A scientist of any scientific discipline (such as biology, chemistry, or medicine) who focuses on conducting research in order to contribute to the knowledge of their field. Most research scientists work for universities, government agencies, non-profit research organizations, and private research companies.

Traditional healer: A person who provides medical treatment and advice based on the traditional healing practices of his or her culture. May include shamans, diviners, acupuncturists, and herbalists. Traditional healers use plants and natural remedies instead of synthetic medications.

Illustrations and Photograph Credits

Photograph of Human Embryonic Stem Cells. Courtesy of Wikimedia, Vojtech.dostal. 25 September 2011. Retouched work; originally published in "Follow the Money – The Politics of Embryonic Stem Cell Research." Russo E, *PLoS Biology* Vol. 3/7/2005, e234. Image author: Nissim Benvenisty.

Photograph of Young Saba Banana Plants. Courtesy of Wikimedia, Obsidian Soul. 15 January 2011.

Comparison of Typical Zygote Development in Twins. Courtesy of Wikimedia, Trlkly. 30 April 2010. Recreated SVG version of original by Wikipedia editor user: ChristinaT3.

Photograph of Identical Twin Babies. Courtesy of Wikimedia, Glow at the Danish language Wikipedia, da.wikipedia. 17 June 2005.

Photograph of Planaria and Lab Procedures. Courtesy of Eric Chudler and Kristi Strauss, University of Washington.

Photograph of Aloe Vera Plant. Courtesy of Wikimedia, 3268zauber. 24 April 2009.

Bibliographic Credits

Barker R, Jain M, Armstrong R, and Caldwell M. "Stem Cells and Neurological Disease." *Journal of Neurology, Neurosurgery, and Psychiatry* 74 (2003): 553-557. Web. 28 January 2013. <http://jnnp.bmj.com/content/74/5/553.short>.

Fields S and Johnston M. "Whither Model Organism Research?" *Science* 307.5717 (2005): 1885-1886. Web. 28 January 2013. <http://www.sciencemag.org/content/307/5717/1885.summary>.

Northwest Association for Biomedical Research. "Plenty of Planaria." *The Science and Ethics of Stem Cell Research, Third Edition.* 2009. PDF file. <http://www.nwabr.org/education/pdfs/STEM_CELL_PDF/Planaria_Teacher.pdf>.

PLANARIA REGENERATION BACKGROUND READING
STUDENT HANDOUT

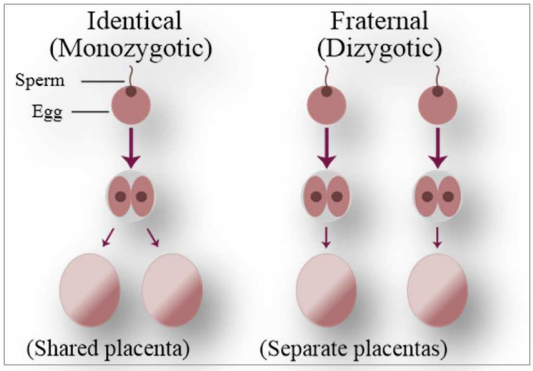
Name:_____ Date:_____ Period:_____

Background Reading for Planaria Regeneration Lab

Identical Twins

Do you know any identical twins? The way that identical twins form is an amazing process that can teach us something about **regeneration**. Early in development, the embryo splits in half, and then each part grows into a whole embryo; each embryo grows into a whole baby. These babies are always the same sex because they are identical—they came from one fertilized egg and share all the same genetics.

In humans this process is only possible in the first three days after **conception**. Human embryos are only flexible enough to divide in half and become twins in the first three days after conception. Older embryos, newborn babies, and adults cannot do this—because our cells are no longer so flexible! Once our cells start to differentiate into different cell types, in most cases they have "committed to their fate" and can't become a different type of cell. So later in development, if you divide the embryo in two, each half cannot regenerate the other half. And certainly, in adults and kids, we can't just cut ourselves in half and watch each half grow into a twin of the other half. But this is what planaria do!

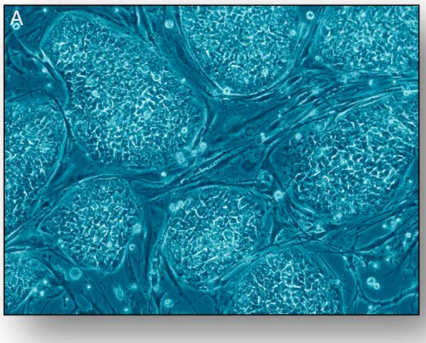


Amazing Regenerators

Planaria are amazing regenerators. You can cut an adult planarian in half and it will grow into two whole identical planaria. Even more amazing, you can cut a tiny fragment off of a planarian and that tiny piece will regenerate a whole new planarian. Because planaria are easy to keep in the laboratory and have this amazing regenerating ability, they are used as **model organisms** to learn about regeneration. Model organisms are non-human organisms that scientists study to better understand something. The hope is that discoveries made on this organism can be used to help understand other organisms, including humans.

Research with Stem Cells

One thing that scientists have learned by studying planaria is that they are such good regenerators because they have a lot of special **stem cells** (the photo below shows human stem cells). Stem cells are undifferentiated cells that can make more of themselves and develop into different cell types (**differentiate**)—but may not be able to differentiate into all cell types.



The stem cells in planaria are called **omnipotent stem cells** because they can become ***any*** other type of cell and thus can grow into an entire organism! Humans have omnipotent stem cells only in the first three days after conception—and this is the only time that you can divide a human in half and it will regenerate into two humans, creating identical twins. Planaria are such good regenerators because they have omnipotent stem cells throughout their entire lives and they have a lot of them—20-30% of all planaria cells are these omnipotent stem cells.

Scientists are currently studying planaria and other model organisms to learn how stem cells can promote health and lead to therapies for disease. Right now, scientists are exploring how to use stem cells to grow new organs for transplantation, to treat neurological diseases, and to help repair spinal cord injuries.

For example, stem cells may be able to help treat Parkinson's disease. Inside the brain of a person with Parkinson's, the nerve cells that normally produce a neurotransmitter called **dopamine** die off. Without dopamine to act as a messenger between the brain and the body, there is a breakdown in communication about muscle activity and movement. This causes tremors, shaking, and stiffness.

Stem cells can be made to differentiate into nerve cells, including neurons that makes the neurotransmitter **dopamine**. These neurons have been transplanted into the brain of model animals with a Parkinson's-like disease; the neurons survive, continue to make dopamine, and reduce symptoms of Parkinson's disease (Barker et. al 2013). Stem cells and model organisms may help us find cures for additional nervous system disorders.

Plants that Heal

Traditional healers around the world use plants to speed healing of cuts, scrapes, and broken bones. Some well-known plants used by different cultures to help the body heal include:

- Astragalus (Huan Qi)
- *Centella* (Gotu kola or ji xue cao)
- Comfrey (called "boneknit" or "healing blade")
- *Ginkgo biloba* (bai guo)
- Hawthorne (Shan zha)
- St. John's wort
- Arnica
- Aloe vera

You may have used one of these plants yourself—perhaps your family has an aloe vera plant in the kitchen. After burning your hand on a hot pan, you quickly tear off a leaf and use the liquid inside to help heal the burn. Or perhaps an acupuncturist has prescribed some of these herbs to help you heal from an accident. You might also think about how stimulants and depressants might affect regeneration. Stimulants (such as coffee, tea, ginger, or ginseng) speed the metabolism and thus may increase the speed of regeneration. However, these may also slow down regeneration by stimulating other body functions and thus taking nutrients and energy away from regeneration.

Scientists are only now studying the effects of some of these plants on healing. In this lesson, you will use one of the decoctions or infusions that you previously made to examine the speed of regeneration in planaria. Perhaps one of the plants you extracted will speed up or slow down the regeneration rate in planaria. Either of these effects could be helpful to scientists as they research ways to use stem cells to cure disease. Your experiment might lead to new information that could be used in treating neurological disease or helping people recover from accidents!

In your lab notebook, respond to these prompts:

1. As a group, choose one plant extract to test during this lab. You will be choosing from the extracts that your class produced during the previous *Infusions and Decoctions Lab*. Your teacher will provide you with specific instructions on how to choose the extract. Record the name of your chosen extract.

2. As a group, develop a hypothesis for your plant extract as to how you think it might affect the speed of regeneration of a planarian. Write down your hypothesis using the "If, Then" format.

Planaria Regeneration Lab: Vocabulary List

Conception: In humans, the fertilization of the ova by the sperm; the union of male and female gametes.

Differentiation: The process of a cell becoming a specific type of cell. Omnipotent stem cells can differentiate into blood cells, nerve cells, or any other type of cell.

Dopamine: A chemical neurotransmitter that is produced by nerve cells in the brain. As a neurotransmitter, dopamine is critical for sending messages between the body and brain about muscle activity and movement.

Meniscus: In the field of chemistry, the meniscus is the curve seen at the top of a liquid inside of a container. When measuring a liquid in a container, such as a graduated cylinder, measure according to the center of the meniscus at eye level. For most liquids, this is the bottom of the concave curve.

Model organism: A non-human organism that scientists study to better understand something. The hope is that discoveries made on this organism can be used to help understand other organisms, including humans. *Lumbriculus*, planaria, mice, fruit flies, and the bacteria *E. coli* are all common model organisms.

Omnipotent cells: Stem cells capable of differentiating into ***any*** other type of cell. A single omnipotent cell is theoretically capable of growing into an entire organism. Also called totipotent cells.

Regenerate: To replace a lost or damaged body part or organ by growing new tissue. Many animals have this ability, including planaria, some lizards, starfish, sea cucumbers, and more.

Stem cells: Undifferentiated cells that can make more of themselves and develop into different cell types (differentiate)—but may not be able to differentiate into all cell types

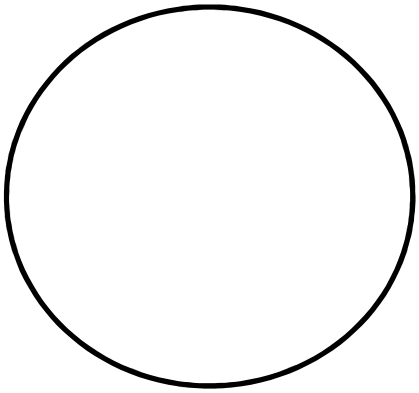
PLANARIA REGENERATION DRAWINGS & OBSERVATIONS
STUDENT HANDOUT

Name:_____ Date:_____ Period:_____

Each time you check your planarian, make a drawing. Be sure to label the eyespots, when they appear.

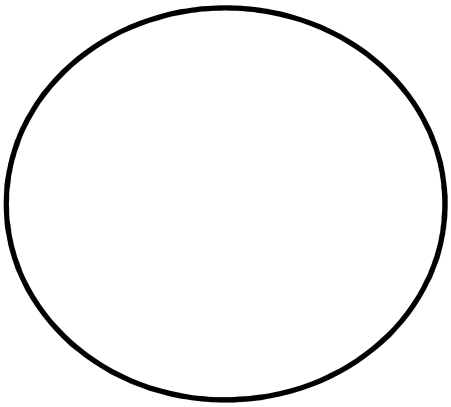
Day _____

Observations:



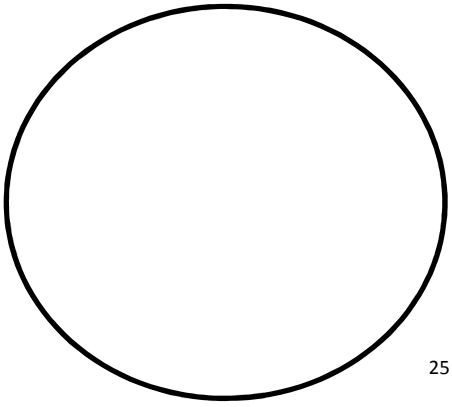
Day _____

Observations:



Day _____

Observations:



PLANARIA REGENERATION LAB PROCEDURE
STUDENT HANDOUT

Name:_____ Date:_____ Period:_____

Lab Procedure for Planaria Regeneration

Decide on which group member will take on each of the following roles:

Recorder, Solutions Master, Surgeon, and Observer.

If you have two people in your group, one person should be the **Solutions Master & Surgeon** and the other person should be the **Recorder & Observer**. If you have three people in your group, one person can be both the **Recorder & Observer**.

Your group will need the following laboratory materials:

Item	Quantity per Lab Group
Goggles and gloves	1 per student
Planaria	4
Petri dish (3 extracts, 1 control, and 1 dish for cutting)	5
Paint brush	1
10 ml graduated cylinder	1
Straight razor blade	1
100 ml beaker • One beaker for conditioned water. • One beaker for "recovery"—the planaria will go here after the experiment to rinse off any extracts before they go back to their aquarium.	2
Conditioned water	~150 ml
Medicine cup (aka "ketchup cup")	3
Plant extract obtained as a result of *Infusions and Decoctions* lab.	1 extract

Set up your lab station:

1. Put on your goggles and gloves.

2. **Recorder:** Use a piece of lab tape and a permanent marker to label your three medicine cups with the name and concentration (10%, 5%, and 1%) of the plant extract you will be testing. For example, your cups might be labeled "Green Tea 10%", "Green Tea 5%", and "Green Tea 1%".

3. **Recorder:** Label your four petri dishes along the edge with your group name and the name and concentration of the plant extract you will be testing (just like the medicine cups). One petri dish should be labeled "Water Control."

4. **Recorder:** Your group should have two 100 ml beakers to label. Using lab tape and a permanent marker, label one "Conditioned Water" (your controlled variable), as shown in the photo. Label the other "Recovery". Make sure there is conditioned water in each of these beakers. See ***Figure 1***.

Figure 1: Label the beakers "Conditioned Water" and "Recovery."

5. **Solutions Master:** Prepare the dilutions of your plant extract and fill your four petri dishes.

    a. **To make a 10% plant extract solution:** Measure 18 ml of conditioned water using the graduated cylinder, remembering to look at the bottom of the **meniscus**. Pour the 18 ml of water into the medicine cup labeled 10%. Then measure 2 ml of your **plant extract** and pour this into the medicine cup labeled 10%. Mix this well, then measure 10 ml of the 10% solution and pour it into the petri dish labeled 10%. Place the lid on the petri dish. Do not open the petri dish again until you are ready to put a planarian inside.

    b. **To make a 5% plant extract solution:** Measure 5 ml of conditioned water using a graduated cylinder and pour it into the medicine cup labeled 5%. Then measure 5 ml of the 10% plant extract solution (in the medicine cup labeled 10%) and pour this into the medicine cup labeled 5%. Mix this well, then pour it into the petri dish labeled 5%. Place the lid on the petri dish. Do not open the petri dish again until you are ready to put a planarian inside.

    c. **To make a 1% plant extract solution:** Measure 9 ml of conditioned water using a graduated cylinder and pour it into the medicine cups labeled 1%. Then measure 1 ml of the 10% plant extract solution (in the medicine cup labeled 10%) and pour this into the medicine cup labeled 1%. Mix this well and pour it into the petri dish labeled 1%. Place

the lid on the petri dish. Do not open the petri dish again until you are ready to put a planarian inside.

d. **To make your controlled variable:** Measure 10 ml of conditioned water using a graduated cylinder and pour it into the petri dish labeled "Water Control". See ***Figure 2***.

Figure 2: Pour 10 ml of conditioned water into the "Water Control" petri dish.

6. **Surgeon:** Use a soft paintbrush to gently remove a planarian from the wall of the beaker or aquarium, as shown in ***Figure 3***. Gently place the planarian into the dry petri dish.

7. **Surgeon:** Use the razor blade to swiftly cut the planarian in half, as your teacher demonstrated. The cut should be made so that you end up with a "head end" and a "tail end" (as shown in ***Figure 4***). Be extremely careful with the razor blade as it is very sharp.

8. **Surgeon:** Place the ***head end*** of the planarian into the recovery beaker and the ***tail end*** into the 10% petri dish.

Figure 3: Use a paintbrush to gently remove one planarian to transfer to the plate.

Figure 4: Use the razor blade to swiftly cut the planarian in half.

9. **Surgeon:** Repeat Steps #5-7 three more times. As before, you will remove a planarian from the beaker, cut the planarian in half, place the head end into recovery. However, now you will place one tail end into the 5% petri dish, one into the 1% petri dish, and one into the water control petri dish. Each dish should only have one planarian tail in it.

10. **Surgeon:** Follow your teacher's instructions about how to dispose of your razor blade. Dispose of the blade immediately after use.

11. **Observer & Recorder:** When your team mate has completed cutting four planaria and placing one in each petri dish, use the dissecting microscope to observe the planaria and draw what you see. Carefully draw and label what you see. For each petri dish, indicate whether you observe any movement or see eye spots.

    HINT: If you do see eye spots, you probably put the head end into the petri dishes and the tail end into the recovery beaker. You can fix this now by using the paintbrush to move the tail end into the petri dishes and the head end into the recovery beaker.

12. **Whole Team:** Follow your teacher's instructions regarding where you should place your planaria and how you should clean up. The paper medicine cups should be thrown away. The graduated cylinders should be washed.

13. **Observer & Recorder:** For the next week or two, you will need to regularly observe the planaria and draw what you see in your lab notebook. Your teacher will provide instructions on how often you will check your planaria. Each time you check your planaria, be sure to date your entry, make a drawing, label any eyespots, and write down your observations. The regeneration is complete when you can see eyespots on your planaria.

PLANARIA REGENERATION RESULTS & CONCLUSIONS
STUDENT HANDOUT

Name:_____ Date:_____ Period:_____

Results

1. In this investigation, the conditioned water was the ***controlled variable***. The ***dependent variable*** was the speed of the planarian's regeneration. The dilutions of the plant extract were the ***independent variable***. Which plant extract did you test?

2. What was your hypothesis for this plant extract?

3. Describe the similarities and differences between the water control (controlled variable) and the different dilutions of the plant extract (independent variable). Did any dilutions cause the death of the planaria?

4. Were there any differences in the speed of regeneration?

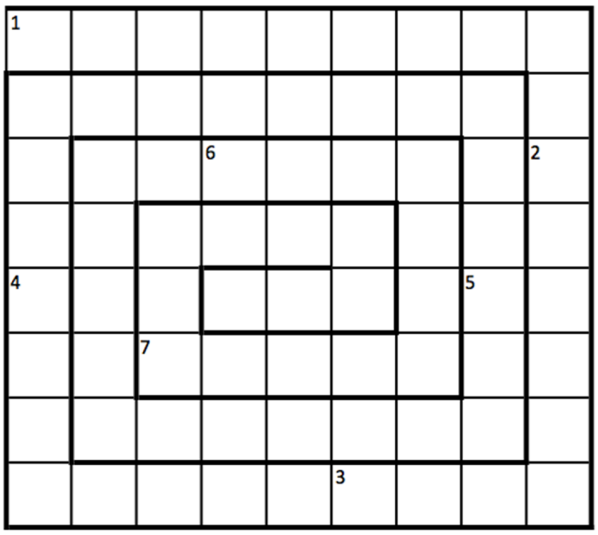
5. Did your observations support or refute your hypothesis? What evidence do you have?

Conclusions

6. Imagine that you are a research scientist studying how plant-based chemicals can help human tissue heal from injury. You are interested in plant-based chemicals that can speed up the normal course of healing after skin is cut. You've heard that banana plants might have these types of properties. Write a paragraph explaining how you would choose to investigate the effects of banana plant on tissue regeneration using planaria as your model organism (since you first need to show that the plant extract has an effect and is safe before doing any testing on humans).
    - What is your hypothesis?
    - How will you set up an investigation to test it?
    - What data will you collect?

PLANARIA REGENERATION LAB VOCABULARY QUIZ
STUDENT HANDOUT

Name:_____ Date:_____ Period:_____



1. In humans, the fertilization of the ova by the sperm; the union of male and female gametes.
2. A chemical neurotransmitter that is produced by nerve cells in the brain. It is critical for sending messages between the body and brain about muscle activity and movement.
3. The curve seen at the top of a liquid inside of a container. For most liquids, this is the bottom of the concave curve.
4. A non-human organism that scientists study to better understand something. The hope is that discoveries made on this organism can be used to help understand other organisms, including humans. (2 words)
5. A stem cell capable of differentiating into *any* other type of cell, theoretically capable of growing into an entire organism. Also called totipotent cells. (2 words)
6. To replace a lost or damaged body part or organ by growing new tissue. Many animals have this ability, including planaria, some lizards, starfish, sea cucumbers, and more.
7. Undifferentiated cells that can make more of themselves and develop into different cell types (differentiate)—but may not be able to differentiate into all cell types. (2 words)