Cells behaving good or bad: An inquiry based activity

Overview

This lesson plan is developed to introduce students on the influence of surfaces onto cellular behavior. Students will start this inquiry by developing testable hypothesis and experimental protocols and progress through the activity by making observations on cellular behavior and developing possible explanations for their observations. This activity is inspired by current ongoing research. At the end of this lesson students will present their findings through a presentation, which is analogous to disseminating information through scientific meetings.

Objectives

Students will learn to identify and develop a researchable question, collect data through conducting experiments, and formulate conclusions. This activity will foster an understanding of the relationship between cellular adhesion and its functions. Moreover, students will gain an introduction to the scientific method, and how to analyze data. Students will be able to describe the function of cellular receptors and how they contribute to cellular shape and behavior.

Grade applicability

9-12

Standards

Arizona State Standards

1SC-P1. Propose solutions to practical and theoretical problems by synthesizing and evaluating information gained from scientific investigations
- PO 1. Evaluate scientific information for relevance to a given problem
- PO 2. Propose solutions to a problem, based on information gained from scientific investigations

1SC-P3. Analyze and evaluate reports of scientific studies
- PO 2. Compare conclusions to original hypotheses
- PO 3. Evaluate validity of conclusions

1SC-P5. Apply the concepts of equilibrium, form and function to a variety of phenomena
- PO 2. Explain how the relationships between form and function are evident in natural and designed systems

1SC-P6. Identify and refine a researchable question, conduct the experiment, collect and analyze data, share and discuss findings
- PO 1. Construct a researchable question
- PO 2. Employ a research design that incorporates a scientific method to carry out an experiment
- PO 3. Analyze experimental data
- PO 4. Communicate experimental findings to others

1SC-D1. Design and complete an advanced scientific investigation, either individually or as part of a team, and formally report results to peers, teachers and others

3SC-P2. Propose and test, using computer software or common materials a solution to an existing problem; or design a product to meet a need, using a model or simulation
- PO 1. Describe a problem or need
- PO 2. Propose a solution to the problem or design a product to meet the need
- PO 3. Design a method of testing the solution or design a model or simulation to test the product
- PO 4. Carry out the test of the solution or product
- PO 5. Evaluate the test results

National Science Standards

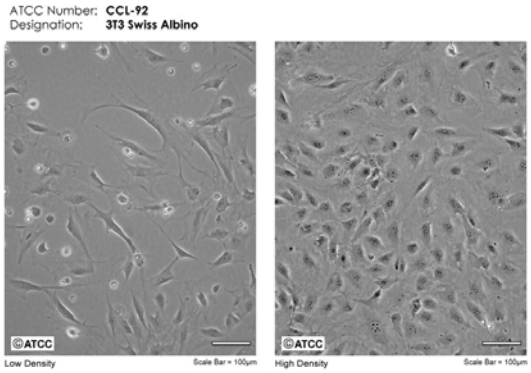
CONTENT STANDARD A: As a result of activities in grades 9-12, all students should develop Abilities necessary to do scientific inquiry

CONTENT STANDARD E: As a result of activities in grades 9-12, all students should develop Abilities of technological design understandings about science and technology.

CONTENT STANDARD G: As a result of activities in grades 9-12, all students should develop understanding of science as a human endeavor, nature of scientific knowledge and understandings about scientific inquiry

Background

3T3 mouse fibroblast cells are commonly used in biotechnology laboratories, due to their ease of culture and hardiness.



3T3 cells can be obtained from several companies, such as American Tissue Typing Company (ATTC), or by contacting a research lab at the local university. These cells were established from mouse embryos. The cells exhibit contact inhibition characteristics, which means that they will grow until they cover a surface and start touching each other, and at that point they will stop growing. Normally, these cells are grown in plastic tissue culture flasks maintained in incubators at 37°C with 5% CO_2. Normally, the cells are manipulated under sterile conditions, in sterile hoods, however since the cells used for this experiment will be incubated for only approximately 15 minutes and the cells will not be preserved for further usage it is acceptable to manipulate the cells under non sterile conditions.

Bovine serum albumin is a plasma protein found in bovine serum that is involved in the clotting process. This protein can be purchased from chemical companies such as Sigma, Fisher Scientific or similar companies. This protein can be absorbed to the surfaces and it provides a surface that is not conducive for cellular attachment.

Gelatin is a mixture of proteins, mostly collagen, than can be adsorbed onto surfaces in a similar fashion to BSA. However, in this case the surface is conductive for cellular adhesion.

The rest of the materials can be purchased from any scientific companies such as Fisher, VWR or Carolina Biologicals.

Time

This activity should be spread over couple of days, allowing students time to develop their own questions, experimental design, discuss their observation and ideas. Also, this activity can be extended by allowing students to develop new experiments once they have made some observations and would like to investigate this principle further. Also, the time could be shorten if they are allowed to make observation only and they are guided through class discussion to explain their observations.

Materials

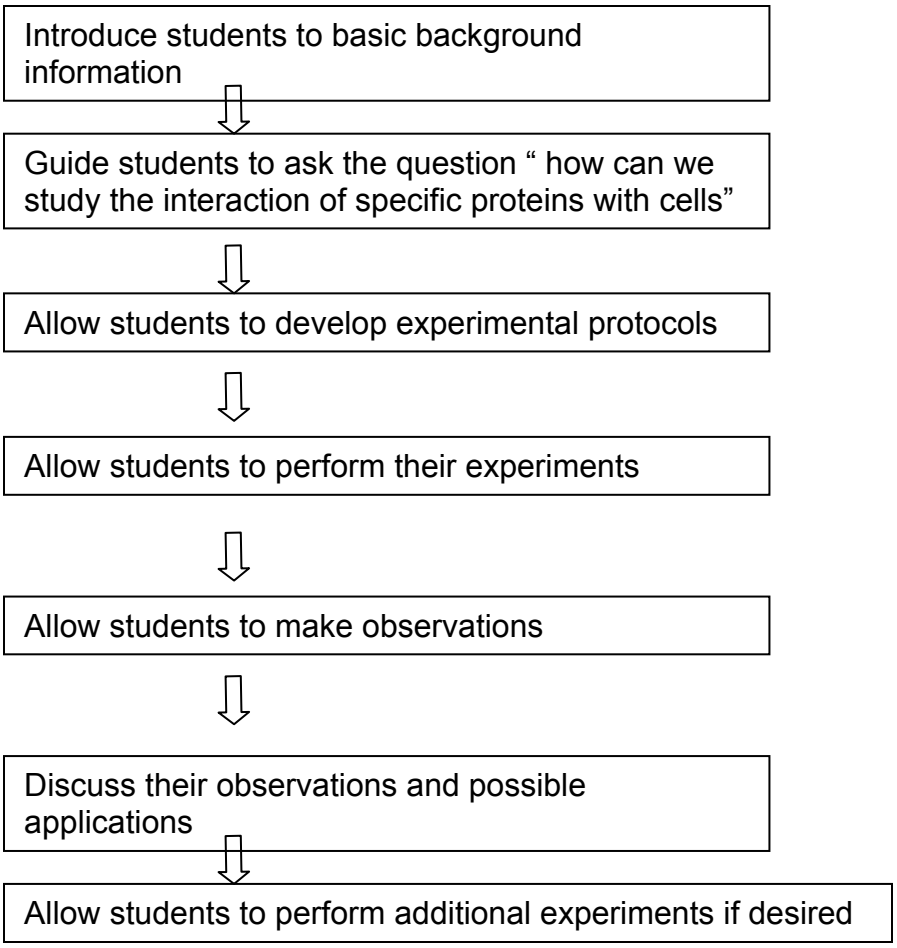
Mouse fibroblast (3T3 cells)
Dulbelco's Minimum Essential Medium
Fetal Bovine Serum
Penicilin/Sterptomycin
L-Glutamine
Trypsin
T-25 cell culture flasks
15 ml conical tubes
Glass slides
Petri Dishes
Gelatin
Bovine Serum Albumin
Phosphate Buffer Saline
Light microscopes
Sterile disposable pipettes

Materials preparation

Teacher should prepare 1% protein solution for each group. To prepare 1% solutions, add 1mg of proteins to 10 ml (the ratio can be scaled up to the necessary volume) of phosphate saline buffer (PBS). Cells will be purchased or cultivated in tissue culture flasks. Before students are ready to use the cells, the teacher will need to harvest the cells from the flask. This is accomplished by removing the growth media (this can be discarded in the sink after mixing it with water) and adding 2ml of trypsin solution to the culture flask. The cells should be allowed to incubate with the trypsin for several minutes (5 to 10 min or until the solution becomes cloudy). Through this process, the cells which grew on the dish will be removed from the surface and send into the trypsin solution. This process is accomplished by snipping off the structures that keep the cells attached to the surface. To inactivate the typsin (which is an enzyme) an equal amount (2ml) will be added to the flask and then the entire volume should be transferred to a 15 ml conical. Under normal laboratory conditions, the cell/media/trypsin mixture would be centrifuged to settle out the cells and then the cells would be re-suspended into fresh media. However, since centrifuges are not readily available, I recommend that the cell/media/trypsin mixture would be diluted with fresh media to create a final volume of 15 ml. This new mixture should be mixed well to distribute cells throughout the volume. This can be accomplished by using a pipette to draw up and expel liquid within the tube several times and thereby mixing the solution. It is not advised to shake of invert the tube since this type of shear stress can damage the cells. Now, you should have a relatively homogenous solution of cells in media with very little trypsin, which can be used by the student in their experiments. Remind students that if they are not using this suspension shortly cells tend to settle and so they should repeat the mixture procedure right before they are going to use the cells. Several groups should be able to use the 15 ml cell suspension.

Procedure

Overview of the entire lesson



Day 1

Introduction to cellular behavior, protein absorption
Discuss the dynamic of cellular behavior and protein interactions
Formulate a specific question that you are interested in examining
Attempt to develop an experimental protocol to answer the question of interest
Check with your teacher for approval of your experimental protocol

Day 2

Perform your experiment
Investigate each slide under the microscope – without removing it from the dish
Make careful observations and record them on your worksheet
Discuss amongst your group the observations
Collect data
Organize and analyze your results and relate it back to your hypothesis (question of interest)

Day 3

Develop a short presentation through which you should communicate the following points:

- The problem that you investigated
- The experimental design that you implemented
- The observations that you collected through the experiment
- The correlation between the experiment outcomes and the question that you set to investigate

[Materials for presentation: white board and colored markers, poster board, presentation easels, colored markers]

Present results to the rest of the class

Possible results

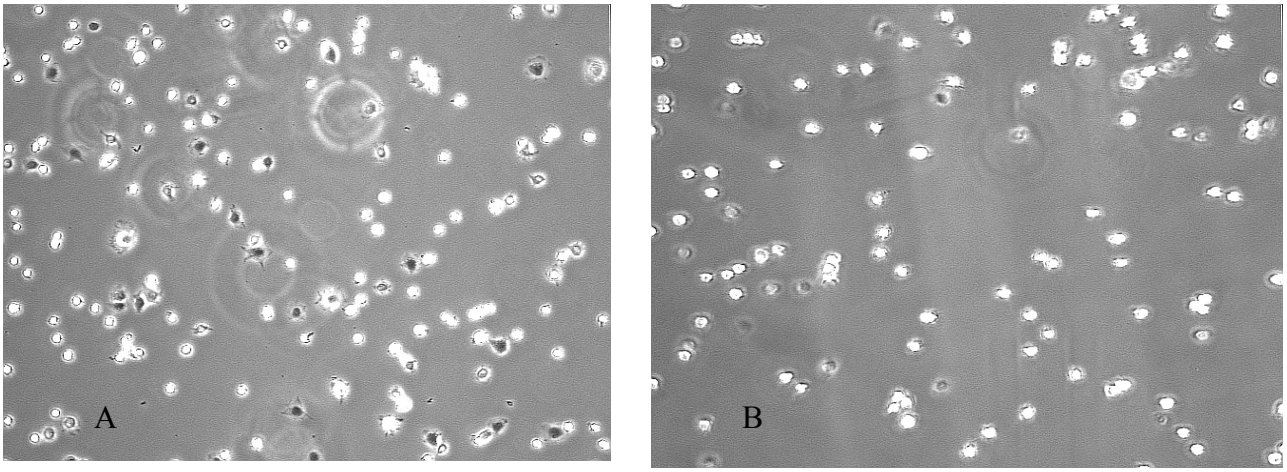


Figure A illustrates cells on the gelatin, notice the cell edge appearance, wavy making cells appear of irregular shape. Compared to figure B where the cells are mostly rounded.

It is very important to know that if the cells are allowed to stay for longer periods of time on the BSA (45 min or longer) they will start demonstrated the same cell appearance as the gelatin case, because the cells will start producing and laying down ECM proteins and so they can adhere to the surface. Also, even at the beginning after cells have settled on the BSA covered slide some will still appear irregular. One explanation for this observation is due to the fact that BSA does not form a complete uniform layer, and so there are empty spaces where the cell can lay their ECM protein and adhere. It is important that you guide students to compare overall cell numbers that exhibit the irregular behavior in comparison with round cellular appearance.

Evaluation and Assessment

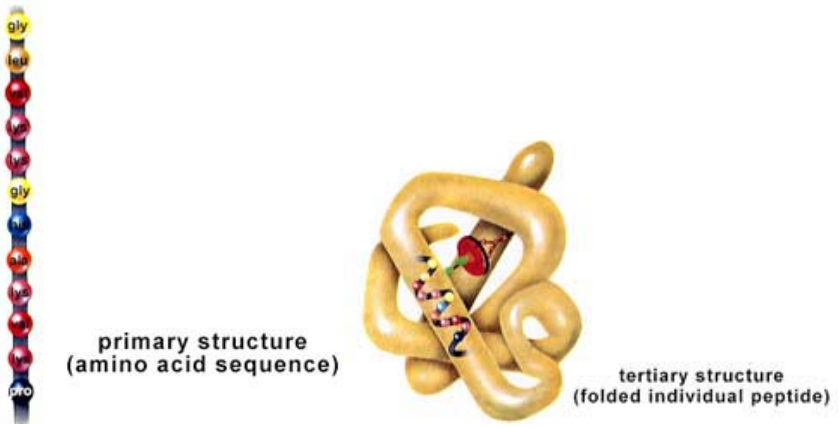
Students should be assessed based on their participation throughout this project. The grading for the experimental outline can be graded according to the following scale:

Grade assignment	Required parts with the experimental outline and presentation
Grade A	Clearly stated objective (hypothesis, problem to investigate), testable and solid hypothesis Steps of the experimental protocol are listed clearly and sequentially Control experiments were performed along with the experimental settings Data were collected at each step of the protocol Data was nicely organized and presented Clearly state conclusion (this might support their hypothesis, or possible they could make other inferences based on the fact that the experiment did not support their initial hypothesis) Presentation is clear, neatly organized and presents all the important steps Students have demonstrated a higher level of thinking by solving the problem given to them
Grade B	Clearly stated objective (hypothesis, problem to investigate), testable but not necessary scientifically solid hypothesis Steps of the experimental protocol are listed sequentially, some steps might be missing Students made an attempt to develop control experiments Data were collected throughout the experiment but some steps were omitted Data was somewhat organized The conclusion is state vaguely but the students demonstrated attempts to summarize their experimental observations and make connection to their initial observations Presentation is clear and organized Students have demonstrated an ability to create clear connection between observations and conclusions
Grade C	Students attempt to state a specific objective (hypothesis, problem to investigate) Steps of the experimental protocol are listed not necessarily sequentially, some steps might be missing Data were collected throughout the experiment but some steps were omitted Data was somewhat organized The conclusion is not stated, but students have made an attempt to establish a connection between observations and conclusion Presentation of the results is organized
Grade D	The students have developed some type of objective, it is not stated too clearly Steps of the experimental protocol are listed but not sequentially, multiple steps might be missing Data were collected throughout the experiment but multiple steps were omitted Data was organized, but not clearly and was not presented in a clear manner There is no conclusion stated, and students made a minimal attempt to establish a connection between observations and conclusion

Guiding information and questions (to be presented and discussed at the teacher's discretion)

The main purpose of this activity is to allow students to develop scientific notions through inquiry. The particular scientific principle under the investigation of this lesson is the interaction of cells with surfaces. Therefore, after students have been introduced or reviewed the necessary notions they will develop a testable hypothesis and a corresponding experimental protocol. Following, I am proposing a series of questions and topics for the introductory discussion:

- Remember, in biology we have four organic molecules that are very important, can you list them.
    - *Proteins, lipids, carbohydrates and nucleic acids.*
- Besides proteins, what else do you find the human body in abundance?
    - *Cells.*
- How are the cells in the body? Floating or attached ?
    - *Attached.*
- If you recall the water strider experiment, we talked a bit about the properties of water such as polarity, and the interaction of other substances with water, such as hydrophilic and hydrophobic. Which of these two environments do you think cells prefer? Why?
    - *Hydrophilic, because the body is mostly composed of water, so cells are exposed to water all the time.*
- So what do you think that might happen when cells come in contact with hydrophilic surfaces?
    - *They like it.*
- So what do you think that might happen when cells come in contact with hydrophobic surfaces?
    - *They will not like it.*
- Now lets talk a bit about proteins? What are proteins made of?
    - *Amino acids.*
- Are amino acids hydrophilic or hydrophobic or both?
    - *Both.*
- What type of environment do you think is inside of body, hydrophobic or hydrophilic?
    - *Hydrophilic.*
- If students have trouble getting to this question, I recommend that you would ask them what is the major component of the human body?
    - *Water.*
- Since amino acids are the building blocks of proteins, what can we say about proteins, are they hydrophobic or hydrophilic?
    - *Probably a combination of both.*
- Since proteins have regions that are hydrophobic and regions that are hydrophilic, what shape do you think that have in the body? In other words where would you find the hydrophobic and hydrophilic regions?
    - *Folded, the hydrophobic region exposed to the inside, and hydrophilic regions to the outside.*

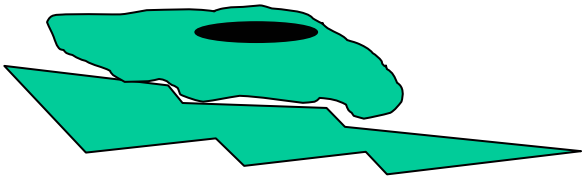


- Which of the two surfaces, hydrophobic or hydrophilic, do you think that cells would like more? Why?
    - *Hydrophilic, because it is the environment in which cells are found.*
- So, how do you think that cells interact with proteins?
    - *Touching, hanging on to them, binding.*
- When you think about the body, do you think that the interaction between cells and proteins takes place in a liquid phase or solid phase?
    - *Solid phase, since most cells are attached to form tissues.*
- Keeping in mind that cells are very small, we need microscopes to see them, how would you describe the surface they are interacting with at that magnification, smooth or rough?
    - *Rough.*

- *If students have a hard time answering this question, I propose that you have them draw a line with a pencil on a piece of paper and allow them to investigate it under the microscope. Students will discover that even though the line appears to be smooth with unaided eye, under investigation at high magnification the surface appears very rough.*

- Would you consider the surface that cells interact with similar to the surface of mountain? Imagine yourself rock climbing, how do hang on? How do you think that cells hold on to the surface?
    - *By using their "fingers" like projections.*

- These finger like projections are called receptors and they interact with the surface by anchoring the cell to the surface. You could imagine these receptors as puzzle pieces, one fits into over the other, usually the receptor fits over the projection, similar to the hand holding onto the rock.



- Will cells interact with all the proteins in the body?
    - *Probably no.*
- The proteins that allow cells to adhere to them, do you know what are they called?
    - *In this case the answer will probably be NO. Cells like to interact with certain type of proteins called Extracellular Matrix Proteins, ECM proteins.*
- Can you think of a way in which we could investigate the relantionship between the cells and the ECM proteins?
    - *Observing how the cells behave when they interact with the specific protein.*
- How can we accomplish this?
    - *Putting cells onto the protein.*
- How can we do this?
    - *Putting the protein on a surface.*
- How can you put proteins on a surface?
- Imagine that you spilled water on the table and you put a paper towel on top, what happens?
    - *The paper towel takes up the water.*
- Can you think of a way though which we can put proteins onto a surface?
    - *Put into the solution to let it take some of proteins onto the surface.*

At this point students should be allowed to develop their experimental protocol, perform experiments and make observations.

- Students are provided petri dishes, glass slides and two different protein solutions, a BSA solution and gelatin solution. Students will be provided with several glass slides and hopefully they will be propose to cover the glass slides in the protein solution to coat them. They should be allowed to determine on their own how they want to coat the slides. The only restriction that needs to be imposed is that once a slide has been immersed into a solution it should be left in there for at least 10 min. After coating students will receive 3T3 cells and they will be added to each surface. After approximately 10-15 min of incubation each student should investigate the slides under the microscope and make observations.

The following paragraphs describe topics of discussion, which should be investigated during the last day of this activity. They provide scientific information, which is not necessarily part of the lesson, but students could be introduced to this notion to show relevance and importance of this lesson. Also, the possibility to link this activity to cutting age research, would provide students with reasons for getting involved in science.

Now that students know that cells can adhere to certain proteins, ask them to think of applications of this phenomenon. To get students into the right directions, ask them where do we find cells and by what means do we interact with them. Hopefully students will respond

that cells are found in the body and that we interact with by introducing transplants (bringing in tissues from other people) or implants (non -biological materials). So, at this point you can have students expand on how this information can be used in this situation. Also, students will be introduced to current ongoing research at Arizona State University in the laboratory of Dr. Caplan, on how they are trying to use fragments of protein that induce cells to stick to them to stop cancer cells from moving around in the body. In this way they are hoping to stop cancer from spreading in the body.