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## Modeling Nuclear Damage from Cell Movement

Cell Movement and Stress Damage Lab

**Purpose:** To quantify the damage that can be done to nuclei as they move by using a model.

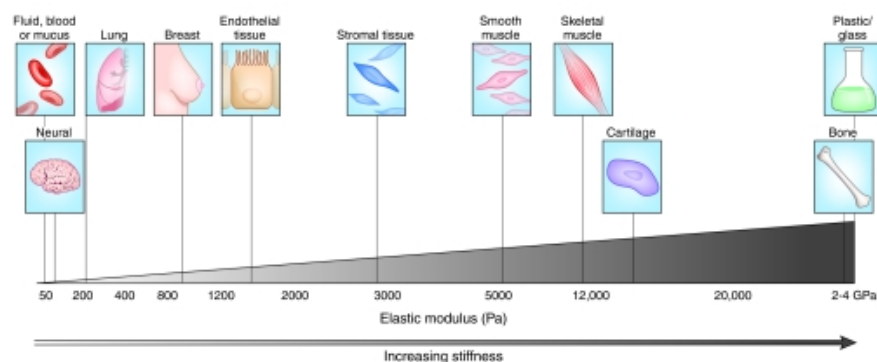
### Introduction:

In biology the extracellular matrix (ECM) is a three-dimensional network of extracellular macromolecules, such as collagen, other proteins and enzymes, and carbohydrates, that provides structural and biochemical support to surrounding cells. Because multicellularity evolved independently in different organisms, the composition of ECM varies between multicellular structures; however, cell adhesion, cell-to-cell communication, and differentiation are common functions of the ECM.

Cells can sense the mechanical properties of their environment by applying forces and sensing the resulting backlash. This helps regulate many important cellular processes including cellular contraction, cell migration, cell proliferation, differentiation and cell death (apoptosis).

Metastasis is a multistep process in which tumor extracellular matrix and cancer cell cytoskeleton interactions are important components. The ECM is physically connected to its environment, through integrins (or linker proteins), to the cell at cell-ECM adhesion sites. These adhesion sites are linked through the cell membrane to

the cytoskeleton (the internal skeleton of the cell, made of fibers of a protein called actin, among other proteins), and this enables the cell to respond to external stimuli in a coordinated manner. Biomechanical cues from the ECM through cell-adhesion proteins like integrins are fundamental for defining the invasive potential of cancer cells, and many of these proteins have been proposed as potential targets for inhibiting cancer cell invasion and thus, metastasis.



Note that the ECM can exist in varying degrees of stiffness, from soft (as in brain) to hard (as in bone); this is primarily dependent on the concentrations of collagen, a rigid protein, and elastin (you guessed it – an elastic protein). The accumulation of collagen fibers results in a stiffer ECM and is considered a typical characteristic of cancerous tumors. This can lead to an intense fibrotic response, known as desmoplasia, and tumor stiffening. Stiffening is not only associated with tumor cells displacing the host tissue and growing in size but also contributes to cell-ECM interactions and can promote tumor cell invasion to surrounding tissues.

,This activity involves exploring the direct damage to DNA that is caused when cells migrate through a stiff ECM – in other words, you’ll learn one potential mechanism for how a stiff environment outside a cell can lead to potentially permanent damage to DNA inside of the cell. Surrounding the nucleus is a structure called the nuclear lamina, which is a dense fibrous network found in most animal cells made of filaments and proteins that surrounds the nucleus and gives the nucleus its shape. This network not only provides mechanical support, but is also important in regulating cellular events such as DNA replication and cell division. Since there is a relationship between the lamina and DNA function, damage to the lamina can contribute to DNA malfunction or incorrect DNA replication, which can lead to cancers.

This activity will simulate blebbing. Blebbing occurs when a cell deforms in shape when it passes through a small space such as the spaces of the extracellular matrix. Imagine what happens when you squeeze a stress ball... it loses its circular shape. In some cases where cells are squeezed, lamins also feel the stress and the nucleus loses its circular shape, leading to DNA damage. The amount of lamins in a cell will change the stiffness of the nucleus and therefore impact damage to the DNA when the cell is migrating. By modeling varying amounts of lamins in a nucleus in this experiment, you will simulate the change in stress the nucleus undergoes during blebbing and visualize the damaging effects on the DNA contained within the cell’s nucleus.

In this lab, you are going to model the movement of different cells, whose nuclei are composed of varying amounts of lamins, through a matrix and measure the DNA damage caused by the forces on the cell.

**To the teacher:** The components of this model are as follows: Balloons will represent the cell nucleus. Students will add pasta pieces (representing nuclear lamins) and hydrogel beads (representing chromatin or DNA). Students will push three different ‘nuclei’ with varying lamin concentrations through a plastic shower curtain ring (representing openings in the ECM). DNA damage will be simulated by ruptured or torn hydrogels beads.

**Materials:**

3- 12” balloons  
empty water bottle

700-1000 hydrogel beads (Sooper Beads \*\*)  
2000 ml beaker

3- plastic shower curtain rings  
handful of pasta (rotini works best, 20-25 pieces)  
scissors  
large tray or box for counting hydrogels

**\*\* NOTE – Sooper Beads (hydrogels) are available here: [Amazon.com](https://amazon.com). or <https://sooperbeads.com/>**

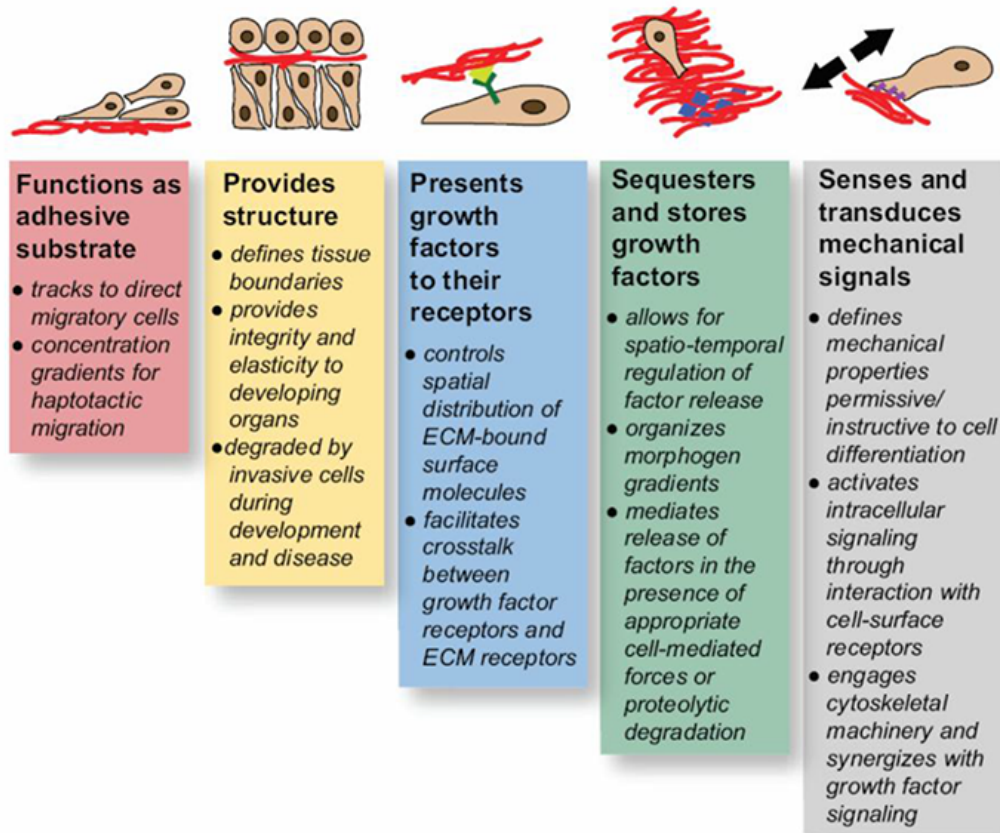
## Procedure

### Day 1

1. Place hydrogel beads into the beaker and cover with water. Allow to sit over night to expand.
2. Go to <https://www.khanacademy.org/science/biology/structure-of-a-cell/cytoskeleton-junctions-and-extracellular-structures/v/extracellular-matrix>  
Or google Extra cellular matrix Khan academy video. Answer the following questions in your lab notebook.

- a. What is the relationship between cells and tissues?
- b. What is the extracellular matrix?
- c. What are the three main components of the ECM? Which one is most abundant in mammals?
- d. What are three roles of the ECM in the cellular environment?
- e. Why is it important for the outside ECM to be connected to the inside of the cell?

## Functions of ECM



3. Read the following article

*Cancer cells with trapped nuclei cut their way through the extracellular matrix*  
by Emmanuel Dornier and Jim C Norman

Link: <https://www.nature.com/articles/s41467-018-06351-6>

After reading the article answer these questions in your lab notebook.

- Why are dendritic cells more likely to deform their nucleus?
- What are nuclear lamina? What are their purpose?
- How do dendritic cells help facilitate their movement through small spaces?
- Examine the picture: What is the difference in how cells behave in a matrix with large pores versus small pores?
- What is the relationship between nuclear deformation and DNA damage?
- How are cancer cells different in terms of nuclear deformation as compared to normal cells?

**Day 2: Lab Modeling Stress on cells**

Create three nuclei by the method demonstrated below.

- Get three 12' balloons, label the first with "O" second with "5" and the third "10"
- Add 5 rotini in to second balloon and 10 rotini into the last balloon. The rotini will represent proteins in the nucleus that will change the stiffness. Slightly blow up balloon and twist top to hold in air. (DO NOT TIE OFF)
- Carefully count out 250 hydrogels each into three smaller beakers, you will be making three nuclei.
- Place one set of 250 hydrogels into an empty water bottle.
- Put balloon on top of filled water bottle and pour hydrogels into balloon (as shown in the picture to the right). Let out extra air from balloon and tie off top.
- Repeat this two more times for the other balloons.



Predict what will happen to each balloon (will there be damage, which would have more, etc.)

A. \_\_\_\_\_  
\_\_\_\_\_

B. \_\_\_\_\_  
\_\_\_\_\_

C. \_\_\_\_\_  
\_\_\_\_\_

7. Take each balloon, one at a time, and place them through the shower ring.
8. Cut open each balloon carefully as not to damage the hydrogels inside and collect the contents in a large tray or box.
9. Recount the hydrogels that are not broken. (ONLY WHOLE HYDROGELS COUNT) and record your data in excel.

Balloon	Whole gels before	Whole gels after
A (0 rotini)		
B (5 rotini)		
C (15 rotini)		

10. Fill in your data on the class data sheet.
11. **Construct a graph depicting the class data.** Put this in your notebook.

#### **Analysis (answer in lab notebook)**

1. What does the shower ring represent?
2. Which nuclei had the most whole hydrogels after passing through?
3. Explain the results in terms of stiffness and stress.
4. Based on the article you read on day 1, which cell is most likely to be the cancer cell and why?
5. Design an experiment that tests the effects of the stress when the nuclei are at a constant stiffness, but the extracellular matrix has a variable stiffness.

## Extension

Name \_\_\_\_\_

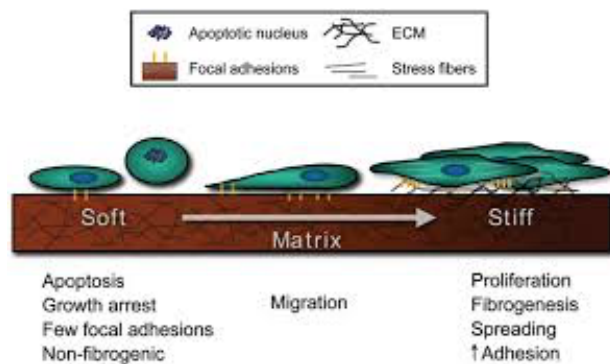
Read the following article for homework and answer the questions.

***Tension on Gut Muscles Induces Cell Invasion in Zebrafish Intestine, Mimicking Cancer Metastasis***

[https://www.eurekalert.org/pub\\_releases/2012-09/uops-tog090712.php](https://www.eurekalert.org/pub_releases/2012-09/uops-tog090712.php)

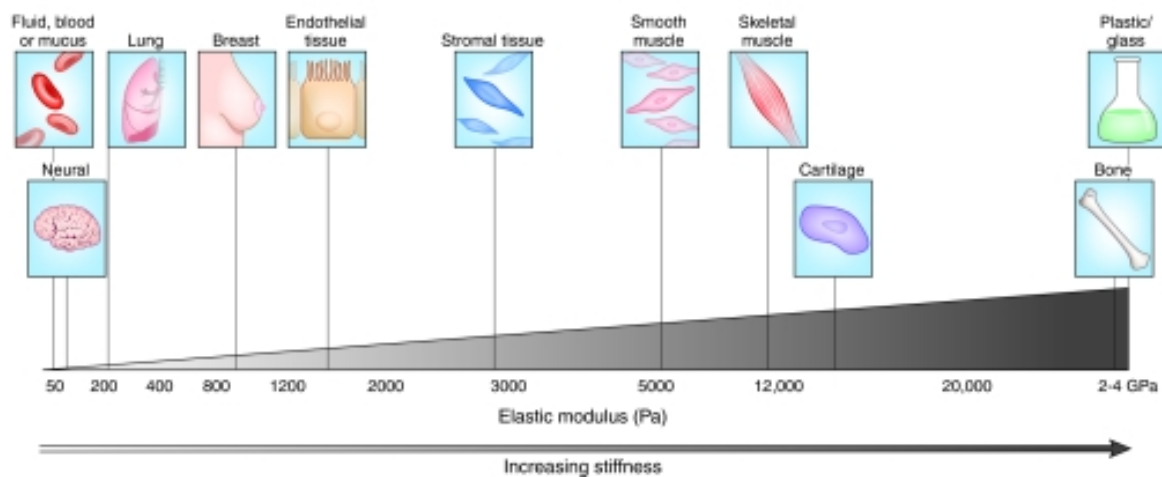
Questions:

1. What changes occur to breast cancer cells when grown on 3-D gels of increasing rigidity?
2. How are the epithelial cells of the zebra fish able to invade the surround tissue?
3. What is meant by an invasive cancer?
4. How is this related to human cancer?
5. Look at the diagram below: what can you say about the difference in the cell on the soft surface and the stiff surface?



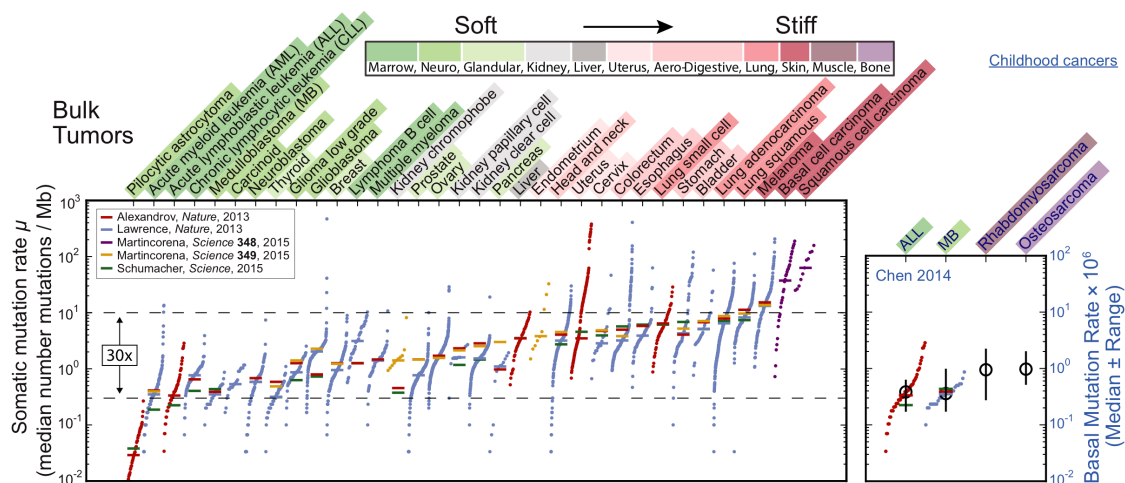
From: Wells 2008

6. Use the diagram below: based on what you have learned in this lesson, explain why breast and lung cancer are more likely to metastasize than bone cancer?



From: Butcher et al., 2009

7. These data show the mutations found in various cancers, which are also varying in tissue stiffness. If the stiffness increases across the x-axis (the top here) from softer to stiffer tissues, what can you conclude about the relationship between mutation rate and tumor stiffness?



From: Irianto et al., 2015

## Acknowledgements

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