Limits to Cell Size (Exploring Surface Area:Volume Relationships)

SC Academic Standards: 4.L.5B; 6.L.4A; 6.L.4B; 7.L.3A; 7.L.3B; H.B.2B; H.B.2C

NGSS DCI: 4-LS1.C; 5-LS2.B; MS-LS1-A; MS-PS3.D; HS-LS1.A

Science and Engineering Practices: S.1A.1; S.1A.2; S.1A.3; S.1A.4; S.1A.5; S.1A.6; S.1A.7

Crosscutting Concepts: Cause and Effect: Mechanism and Explanation; Structure and Function; Scale, Proportion, and Quantity; and Systems Models.

Focus Questions(s): What limits the size of a cell?

Conceptual Understanding: Cells are the most basic unit of any living organism. All organisms are composed of one (unicellular) or many cells (multicellular) and require food and water, a way to dispose of waste, and an environment in which they can live in order to survive.

Transport processes which move materials into and out of the cell serve to maintain the homeostasis of the cell.

Background: All organisms need to exchange substances such as food, waste, gases and heat with their surroundings. These substances must **diffuse** between the organism and the surroundings. The rate at which a substance can diffuse is given by **Fick's law**:

$$\text{Rate of Diffusion} \propto \frac{\text{surface area} \times \text{concentration difference}}{\text{distance}}$$

The rate of exchange of substances therefore depends on the organism's surface area that is in contact with its surroundings. A small organism, like an amoeba, has a large surface area:volume ratio, and so it can accomplish all the exchange it needs by simple diffusion across its body surface. However, a large organism, like a mammal, has a much smaller surface area: volume ratio, so it cannot accomplish all the exchange it needs in this way (a large surface area:volume ratio is preferable for carrying out exchange of substances). Such large organisms need special respiratory organs such as lungs for taking in oxygen and removing carbon dioxide.

The requirements for materials depends on the volume of the organism, so the ability to meet the requirements depends on the surface area : volume ratio. As organisms get bigger their volume and surface area both get bigger, but volume increases much more than surface area. Think of it this way: a bacterium, with a length of 1 mm and a surface area (SA) of $6 \times 10^{-12}m^2$ has a volume (V) of $10^{-18}m^3$ will have a SA:V ratio of 6,000,000. A fly, with a length of 10 mm and a surface area of $6 \times 10^{-4}m^2$ has a volume of $10^{-6}m^3$ and a SA:V ratio of 600. But a dog, with a length of 1 m and a SA of 6 m^2 has a volume of $1m^3$ and a SA:V ratio of 6.

So as organisms get bigger their surface area/volume ratio gets smaller. A bacterium is all surface with not much inside, while a whale is all insides with not much surface. This means that as organisms become bigger it becomes more difficult for them to exchange materials with their surroundings. In fact this problem sets a limit on the maximum size for a single cell of about 100 mm. In anything larger than this materials simply cannot diffuse fast enough to support the reactions needed for life. Very large cells like birds' eggs are mostly inert food storage with a thin layer of living cytoplasm round the outside.

It gets more complicated. If you have two cells that are different shapes – one a cube, and one long and skinny – the long skinny one can still have a SA:V ratio that is sufficient and has a short enough distance from the center of the cell to the cell surface for the cell to be ok, that is, to have sufficiently rapid rates of diffusion. In long skinny cells, like a nerve cell / axon, diffusion of oxygen across the cell membrane is rapid enough for the cell to thrive. But a cube like cell of the same volume wouldn't be able to get oxygen into the center of the cell quickly enough (Table 1).

Organisms also need to exchange heat with their surroundings, and here large animals have an advantage in having a small surface area/volume ratio: they lose less heat than small animals. Large mammals keep warm quite easily and don't need much insulation or heat generation. Small mammals and birds lose their heat very readily, so need a high metabolic rate in order to keep generating heat, as well as thick insulation. So large mammals can feed once every few days while small mammals must feed continuously. Human babies also lose heat more quickly than adults, which is why they need warm hats.

So how do organisms larger than 100 mm exists? All organisms larger than 100 mm are multicellular, which means that their bodies are composed of many small cells, rather than one big cell. Each cell in a multicellular organism is no bigger than about 30mm, and so can exchange materials quickly and independently. Humans have about 10^{14} cells.

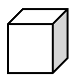
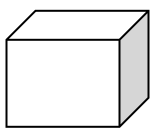
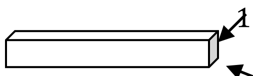
Hypothetical Cells	Surface area	Volume	Surface-area-to-volume ratio	Distance from center of cell to nearest cell surface
 10 µm	6 x 100 μm^2	10^3 μm^3	.6/µm	5 µm
 100 µm	6 x 10^4 μm^2	10^6 μm^3	.06/µm	50 µm
 10µm, 10 µm, 10,000 µm	[2 x 100] + 4 x 10^5 μm^2	10^6 μm^3	~.4/µm	5 µm

Table 1. Hypothetical cells and their SA:V ratio and distance from center of cell to cell surface.

In this lab we will explore surface area:volume relationships as we watch diffusion of an acid (vinegar) into a blue agar block. The blue is bromothymol blue, which is an indicator solution for acid (acid makes it turn yellow). We will use agar blocks as model cells. A **model** is a simplified representation of a complex biological structure or process. A model focuses on a few key features in order to help us understand a biological structure. Because a model is simpler than the biological structure it represents, a model does not demonstrate all the features of the actual biological structure.

<u>**Materials**</u>: square cake pan, Tovolo_Perfect cube ice cube trays to make agar blocks ($15 on Amazon.com for set of 2 (makes 30 cubes)), agar (Carolina Biological, Agar powder (not agarose), 100 g for $20), white vinegar, powdered bromothymol blue (Carolina Biological 5g, $20), water, ruler, butter knife or thin plastic knife (to cut cleanly through jello-like material).

TEACHER notes: Make agar cubes 2-3 days in advance:

- Mix 15g agar in 1 liter water. This is **AGAR**, not agarose. (Note: 15g is thicker than you would make for growing bacteria, because you want the agar stiffer and tougher for handling.) It is better to use plain agar and not LB (nutrient) agar, so it has less of a chance to grow bacteria.

- Boil slowly in microwave or hot water bath until agar is melted (granules will disappear). Watch for and avoid boil-over.

- Remove from heat. Add 0.1 g powdered bromothymol blue and mix. If the mixture is not dark blue, then add more bromothymol blue. If the mixture is green or yellow, you will need to stir in drops of NaOH (or another base) until it turns blue. Wear safety goggles and gloves when handling NaOH.

- Pour the agar into trays. For the initial lab, I make the agar in rectangular trays, like a wide silverware tray or a square Pyrex dish -- enough to be at least 2cm deep -- and slice chunks for the students to cut from. (I use the tops of my microcentrifuge storage boxes most of the time).

- For the actual contest, I mold them in Tovolo perfect cube ice cube trays from Amazon.com, and use a spatula or butter knife to remove the gelatinous cubes. Make enough for 1-2 ice cubes per student or student group. Let agar harden at room temperature or in refrigerator. Cubes can be made a couple of days in advance (not too much more than that or the blue agar starts turning green, then yellow). Cover with plastic wrap to keep from drying out and store in refrigerator, otherwise it will get a lot of bacterial growth quickly, especially if you use nutrient agar.

Previous Knowledge: (chemistry): Bromothymol Blue is an indicator for pH – it can also indicate CO_2 presence (which lowers pH when CO_2 combines with certain solutions to make carbonic acid). When pH lowers, Bromothymol blue goes from blue to green to yellow.

Previous Knowledge (math): Students have to calculate volume, surface area, and SA:V ratio. The surface area of a cube with length of side L is LxL X6 ($6L^2$), while the volume is L^3. You'll notice that 2x2x2 and 1x1x8 have same volume, but different surface areas.

Calculating the ratio of surface area : volume for organisms

- Look at surface area and volume values
- Check they are in the same units
- Divide the surface area value by the volume value.
- The answer : 1 is the ratio of surface area to volume.

  - e.g. Surface area = 54 cm^2
  - Volume = 27 cm^3
  - Surface area to volume = 54/27 = 2
  -
  - Therefore 2 : 1 is the ratio of surface area to volume.

Procedure:

Part 1: investigating diffusion

1. Cut four cubes of the blue agar block. They should be the following sizes:

0.5 cm x 0.5 cm x 0.5 cm	**1cm x 1cm x 1cm**
2cm x 2cm x 2cm	**1cm x 1cm x 8cm**

2. Pour 30 ml of white vinegar (a weak acid) into a 100 ml beaker. Gently place the blocks into the acid all at the same time. Record the start time. You will wait for 5 minutes before taking the blocks out.

Start Time: ________________

3. After 5 minutes take all blocks out of the vinegar solutions and CUT them in ½ with a straight edge or plastic knife. Quickly measure the depth to which the acid diffused (your block will now be yellow on the outside and blue in the center – measure the distance, in mm, from the agar block surface to the start of the blue line). Also measure the shortest length of blue rectangle in the center of the agar block.

Did some blocks turn totally yellow? If it is all yellow, the depth of diffusion would be half of the distance across the cube, and shortest length of blue inside the cube would be zero.

(Teacher Notes: notice the yellow/acid should have moved into all the cubes to the same depth, due to diffusion (its at the same rate regardless of cube size – the cubes are made from the same material (molecular weight), the acid and agar are the same temperature regardless of block size, so diffusion rate is equal – later we will look at different temperatures of acid, and the colder the acid the slower diffusion should be, so the depth to which the yellow meets blue will be fairly shallow/small). The bigger cubes have much more blue cube yet to turn yellow. They have a bigger inside! It will take longer foe the bigger cubes to turn completely yellow. You can, if you want, do a simple **extension** where you simply time how long it takes for the cubes to turn entirely yellow.

Cube Size	**Volume (V) = LxWxH**	**Surface Area of cube = (SA) = 6(S^2) SA of rectangle = 4x(LxW) + 2x (LxW)**	**Surface Area / Volume ratio = SA/V : 1**	**Depth of Diffusion (mm) after 5 minutes)**	**Shortest Length of blue remaining in the agar square**
0.5 cm x 0.5 cm x 0.5 cm					
1 cm x 1 cm x 1 cm					
2 cm x 2 cm x 2 cm					
1 cm x 1 cm x 8 cm					

Table 1. SA:V relationships and diffusion for model cells (agar blocks)

1. What do the different sized cubes represent?
2. What does the acid represent?
3. Why do we use the indicator?
4. How do you know diffusion is occurring?
5. As the volume of the cell gets <u>smaller</u> what happens to the surface area to volume ratio?
6. As SA:V ratio gets <u>larger</u>, what happens to the speed of diffusion?
7. Why are cells so small? (what is the advantage)?

Part 2: Investigating the effects of temperature on rates of diffusion

Question: What is the effect of temperature on the rate of diffusion?

Hypothesis: As temperature increases the rate of diffusion will also increase

Null Hypothesis: temperature has no effect on the rate of diffusion

Prediction: the rate of diffusion in the beaker with the warm vinegar will be the fastest, and slowest in the beaker of cold vinegar.

Procedure 2 :

1. Obtain 3 small beakers. Beaker #1 should have 300 ml cold vinegar (placed in freezer for 1 hour – check to make sure it doesn't freeze solid!); Beaker #2 should have 300 ml room temperature vinegar; Beaker #3 should have 300 ml warm vinegar (microwave 1-2 minutes)

2. Measure the temperatures of the vinegar in each beaker and record this on your data table.

3. Cut 4 blue agar cubes, each 1 cm x 1 cm x 1 cm

4. Drop them into the beakers simultaneously. Remove all after 10 minutes.

5. Cut each block in ½ and Measure depth of diffusion after 10 minutes.

Beaker #	**Temperature of Vinegar**	**Total Depth of Diffusion (mm) after 10 min)**	**Rate of Diffusion (depth / time)**
1 (cold vinegar)			
2 (room temperature vinegar)			
3 (warm vinegar)			

Table 2: Rates of diffusion for agar block cells in different temperatures of vinegar

6. Explain how temperature affects the rate of diffusion.

<u>Data Analysis:</u> You can graph Part 2 with Time on the X- axis and Depth of diffusion on the Y. Your graph will have three lines, one for each temperature. The independent variable is Time, the dependent is depth of diffusion, and the treatments are the 3 temperatures. You should see the slowest diffusion in the cold vinegar, because molecules that are moving slowly register as cold, and slow moving molecules will diffuse more slowly. (Remember, temperature is actually a measure of how fast the molecules are moving – not "heat").

<u>Reflection Questions:</u>

- **Which is better for quick diffusion of materials into/out of a cell- a large surface area:volume or a small SA:V ratio?** (larger)

- **How can a cell have a large volume but still maintain an adequate speed of diffusion of materials into / out of the cell?** (cells that are very slender, like the long axon of a neuron, still have a short distance from center of cell to cell surface)

- **What are some disadvantages of a large cube shaped cell versus a small cube shaped cell?** (the distance from the center of the cell to the cell surface is a lot longer in a large cell, so it takes a lot longer for diffusion of materials (e.g., it takes only 15 milliseconds for O_2 to diffuse to the center of a sphere with a diameter of 20 μm, but it would take 265 days for O_2 to diffuse to the center of a sphere the size of a basketball; diffusion is very slow over long distances due to collisions which repeatedly change the direction of molecular motion).

  Also, the surface-area-to-volume ratio much lower, so ratio of supply to demand much lower for substances like O_2 that diffuse across the cell surface (algebraically, if r = the radius of a sphere or the length of the side of a cube, the surface-area-to-volume ratio is proportional to $r^2/r^3 = 1/r$, which demonstrates that for these shapes surface-area-to-volume ratio decreases as cell size increases)).

  In accord with these limitations of diffusion, most cells are tiny (e.g. prokaryotic cells typically 1-10 μm in diameter and most eukaryotic cells 10 - 100 μm.

- **How is it that we have many organisms that reach a large body size – how are they able to maintain large SA:V ratios so rates of diffusion are high?** (have a multicellular body, with many many small cells)

EXTENSION: STEM Challenge: Race to Design a cell with maximum volume and mass but minimal diffusion time.

The next day students are given an ice cube block of the agar and they must design their own cell to maximize volume & mass, but minimize diffusion time. This process allows students to confront a lot of misconceptions of cell design.

RULES:
1. No donut-like holes through the agar cell -- cell membranes cannot sustain that shape.
2. No poking, prodding, touching beaker containing agar cell in vinegar.
3. Students mass agar at conclusion of race...cell must not break when handled. Disqualification if cell breaks upon massing (although you can be a bit lenient here).
4. Winner = highest ratio of **mass divided by time**.
5. We sometimes run a second race after a trial run, so students can improve designs. I make a lot of agar just in case. It's a fun learning day!

EXTENSION: You can get three balloons – fill the first with 500 ml of water. With the next balloon, add 1000 ml water, and add 1500 ml to the third balloon. Measure circumference of each balloon and calculate surface area and volume. What is the relationship between surface area and volume? Volume should be gaining a lot more than the surface area.

Circumference = 2 pi r
Surface Area of a Sphere = 4 *pi* r ^{2}
Volume = (4/3) pi r ^{3}

Models and Explanations: In this lab we explored surface area:volume relationships as we watched diffusion of an acid (vinegar) into a blue agar block. The blue is bromothymol blue, and indicator solution for acid. We used agar blocks as model cells. The primary objective is to demonstrate the potential limitation on cell size. **A student who demonstrates understanding** of these concepts can explain why multicellular organisms are made of many many small cells, and not a few large ones. This student understands that diffusion/osmosis (diffusion of water) is the main way nutrients and gasses get transported across a cell membrane, and that the surface area of the cell membrane limits how much diffusion can take place, and thus how much nutrients /gasses/wastes can be transported across the membrane. Diffusion is a fairly slow process and a cell that relies primarily on diffusion to transport essential molecules into and throughout its interior – and to carry waste products out – could conceivably grow too large for this process to work efficiently.

Successful students will be able to use the SA/V ratio by first predicting the outcome of a hypothetical experiment: which blue agar cube in vinegar will show complete color change to yellow first? This student will also be able to explain why, if you cut the blue agar blocks that have been soaked in vinegar for 5 minutes, what you see is that the penetration depths are the same, regardless of cube size, and the diffusion rates are equal for all 3 cubes BUT what is dramatically different is how much of the interior volume of each cube has been affected. Math inclined students can calculate the percentage of each cell's interior volume that has turned yellow. This gives a fairly good indication of which cell is most likely to "survive". The key characteristic of the blocks is not the total surface area but the surface area to volume ratio – the table will show that cube size and SA / V ratio are inversely proportional and the cube with the highest ratio will be most efficient.

A successful student will conclude that smaller cells are better able to move materials in and out. A cell could eventually reach a size at which materials could not diffuse in (or out) fast enough to meet requirements – and wastes could build up to toxic levels. **Further**, **a successful** student can describe shapes of cells that might help to maximize surface area (round versus folded or branched, for example).

Bibliography:

The American Biology Teacher, Vol. 76(4): 265-269 does something similar, outlined in the article "A simple inquiry-based lab for teaching osmosis" by John Taylor. This lab uses potatoes instead of agar blocks, and has students carve a potato into a 20 gram piece designed so that it will maximize diffusion - as evidenced by the potato gaining weight when placed in salt water for ten minutes. This write up also includes a section where students guess which bucket contains the saltiest water based on how much weight a potato gains.

Examstutor.com (n.d.) Retrieved September 11,2014 from http://www.examstutor.com/biology/resources/studyroom/organs_and_systems/gas_exchange/

Foglia, K. (2010). Explore Biology – Limits to cell size lab / AP Biology. Retrieved September 10, from http://www.explorebiology.com/apbiology/labs/lab42.html

Gilbert, J. (2004). Biology Mad (Diffusion). Retrieved September 10, 2014, from http://www.biologymad.com/master.html?http://www.biologymad.com/cells/cells.htm

Taylor, J. (20140. A simple inquiry-based lab for teaching osmosis. The American Biology Teacher, Vol. 76(4): 265-269.

Waldron, I. Serendip. (2003-2014). University of Pennsylvania, retrieved September 12, 2014 from
http://serendip.brynmawr.edu/sci_edu/waldron/#diffusion

Student worksheet:

Each cell is surrounded by a **selectively permeable membrane** which regulates what gets into and out of the cell. A selectively permeable membrane allows some types of molecules and ions to diffuse across the membrane and prevents other types of molecules and ions from crossing the membrane. For example, oxygen (O_2) can cross the selectively permeable membrane that surrounds each cell, but large molecules cannot.

Most cells are between 2 micrometers and 200 micrometers—too small to be seen with naked eye. Remember, a micrometer is 1 millionth of a meter! Why can't cells ever become larger than that? Why don't we regularly find one-celled organisms the size of small multicellular animals, like frogs or even flies?

One of the core principles that governs the efficiency of diffusion is the ratio of surface area to volume (SA:V). Surface area is the amount of cell membrane available for diffusion. So for a cell, surface area actually represents how much diffusion that can happen at one time. Whereas volume is the amount of cytoplasm contained within the cell membrane. So for a cell, volume is how long it takes to get from the membrane to the center of the cell by diffusion.

As organisms get bigger the ratio of surface area/volume gets smaller. A bacterium is all surface with not much inside, while a whale is all insides with not much surface. This means that as organisms become bigger it becomes more difficult for them to exchange materials with their surroundings. In fact this problem sets a limit on the maximum size for a single cell of about 100 mm, because diffusion is too slow over longer distances. In anything larger than this materials simply cannot diffuse fast enough to support the reactions needed for life.

In this lab we will explore surface area:volume relationships as we watch diffusion of an acid (vinegar) into a blue agar block (our model of a cell). The blue is bromothymol blue, an indicator solution for acid, which turns yellow under acidic conditions. We will time how long it takes for the acid (vinegar) to diffuse into the cell, and then calculate rates of diffusion.

Part 1: investigating diffusion

Question:

Hypothesis:

Prediction:

Cube Size	Volume (V) = LxWxH	Surface Area of cube = (SA) = $6(S^2)$ SA of rectangle = 4x(LxW) + 2x (LxW)	Surface Area / Volume ratio = SA/V : 1	Depth of Diffusion (mm) after 5 minutes)	Shortest Length of blue remaining in the agar square
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- Why are cells so small? (what is the advantage)?
- Which is better for quick diffusion of materials into/out of a cell- a large surface area:volume or a small SA:V ratio?
- How can a cell have a large volume but still maintain an adequate speed of diffusion of materials into / out of the cell?
- How is it that we have many organisms that reach a large body size – how are they able to maintain large SA:V ratios so rates of diffusion are high?

Part 2: investigating the effects of temperature on the rates of diffusion

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Prediction:

Beaker #	Temperature of Vinegar	Total Depth of Diffusion (mm) after 10 min)	Rate of Diffusion (depth / time)
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GRAPH depth of diffusion vs. time. Which is the independent variable? ______________
Dependent? _______________________ Controlled variables? _______________________________