BIO-372 “MICROBIOLOGY” EXERCISES (WEEK 2)

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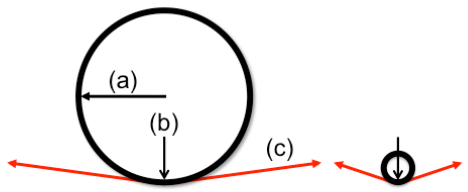
EXERCISE 1 "BIOMECHANICS OF THE BACTERIAL CELL ENVELOPE" :

Imagine two spherical cells, one large and one small.



1. Write the equation for Laplace’s Law:

$T = P * r$

and define each term below, referring to the diagram above. Do not peek at the slides until you’ve made your best effort to write everything from memory!

(a) = r = radius of curvature (in m)
(b) = P = pressure (in $kg * m^{-1} * s^{-2}$)
(c) = T = tension (in $kg * s^{-2}$)

2. If the internal pressure is the same in both cells, then the cell wall tension
☐ A. will be higher in the small cell.
☒ B. will be higher in the large cell.
☐ C. will be the same in both cells.
Explain your answer:

According to Laplace’s Law, $T = P * r$ where T is tension, P is pressure, and r is the cell’s radius of curvature. Thus, it follows mathematically that, at a defined internal pressure, as r increases T also must increase in order to prevent the cell from expanding. Geometrically, tension in the cell wall can be considered as a resultant comprising a “vertical” component (acting opposite to the direction in which pressure is exerted) and a “horizontal” component (acting perpendicular to the direction in which pressure is exerted). Only the “vertical” component is useful to balance internal pressure acting on the cell wall. In small cells, the “vertical” (useful) component of cell wall tension is large and the “horizontal” (useless) component is small. In large cells, the “vertical” (useful)component of cell wall tension is small and the “horizontal” (useless) component is large. Thus, at a defined internal pressure, in large cells the overall tension (vertical + horizontal components) in the cell wall must be large in order to achieve a vertical component equal but opposite to the load exerted by the internal pressure. At the same internal pressure, in small cells a smaller overall tension in the wall is sufficient to achieve a vertical component equal but opposite to the load exerted by the internal pressure.

3. If the cell wall tension is the same in both cells, then the internal pressure
☒ A. will be higher in the small cell.
☐ B. will be higher in the large cell.
☐ C. will be the same in both cells.
Explain your answer:

According to Laplace’s Law, $T = P * r$ where T is tension, P is pressure, and r is the cell’s

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radius of curvature. Thus, it follows mathematically that, at a defined cell wall tension, the pressure will be higher in the smaller cell. Geometrically, tension in the cell wall can be considered as a resultant comprising a "vertical" component (acting opposite to the direction in which pressure is exerted) and a "horizontal" component (acting perpendicular to the direction in which pressure is exerted). Only the "vertical" component is useful to balance internal pressure acting on the cell wall. In small cells, the "vertical" (useful) component of cell wall tension is large and the "horizontal" (useless) component is small. In large cells, the "vertical" (useful) component of cell wall tension is small and the "horizontal" (useless) component is large. Thus, at a defined pressure, tension will be lower in the small cell and higher in the large cell. If the cell wall tension is the same, as posited here, then the internal pressure in the small cell must be larger than the internal pressure in the large cell.

4. Starting from the same internal pressure, if the pressure is gradually increased then
☐ A. the small cell will rupture first.
☒ B. the large cell will rupture first.
☐ C. both cells will rupture at the same time.
Explain your answer:

According to Laplace's Law, $T = P * r$. Therefore, it follows mathematically that, at a defined pressure the cell wall tension will be small in the small cell and large in the large cell. The geometric argument is given above. As the pressure increases, eventually the tension in the cell wall will exceed the material strength of the cell wall, resulting in rupture of the cell. Since at any defined value of P the value of T is larger in the large cell than in the small cell, as pressure increases the large cell will rupture first. If pressure continues to increase, the small cell will eventually rupture as well, when the value of T reaches the same value that caused the large cell to rupture.

5. Imagine two cells with equal size (volume), a sphere-shaped cell and a cube-shaped cell. Starting from the same internal pressure, if the pressure is gradually increased then
☐ A. the sphere-shaped cell will rupture first.
☒ B. the cube-shaped cell will rupture first.
☐ C. both cells will rupture at the same time.
Explain your answer:

In the sphere-shaped cell every "element" of the cell wall is geometrically equivalent. Thus, the cell wall tension caused by the internal pressure is equally distributed everywhere. In the cube-shaped cell, nearly every element of the cell wall is "horizontal" to the direction of pressure and is therefore useless to oppose it, for reasons discussed above. Consequently, the pressure-driven load on the cell wall is transferred to the edges and especially to the corners. Thus, at any given pressure, because the pressure-driven load is concentrated on the edges and corners of a cube-shaped cell the tension in these elements of the cube-shaped cell wall will be higher than the tension in any element of the sphere-shaped cell. Consequently, as the pressure increases the cube-shaped cell will rupture before the sphere-shaped cell, most likely at the corners.

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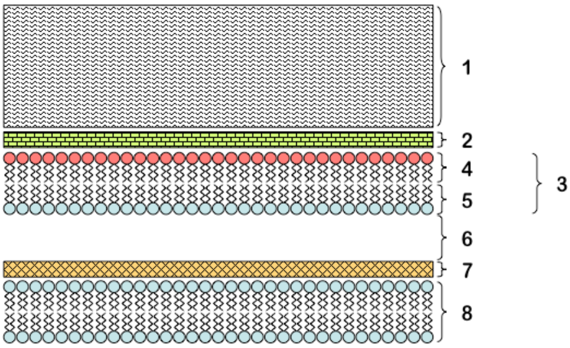
EXERCISE 2 "BIOMECHANICS OF THE BACTERIAL CELL ENVELOPE" :

Here is a schematic diagram of a "generic" bacterial cell wall :



Identify each layer (1-8) and briefly explain its biological function(s).

1. Name: Capsule. Composed of polysaccharides.
Function(s): Prevents dehydration by binding and retaining water (this is why colonies of encapsulated bacteria are "slimy"). Helps the bacteria to evade attack by the complement system and antibodies.

2. Name: S-layer. Composed of a two-dimensional crystal of a single protein. This layer is found in some (but not all) bacteria and archaea.
Function(s): Mechanical support. Contributes to cell shape. Serves as an "anchoring site" for some extracellular proteins.

3. Name: Outer membrane. It is a lipid bilayer. Present in Gram-negative bacteria but not in Gram-positive bacteria.
Function(s): Selective permeability barrier. Also serves as an "anchoring site" for some extracellular (outer membrane) proteins.

4. Name: Outer leaflet of outer membrane. Composed mostly of lipopolysaccharide (LPS).
Function(s): Selective permeability barrier. Evasion of the complement system. The lipid A component of LPS is strongly immunostimulatory and is largely responsible for the clinical signs and symptoms of Gram-negative septic shock.

5. Name: Inner leaflet of outer membrane. Composed of "typical" phospholipids like phosphatidylethanolamine and phosphatidylglycerol.
Function(s): Permeability barrier.

6. Name: Periplasmic space between the inner and outer membranes. Present in Gram-negative bacteria but not in Gram-positive bacteria.
Function(s): Although depicted as "empty" in the diagram, the periplasmic space is actually filled with proteins that have extracellular functions, e.g., enzymes that inactivate antibiotics, proteins involved in nutrient uptake, etc.

7. Name: Peptidoglycan. Composed of long strands of glycans (sugar backbone) crosslinked with short peptides.
Function(s): Mechanical support and cell shape. It is the main stress-bearing "fabric" that protects bacterial cells against osmotic rupture and other mechanical insults.

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8. Name: Inner (cytoplasmic) membrane). It is a lipid bilayer composed of "typical" phospholipids like phosphatidylethanolamine and phosphatidylglycerol. Similar to the cell membrane of eukaryotic cells.
Function(s): Permeability barrier. Also serves as a "binding site" for membrane-associated proteins involved in a variety of functions (e.g., motility, nutrient uptake, etc.). Also serves to maintain a proton motive force (PMF) across the cytoplasmic membrane, which can be used either directly as a power source or indirectly by "storage" of energy in the form of ATP.

9. On the basis of the diagram, is this organism ☐ Gram-positive or ☒ Gram-negative?
Explain:

Only Gram-negative bacteria have an outer membrane. Also the peptidoglycan is depicted as a thin layer, which is characteristic of Gram-negative bacteria, whereas Gram-positive bacteria have thick (multi-layered) peptidoglycan. Finally, teichoic acids are not present in the peptidoglycan layer in the diagram; teichoic acids are characteristic of Gram-positive bacteria but they are not found in Gram-negative bacteria.

10. In class we discussed some mechanisms that layers #1 and #4 contribute to evasion of the host complement system.

Explain (layer #1):

The capsule acts as a physical "shield" to block binding of antibodies and complement proteins to structures on the bacterial cell surface (under the capsule). Variation of the sugars in the capsule, both between species and between strains of the same species, can also help encapsulated bacteria to avoid binding of antigen-specific (i.e., sugar-specific) antibodies. The sugars that comprise the capsule are poorly immunogenic and, in some cases, are non-immunogenic because they are the same sugars found in human tissues ("molecular mimicry") [an example is hyaluronic acid, but you don't need to memorize this]. This example of "molecular mimicry" means that the bacteria are covered with a "blanket" of molecules that the immune system recognizes as "self" rather than "non-self" (like the "wolf in a sheep's skin").

Explain (layer #4):

The lipopolysaccharide (LPS) includes a highly variable "O-antigen" on the cell-distal end of the molecule. (1) Antigenic variation of the O-antigen both between species and between strains of the same species allows the bacteria to evade attack by antigen-specific antibodies. (2) In some bacteria, the O-antigen is extremely long, which helps the bacteria to evade killing by the complement system "membrane attack complex" (MAC) by causing the MAC to assemble at a distance that is too great for it (the MAC) to punch holes in the bacterial cell wall. (3) In some bacteria, the molecules that comprise the O-antigen are non-immunogenic because they are the same sugars found in human tissues ("molecular mimicry"). [An example is sialic acid, but you don't need to memorize this.] This example of "molecular mimicry" means that the bacteria are covered with a "blanket" of molecules that the immune system recognizes as "self" rather than "non-self" (again, like the "wolf in a sheep's skin").

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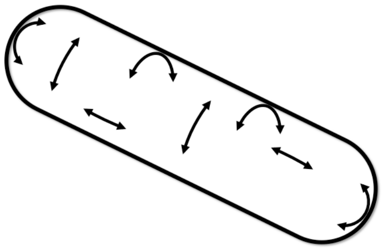
EXERCISE 3 "BIOMECHANICS OF THE BACTERIAL CELL ENVELOPE" :

The schematic depicts the distribution of stresses in the bacterial cell wall under positive internal osmotic pressure:



1. Write the equations that describe cell wall stress in the same direction as the long axis of the cell:

$\sigma_L = P * r * (2t)^{-1}$

and in the same direction as the short axis of the cell:

$\sigma_H = P * r * t^{-1}$

and define each term below. Do not peek at the slides until you’ve made your best effort to write everything from memory!

P = pressure in kg * m^{-1} * s^{-2}
r = radius of curvature in m
σ_H = hoop stress in kg * m^{-1} * s^{-2}
σ_L = longitudinal stress in kg * m^{-1} * s^{-2}
t = cell wall thickness in m

2. Imagine a rod-shaped cell with no peptidoglycan in which internal pressure increases until the cell ruptures. The cell would rupture along:

☐ A. Its short axis.
☒ B. Its long axis.
☐ C. Either axis with equal probability.

Explain:

Because the “hoop stress” (σ_H) is roughly twice the “longitudinal stress” (σ_L). According to Newton’s first law, an object will remain at rest or in uniform motion in a straight line unless acted upon by an external force. If a cell experiences internal turgor pressure (as most bacteria do in most environments, because the interior is usually hypertonic relative to the exterior), then the turgor pressure must be balanced by the tension in the cell wall to prevent the cell from expanding and bursting.

Consider first the longitudinal stress (σ_L) aligned with the cell’s long axis by making a “slice” along the cell’s short axis:

$\sigma_L * 2\pi r * t = P * \pi r^2$

In this equation, the tension in a “slice” of the cell wall (left of the = sign) balances the turgor pressure acting on the same “slice” of the cell wall (right of the = sign). Solving for σ_L:

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$\sigma_L = P * r * (2t)^{-1}$

Consider next the hoop (circumferential) stress aligned with the cell's short axis by making a "slice" along the cell's long axis:

$\sigma_H * 2L * t = P * 2r * L$

In this equation, the tension in a "slice" of the cell wall (left of the = sign) balances the turgor pressure acting on the same "slice" of the cell wall (right of the = sign). Solving for σ_H:

$\sigma_H = P * r * t^{-1}$

In conclusion, at a specific pressure (*P*) the hoop stress is twice as big as the longitudinal stress. Thus, if the pressure increases until the cell ruptures, it will rupture along its long axis (like an overcooked sausage).

3. In order to provide the best mechanical support against internal pressure, peptidoglycan cables should be oriented:
☒ A. In the same direction as the short axis of the cell.
☐ B. In the same direction as the long axis of the cell.
☐ C. In the same direction as both axes of the cell (long and short).
☐ D. Randomly.
Explain why:

Since the hoop stress is twice the longitudinal stress, as the pressure increases the cell will eventually rupture along the cell's long axis (see above). In order to prevent the cell from rupturing along its long axis, the peptidoglycan cables should be oriented along the cell's short axis so that the supporting elements (cables) are oriented in the same direction as the hoop stress. If the cables were oriented in the opposite direction, along the cell's long axis, then they would be oriented perpendicular to the hoop stress and [as we discussed in class in Week02] the tension in the cell wall is only useful if it is oriented along the same axis as the stress it is counteracting.

4. If you increase the thickness of the cell wall while keeping the internal pressure the same, the tensile stress in the cell wall will:
☐ A. Increase.
☒ B. Decrease.
☐ C. Stay the same.
Explain:

Tensile stress in the cell wall is inversely proportional to cell wall thickness ($\sigma \propto t^{-1}$). This is true along both the long axis and short axis of the cell:

$\sigma_L = P * r * (2t)^{-1}$

$\sigma_H = P * r * t^{-1}$

Thus, at a specific pressure (*P*), as the cell wall gets thicker the tension required to balance the pressure gets smaller; conversely, at a specific pressure (*P*), as the cell wall gets thinner the tension required to balance the pressure gets bigger.

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5. If the width (i.e., the length of the short axis) of a cell increases by 2-fold, the resistance of the cell to rupture caused by osmotic pressure will:

- ☐ A. Increase by 2-fold.
- ☐ B. Increase by 4-fold.
- ☒ C. Decrease by 2-fold.
- ☐ D. Decrease by 4-fold.

Explain:

At a given internal pressure, the hoop stress in the cell wall is given as $\sigma_H = P * r * t^{-1}$, where σ_H is the hoop stress, *P* is the internal pressure, r is the radius of the cell along the short axis, and t is the thickness of the cell wall. Thus, if the width of the cell increases by 2-fold then the radius of the cell increases by 2-fold and the hoop stress also increases by 2-fold. A 2-fold increase in the hoop stress means that the "wide" cell will be 2-fold more vulnerable to osmotic rupture compared to a "this" cell (i.e., a cell with a smaller width).