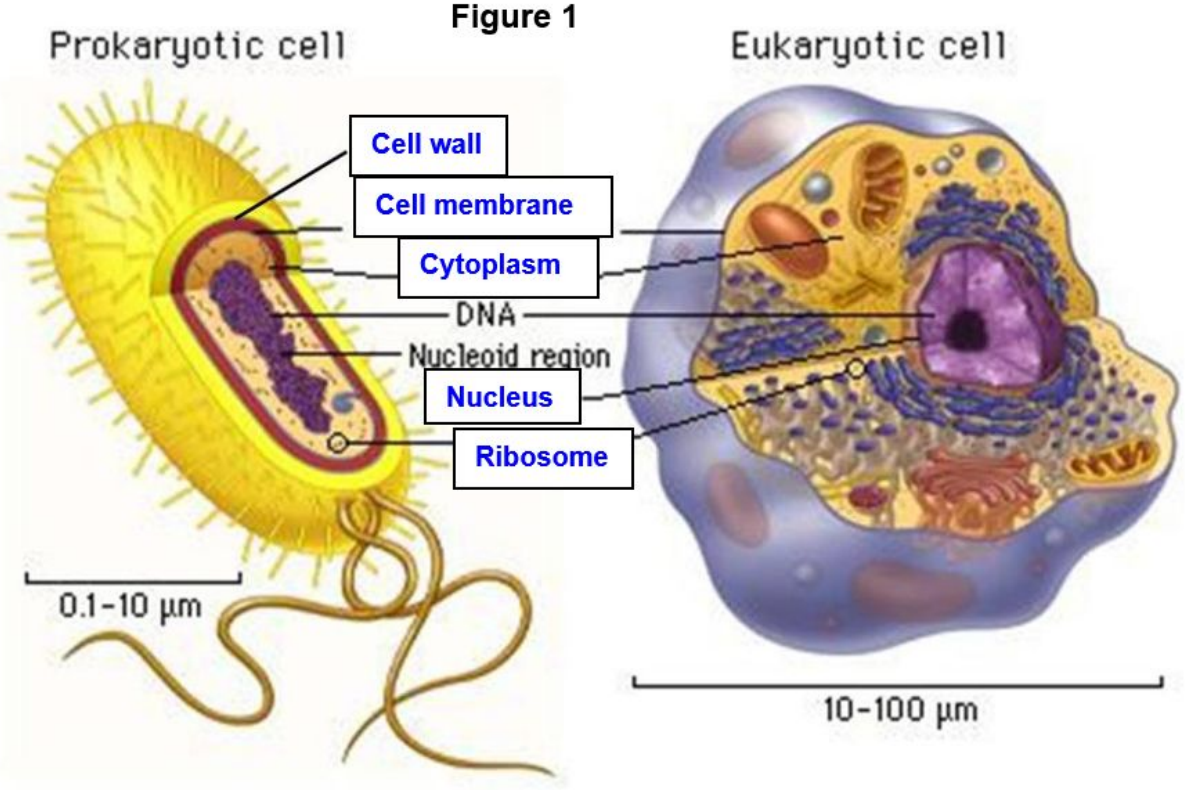
A221: Microbiology
Problem 6: Check it out! Part IV
WORKSHEET

Question 1

Figure 1 depicts the structural features of prokaryotic and eukaryotic cell.

a) Fill in the blanks to indicate the components that make up prokaryotic and eukaryotic cell.

Figure 1



b) What are the major differences between prokaryotic and eukaryotic cell that is observable in the diagram?

→ Different shape

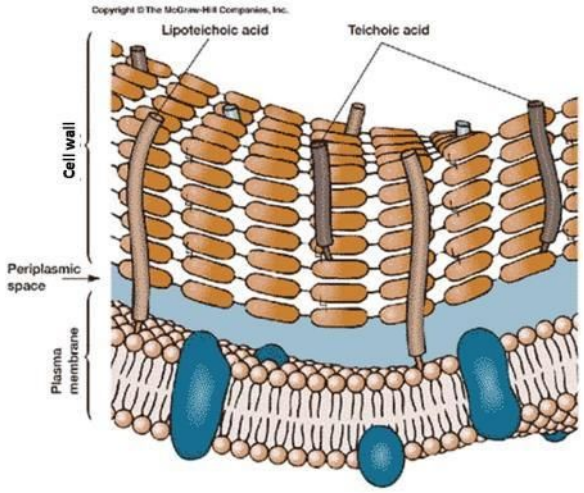
→ Different structures/components:

- Prokaryote - Flagellum, Pili & Nucleoid
- Eukaryote - Nucleus, Mitochondria

Prokaryotic cell lack a nucleus and membranous organelles. In addition, prokaryotic cell also have cell wall and flagella that are absent in eukaryotic cell (eg. animal cell)

c) Figure 2 shows a picture of the boundary layer of bacteria. Bacterial cell wall account for a number of important bacterial characteristics.

Figure 2



i. What is the major function of bacterial cell wall?
Protects the bacterium from external damage, prevent cell from bursting due to osmotic pressure thus maintaining its shape.

It helps in the determination of the bacterial shape. It also provides a strong structural support to keep bacteria from bursting or collapsing because of changes in osmotic pressure

ii. Does the cell wall of all bacteria have the same thickness?
No, it depends on external factors such as the growth environment of the bacteria.

No, they may not have the same thickness. Some bacteria have a thicker cell wall while others may have a thinner one

iii. How are bacteria group accordingly to the characteristic of their cell wall?
They are classified by gram positive (thick cell wall) and gram negative (thin cell wall).

iv. What is the major component that makes up bacterial cell wall?
Peptidoglycan, which is made up of a repeating framework of long glycan chains cross-linked by short peptide fragment

v. How does the cell wall of the bacteria groups (Part ciii) differ from each other?
One has thicker cell wall one has thinner cell wall.

The Gram-positive cell wall is characterized by the presence of a very thick peptidoglycan layer. embedded in the gram-positive cell wall are polyalcohols called teichoic acids, some of which are lipid-linked to form lipoteichoic acids. because lipoteichoic acids are covalently linked to lipids within the cytoplasmic

membrane they are responsible linking the peptidoglycan to the cytoplasmic membrane.

The gram-negative cell wall contains a thin peptidoglycan layer adjacent to the cytoplasmic membrane. In addition to the peptidoglycan layer, the gram-negative cell wall also contains tan outer membrane composed by phospholipids and lipopolysaccharides, which face into the external environment.

vi. Based on your understanding on the components that makes up the cell wall of the different groups of bacteria, what is the overall charge of the bacterial cell wall of these groups? Which component is responsible for the overall charge of the respective cell wall?
The overall charge of the bacterial cell wall is negative charge. The components responsible for the overall charge of the cell wall are the wall teichoic acid and lipopolysaccharides and the major component of the cell wall peptidoglycan.

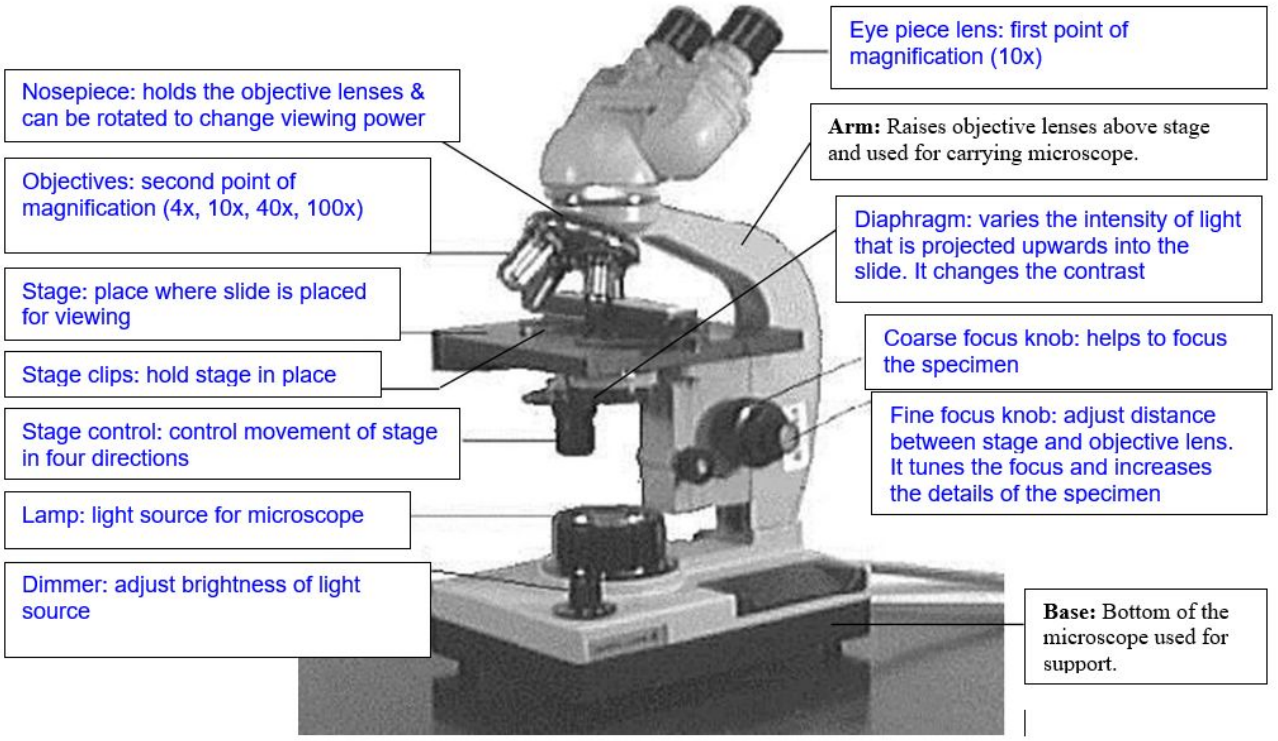
Gram-positive cell wall and overall negative charge due to the presence of phosphodiester bonds between teichoic acid monomers.

Gram-negative cell wall has an overall negative charge as the lipopolysaccharides are highly negatively charged.

Question 2

Bacteria are too small to be observed with naked eyes. They can only be observed when magnified under microscope.

a) With the help of references in pre-reading, label the parts of the microscope below and state their respective functions in Figure 3 below.



b) Which part of the light microscope is responsible for magnification of bacteria?
Objective lens and ocular lens.

c) How many times can bacteria be magnified when viewed under light microscope?
100x, 400x and 1000x. Objective can be 10x, 40x and 100x, plus ocular lens 10x.

d) At which objective is it necessary to add a drop of oil between the tip of the lens and the specimen on the slide? Why is this necessary?
Oil is necessary as it is used to increase the resolving power of a microscope. This is achieved by immersing both the objective lens and the specimen in a transparent oil of high refractive index, thereby increasing the numerical aperture of the objective lens 100X (oil)

At 100x objective. because oil has the same optical qualities as glass, it prevents the refractive loss that normally occurs as peripheral light passes from the slide into the air. the oil will form a continuous medium to transmit a beam of light from the condenser to the objective and effectively increase the numerical aperture.
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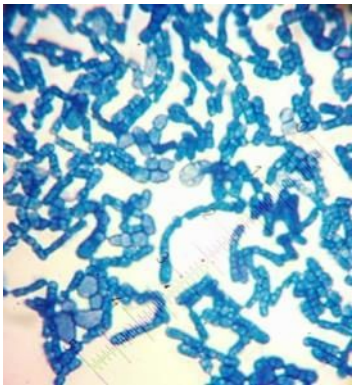
The immersion of oil helps to increase the refractive index of the medium thereby increasing the resolution to distinguish between bacteria that are close together.
When the space between the lens and specimen is replaced with immersion oil, many of the light rays that's previously did not enter the lens due to refractions and reflection will now be able to do so.

Question 3

There are different ways in which microorganisms can be observed under microscope.

Figure 4A

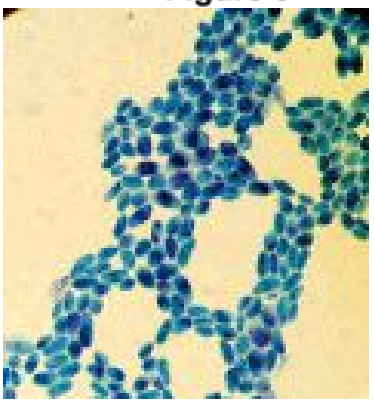
Figure 4B



a) Figure 4A and 4B shows the microscopic view of unstained and stained microorganism respectively. In which figure can microorganism be better observed?
Figure 4B

b) The bacteria in figure 5 below are stained with via simple staining method, using methylene blue.

Figure 5



i. What is the charge of methylene blue, which is a basic dye.
Positive charged

ii. Speculate the mechanism in which methylene blue stains bacterial cell.
Peptidoglycan. Methylene blue has positive charge ions which interact with the negatively charged cell wall, this causes the cell wall to be stained.

The positively charged dye will bind to the negatively charged cell wall, hence staining the cell

iii. Will simple staining enable the differentiation between the different groups of bacteria you have stated in Question 1ciii? If not, suggest how can the different groups of bacteria be differentiated?
Use gram dye

Simple staining will stain all bacteria indiscriminately. in order to differentiate between the groups of bacteria (Gram-positive and Gram-negative), differential staining is required. Gram staining is the most universal diagnostic staining for bacteria.

iv. What is the mechanism of the staining method you have suggested in Part iii.
Gram straining is based on **the ability of bacteria cell wall to retaining the crystal violet dye during solvent treatment**. The cell walls for gram- positive microorganisms have a higher peptidoglycan and lower lipid content than Gram-negative bacteria. Bacteria cell walls are stained by the crystal violet. Iodine is subsequently added as a mordant to form the crystal violet-iodine complex so that the dye cannot be removed easily. This step is commonly referred to as fixing the dye. However, subsequent treatment with a decolorizer, which is a mixed solvent of ethanol, dissolves the lipid layer from the Gram-negative cells. The removal of the lipid layer enhances the leaching of the primary stain from the cells into the surrounding solvent. In contrast, the solvent dehydrates the thicker Gram-positive cell walls, closing the pores as the cell wall shrinks during dehydration. As a result, the diffusion of the violet-iodine complex is blocked, and the bacteria remained stained. Finally, a counterstain (safranin) is applied to the smear to give decolorized Gram-negative bacteria a pink colour.

Going Further (Optional)

Bacterial endospores are highly resistant to hostile physical and chemical conditions. The endospores have a tough outer covering that are highly resistant to heat and chemicals. Endospores are very impermeable to dyes. In order to stain endospores specifically, special endospore staining procedures must be used.

Briefly describe how endospores can be stained.

Endospores are resistant to heat, UV radiation and chemicals because they are comprised of a tough proteinaceous covering called keratin.

A differential staining technique (the **Schaeffer-Fulton method**) is used to distinguish between the vegetative cells and the endospores. A primary stain (**malachite green**) is used to stain the endospores. Because endospores have a keratin covering and resist staining, the malachite green will be forced into the endospores by heating. In this technique heating acts as a mordant.

Water is used to decolorize the cells; as the endospores are resistant to staining, the endospores vegetative cells will lose the stain. The addition of a **counterstain or secondary stain (safranin)** is used to stain the decolorized vegetative cells. When

visualized under microscopy the cells should have 3 characteristics: the vegetative cells should appear pink, the vegetative cells that contain endospores should stain pink while the spores should be seen as green ellipses within the cell.