Microbiology

Morphology & Ultra-Structure of Microorganism

Prof. Dr. Batool Hassan Al-Ghurabi

References

- **1. Essential Microbiology for Dentistry**
- by Lakshman Samaranayake
- 2. Microbiology and Immunology
- by Richard Hunt

Why is microbiology important in dentistry?

Microbiology

- Microbiology: the study of organisms too small to be seen without magnification.
- Micro too small to be seen with the naked eye
- Bio life
- logy study of
 - Microorganisms are ubiquitous.
 - Microorganisms include:
 - Obacteria
 - **O**viruses
 - Ofungi
 - **O**Parasite {protozoa &helminthes (worms) }
 - Oalgae

History of the Study of Microorganisms

- 1665 Robert Hooke
 - "little boxes" "cells"
 - -Cell Theory
- The cell theory states that:
 - -All living things are made of one or more cells.
 - -Cells are the basic unit of structure and function in living things.
 - -All cells come from other cells.

Antoni van Leeuwenhoek 1674

- 1st person to actually see living microorganisms



Cell Types

There are several classes of living organisms

- Based on the organization of their cellular structures, all living cells can be divided into two categories:
 - 1. Cell that have

membrane-bound organelles

- Called Eukaryotic Cells

- 2. Cells that <u>do not</u> have membrane-bound organelles
- called prokaryotic cells
- Eukaryotic cell types Animals, plants, fungi, protozoa, and algae
- Prokaryotic cell types -
 - Unicellular organisms such as bacteria and blue green algae

<u>Similarities</u>

- All cells are surrounded by a *plasma membrane*.
- The semi-fluid substance within the cell is called "cytosol", containing the cell organelles.
- All cells contain chromosomes which have genes in the form of DNA.
- All cells have tiny organelles called "*Ribosomes*" that make proteins.

- A major difference between prokaryotic and eukaryotic cells is the location of chromosomes.
- In an eukaryotic cell, chromosomes are contained in a membrane-enclosed organelle, the *nucleus*.
- In a prokaryotic cell, the DNA is concentrated in the nucleoid without a membrane separating it from the rest of the cell.

- Prokaryotic Cells
- much smaller (microns) and more simple than eukaryotes
- large surface-to-volume ratio : nutrients can easily and rapidly reach any part of the cells.



Schematic of typical animal (eukaryotic) cell, showing subcellular components.

Organelles:

(1) nucleolus (2) nucleus (3) ribosome (4) vesicle (5) rough ER
(6) Golgi apparatus (7) Cytoskeleton (8) smooth ER (9) mitochondria (10) vacuole (11) cytoplasm (12) lysosome (13) centrioles



Differences between prokaryotic & eukaryotic cells

Definitions / Description	Eukaryotic Cell	Prokaryotic Cell
Organisms:	Plants, <u>animals</u> and fungi have eukaryotic cells.	Only bacteria and cyanobacteria have <u>prokaryotic cells</u> .
Cell wall:	No (animals); Yes (plants)	Yes
Centrioles:	Yes (all animals and some lower plant forms)	No
Cilia and Flagella:	Yes, simple	Yes, complex
Golgi Complex:	Yes	No
Lysosomes:	Common in animals; Not present in plants	No
Peroxisomes:	Yes	No
Nucleus:	Yes	No
Plasma membrane:	Yes	Yes
Chromosomes:	Several chromosomes	One long DNA strand
Ribosomes:	Yes	Yes
Endoplasmic Reticulum:	Present	Absent

- Size of Bacteria
- Unit of measurement in bacteriology is the micron / micrometre (µm)
- Bacteria of medical importance
- $0.2 1.5 \ \mu m$ in diameter
- $3-5 \ \mu m$ in length

- Shape of Bacteria
- Cocci spherical/ oval shaped (major groups)
- Bacilli rod shaped
- Vibrios comma shaped
- Spirilla rigid spiral forms
- Spirochetes flexible spiral forms
- Actinomycetes branching filamentous bacteria

Arrangement of bacteria: Cocci



Cocci in pair – Diplococcus



Coccus

Tetrad – groups of four

Cocci in chain - Streptococci



Cocci in cluster - Staphylococci

Arrangement of bacteria: Bacilli

Arrangements of Bacilli



Other shapes of bacteria



Comma shaped



Spirilla



Spirochetes

Anatomy of Bacterial Cell



- Anatomy of Bacterial Cell
- A. <u>Outer layer</u> two components:
- 1. Rigid cell wall
- 2. Cell membrane or Plasma membrane
- present beneath cell wall
- <u>Cytoplasm</u> gel-like substance enclosed within the cell membrane contains cytoplasmic inclusions, ribosomes, mesosomes and nucleoid
- B. <u>Additional structures</u> plasmid, slime layer, capsule, flagella, fimbriae (pili) and spores.

Structure & Function of Cell Components

CELL WALL

- Outermost layer, encloses cytoplasmic membrane
- 1. Confers shape and rigidity
- Peptidoglycan is responsible for the rigidity of the bacterial cell wall and for the determination of cell shape.



3. Composed of Mucopeptide (peptidoglycan/ murein): formed by N-acetyl glucosamine (NAG) & N-acetyl muramic acid (NAM) alternating in chains, held by peptide bonds.



- 4. Can not be seen by direct light microscopy and do not stain with simple stains.
- 5. Carries bacterial antigens important in virulence & immunity.
- Chemical nature of the cell wall helps to divide bacteria into two broad groups – Gram positive & Gram negative .
- 7. Gram +ve bacteria have simpler chemical nature than Gram–ve bacteria.
- 8. Several antibiotics may interfere with cell wall synthesis
 - e.g. Penicillin, Cephalosporins

- Gram positive cell wall
- The Gram-positive cell wall is composed of a thick, multilayered **peptidoglycan** sheath outside of the cytoplasmic membrane.
- **Teichoic acids** are linked to and embedded in the peptidoglycan, and **lipoteichoic acids** extend into the cytoplasmic membrane



- Gram negative cell wall
- The Gram-negative cell wall is composed of an outer membrane linked to thin single-layered peptidoglycan by lipoproteins.
- The peptidoglycan is located within the periplasmic space that is created between the outer and inner membranes.
- The outer membrane includes *<u>porins</u>, which allow the passage of small hydrophilic molecules across the membrane, and **<u>lipopolysaccharide</u> molecules that extend into extracellular space.







A comparison between Gram positive and Gram negative cell wall

ltem	Gram positive	Gram negative
Peptidoglycan layer	Thick (multilayered)	Thin (single-layered)
Teichoic acids	Present	Absent
Periplasmic space	Absent	present
Lipopolysaccharide (LPS) content	Virtually none	High
Lipid and lipoprotein content	Low	High
Resistance to physical disruption	Low	High
Inhibition by basic dyes	Low	High
Susceptibility to anionic detergents	Low	High
Resistance to drying	Low	High
Gram reaction	Retain crystal violet dye and stain dark violet	Can be decolorized to accept counter stain

Cytoplasmic (Plasma) membrane

- Thin layer 5-10 nm, separates cell wall from cytoplasm.
- Acts as a semi-permeable membrane: controls the inflow and outflow of metabolites.
 - Composed of lipoproteins with small amounts of carbohydrates.



Cytoplasm

- Colloidal system of variety of organic & inorganic solutes in viscous watery solution
- = Cytoplasmic Components
- 1. Ribosomes: place of protein synthesis (70 S)
- 2. Mesosomes:



(c) Complete 70S ribosome

- 1. Multi-laminated structures formed as invaginations of plasma membrane.
- 2. Principal sites of respiratory enzymes.
- 3.Coordinate nuclear and cytoplasmic division during **binary fission**
- 4. More prominent in Gram +ve bacteria

3. Intracytoplasmic inclusions:

Reserve of energy and phosphate for cell metabolism e.g. Metachromatic granules in *diphtheria bacilli*

4. Nucleus:

- No nucleolus
- No nuclear membrane
- Oval or elongated bodies generally 1 per cell
- Genome single, circular double stranded DNA (one chromosome).
- Divides by binary fissio



=Additional Organelles

1. Plasmid

- Extra-nuclear genetic elements consisting of DNA
- Transmitted to daughter cells during binary fission
- May be transferred from one bacterium to another by conjugation
- Not essential for life of the cell
- Confer certain properties e.g. drug resistance because the plasmid carries some genes responsible for drug resistance .



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2. Capsule & Slime layer:

- Viscous layer secreted around the cell wall.
- Polysaccharide / polypeptide in nature

a) Capsule – sharply defined structure, antigenic in nature

- Protects bacteria from lytic enzymes
- Inhibits phagocytosis
- Stained by negative staining using India Ink
- Can be demonstrated by Quellung reaction (capsule swelling reaction).



b) Slime layer – is loosely associated with the bacterium and can be easily washed off, whereas a capsule is attached tightly to the bacterium and has definite boundaries.

3. Flagella

- Long filamentous surface appendages
- Organs of locomotion
- Composed of proteins called flagellins
- The number and distribution of flagella on the bacterial surface are characteristic for a given species hence are useful in identifying and classifying bacteria
- Flagella may serve as antigenic determinants (e.g. the H antigens of flagella in Gram-negative enteric bacteria)
- Presence shown by motility e.g. hanging drop preparation test.
- Each flagellum consists of 3 parts:
- 1. Filament
- 2. Hook
- 3. Basal body



Types of flagellar arrangement







Lophotrichous – tuft of flagella at one pole

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Amphitrichous – flagella at both poles





Peritrichous – flagella all over

Amphilophotrichous – tuft of flagella at both ends

4. Fimbriae/ Pili

- Thin, hair like appendages on the surface of many Gram negative bacteria
- Acts as organs of adhesion (attachment) allowing bacteria to colonize environmental surfaces or cells and resist flushing
- Made up of proteins called pilins.
- Pili can be of two types

A. Common pili - short and abundant

B. Sex pili - very long pili and small number (one to six), helps in conjugation (process of transfer of DNA)

5. Spores :

- Highly resistant resting stages formed during adverse environment (depletion of nutrients).
- Formed inside the parent cell, hence called **Endospores**
- Very resistant to heat, radiation and drying and can remain dormant for hundreds of years.
- Formed by bacteria like *Clostridium* and *Bacillus*



Shape & position of bacterial spore

