Electron Microscope Emerging a Powerful Tool for Research in Biotechnology

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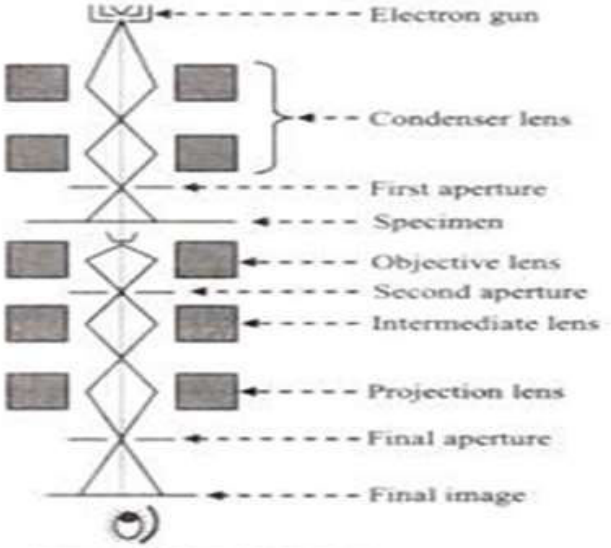
Infectious diseases of animals are important especially when they are capable of infecting humans. Such diseases are called zoonoses. Based on their aetiologies, zoonoses may be bacterial, viral, mycotic or parasitic. Zoonotic infections occur worldwide and often spread to humans through their companion domestic animals as well as through wild animals. As a result of our interconnectedness, infectious diseases emerge more frequently, spread greater distances, pass more easily between humans and animals, and change rapidly into new and more virulent strains. Therefore, electron microscope is an important tool for biomedical investigations.

The electron microscope a high energy beam of electrons is shone through a very thin sample, and the interactions between the electrons and the atoms can be used to observe features such as the crystal structure and features in the structure like dislocations and grain boundaries. It has already been known that, the smaller is the wavelength of light, the greater is its resolving power. The wavelength of green light (=0.55μ) is 1, 10,000 times longer than that of electron beam (=0.000005μ or 0.05 Å; 1μ = 10,000 Å). That is why, despite its smaller numerical aperture, an electron microscope can resolve objects as small as 0.001μ (=10 Å), as compared to 0.2μ by a light microscope. Thus, the resolving power of an electron microscope is 200 times greater than that of a light microscope. It produces useful magnification up to X 400,000, as compared to X 2000 in a light microscope. Thus, the useful magnification is 200 times greater in an electron microscope than that in a light microscope.

There are two types of electron microscopes as described below :

(1) Transmission Electron Microscope (TEM) :

In this microscope, an electron beam from an electron gun is transmitted through an ultra-thin section of the microscopic object and the image is magnified by the electromagnetic fields. It is used to observe finer details of internal structures of microscopic objects like bacteria and other cells. The specimen to be examined is prepared as an extremely thin dry film or as an ultra-thin section on a small screen and is introduced into the microscope at a point between the magnetic condenser and the magnetic objective.



TRANSMISSION ELECTRON MICROSCOPE

The point is comparable to the stage of a light microscope. The magnified image may be viewed on a fluorescent screen through an airtight window or recorded on a photographic plate by an in-built camera. Modern variants have facility to record the photograph by digital camera.

(2) Scanning Electron Microscope (SEM) :

In a scanning electron microscope, the specimen is exposed to a narrow electron beam from an electron gun, which rapidly moves over or scans the surface of the specimen. This causes the release of a shower of secondary electrons and other types of radiations from the specimen surface. The intensity of these secondary electrons depends upon the shape and the chemical composition of the irradiated object. These electrons are collected by a detector, which generates electronic signals. These signals are scanned in the manner of a television system to produce an image on a cathode ray tube (CRT). The image is recorded by capturing it from the CRT. Modern variants have facility to record the photograph by digital camera. This microscope is used to observe the surface structure of microscopic objects.

It has both transmission and scanning electron microscope functions.

Limitations of Electron Microscopes :

The limitations of electron microscopes are as follows :

(a) Live specimen cannot be observed.

(b) As the penetration power of electron beam is very low, the object should be ultra-thin. For this, the specimen is dried and cut into ultra-thin sections before observation.

Principle of Electron Microscope :

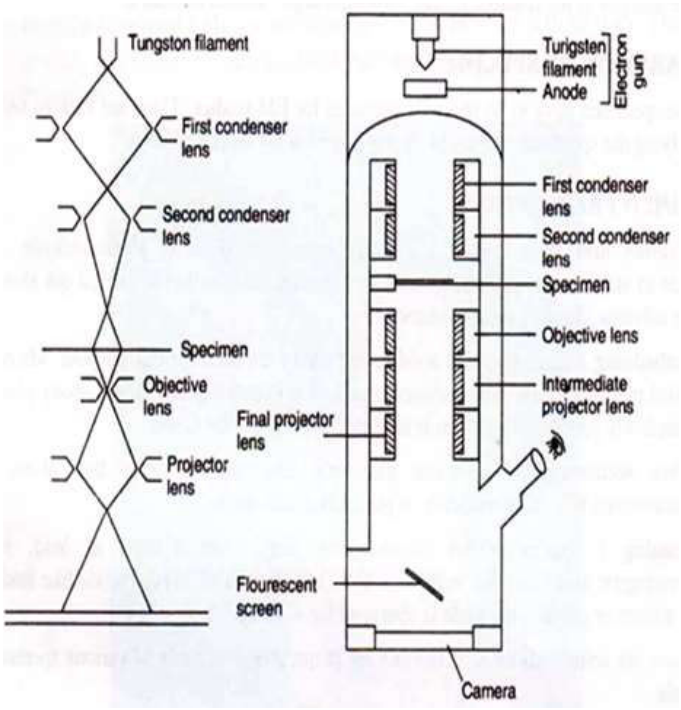
Electrons are subatomic particles, which orbit around the atomic nucleus. When atoms of a metal are excited by heat energy, electrons fly off from the atom. In electron microscope, tungston is heated by applying a high voltage current, electrons form a continuous stream, which is used like a light beam.The lenses used in EM are magnetic coils capable of focusing the electron beam on the specimen and illuminating it. The strength of the magnetic lens depends upon the current that flows through it. Greater the flow of the current, greater will be strength of the magnetic field. The electron beam cannot pass through the glass lens.

Components of Transmission Electron Microscope :

EM is in the form of a tall column which is vertically mounted.

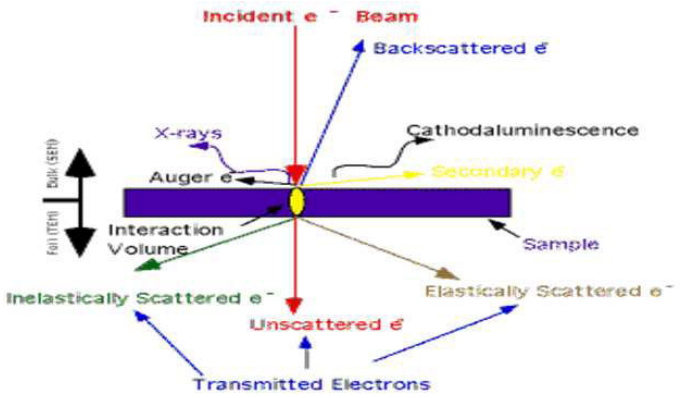
It consists of the following main components :

1. Electron gun
2. Electromagnetic lenses—three sets.
3. Image viewing and recording system.



Electron gun is a heated tungsten filament, which generates electrons. Condenser lens focuses the electron beam on the specimen. A second condenser lens forms the electrons into a thin tight beam.To move electrons down the column, an accelerating voltage is applied between tungsten filament and anode. Now most EMs use accelerating voltages between 100 kV-1000 kV. Electrons also function as a source of illumination for the specimen. High velocity electrons pass into the system of condenser lenses, which focus them on the specimen. The specimen to be examined must be extremely thin, at least 200 times thinner than those used in optical microscope. Ultra thin sections of 20-100 nm are cut. The specimen holder is an extremely thin film of carbon or collodion held by a metal grid.

Scattering of Electrons



The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen. The denser regions in the specimen scatter more electrons and therefore appear darker

in the image since fewer electrons strike that area of the screen. In contrast, transparent regions are brighter. The electron beam coming out of the specimen passes down the second of magnetic coils called objective lens, which has high power and forms the intermediate magnified image. Finally, a third set of magnetic lenses called projector (ocular) lenses produce the final further magnified image. Each of these lenses acts as image magnifier all the while maintaining an incredible level of details and resolution. His whole image remains in focus. This image is projected on a fluorescent screen. Below the fluorescent screen is a camera for recording the image. These lenses provide immense magnification and resolution.

As the EM works in vacuum, the specimen should be completely dry. Air molecules present in the column of EM scatter the electrons causing flicker in the electron beam. Vacuum is created in two steps. Firstly, a mechanical vacuum pump is used to create vacuum. Secondly, a diffusion pump uses a fast downward moving liquid, either oil or mercury which traps air and gas in the column. In this way, ultra high vacuum is created, It consists of a tungsten filament or cathode that emits electrons on receiving high voltage electric current (50,000-100,000 volts). Near the top of the tube is an anode which attracts electrons.

(b) Ray tube (Microscope Column) :

It is a high vacuum metal tube (2mt. high) through which electrons travel.

(c) Condense lens :

It is the electromagnetic coil which focuses the electron beam in the plane of the specimen.

(d) Objective lens :

It is the electromagnetic coil which produces the first magnified image formed by the objective lens and produces the final image.

(e) Projector lens :

It is also an electromagnetic coil which further magnifies the first image formed by the objective lens and produces the final image. Each electromagnetic coil has a coil of wire encased by a soft iron casing.

(f) Fluorescent Screen or Photographic Film :

Since unaided human eye cannot observe electrons, therefore, a fluorescent screen is used for viewing the final image of the specimen. The final image can be captured on photographic film and die photograph obtained is called an electron micrograph.

Sample preparation for Transmission electron microscopy (TEM)

In the TEM electrons is transmitted through an ultrathin sections, of the specimen The image is formed from the electrons transmitted through the specimen, magnified and focused by an objective lens and appears on a fluorescent screen, plus a monitor, or to be detected by a sensor such as a CCD camera. Biological materials contain large quantities of water. To be able to view it in the electron microscopy, the first stage in preparing is the fixation, one of the most important and most critical stages. We need to fixed it in a way that the ultrastructure of the cells or tissues remain as close to the living material as possible. The sample is then dehydrated through an acetone or ethanol series, passed through a “transition solvent” such as propylene oxide and then infiltrated and embedded in a liquid resin such as epoxy and LR White resin. After embedding in the resin, block were made and then thin sectioned by a process known as ultramicrotomy, sections of 50-70 nm thickness are collected on metal mesh ‘grids’ and stained with electron dense stains before observation in the TEM. Sectioning the sample allows us to look at cross-sections through samples to view internal (ultra)structure. Many modifications to the basic protocol can be applied to achieve many different goals, immunogold labeling for example; the in situ localization of specific tissue constituents using antibody and colloidal gold marker systems. Every sample is different. Please consult with the EM Staff before starting a project. Support film on TEM grids Formvar film is useful for the support of ultrathin sections on the finer mesh grids. Using of support film are ideal for those applications requiring large viewing areas without grid bar interference. These films must be strong, clean and remain attached to the specimen grids during specimen preparation. A Formvar film covered with a “light” layer of carbon will help to stabilize the film when the film is exposed to the electron beam. Sectioning with ultramicrotome Materials for TEM must be specially prepared to thicknesses which allow electrons to transmit through the sample, much like light is transmitted through materials in conventional optical microscopy. Because the wavelength of electrons is much smaller than that of light, the optimal resolution attainable for TEM images is many orders of magnitude better than that from a light microscope. The block is cut into semithin sections (1 µm) with a glass knife, using an ultramicrotome. The sections are then stained with Toluidine Blue for LM for orientation, and for selecting of a small area for ultrathin sectioning. Ultrathin sections are made at 50 70 nm using a diamond knife and placed/collected on a grid of metal. Positive staining Side 2 Details in light microscope samples

can be enhanced by stains that absorb light; similarly TEM samples of biological tissues can utilize high atomic number stains to enhance contrast. The stain absorbs electrons or scatters part of the electron beam which otherwise is projected onto the imaging system. Uses heavy metals such as lead and uranium to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to electrons (weak phase objects). Heavy metal salts attach to various organelles or macromolecules within the sections to increase their electron density and they appear dark against a lighter background. Uranyl ions react strongly with phosphate and amino groups so that nucleic acids and certain proteins are highly stained. Lead ions bind to negatively charged components and osmium reacted areas (membranes). Grids are stained with heavy metals, such as uranyl acetate and lead citrate. The grids, with the specimen side down, remain in 4% uranyl acetate for 25 minutes and are then rinsed in a series of four beakers of pure water. After rinsing, the grids are then stained with 1% lead citrate for 5 minutes, rinsed again in pure water, and stored in a grid box.

The specimen have to be specially prepared for EM studies. There are various techniques for studying the specimen under EM. Some of which are discussed here.

Fixation and Dehydration :

The specimens are fixed in glutaraldehyde, osmium tetroxide to stabilize the cell structure. After fixation, dehydration is carried out slowly with organic solvents like acetone and ethanol.

Embedding :

Resins such as araldite and epoxy are used for this purpose. Microbes are embedded in plastic resin. The specimen is soaked in un-polymerized, liquid epoxy plastic until it is completely permeated and then is hardened to form a solid block.

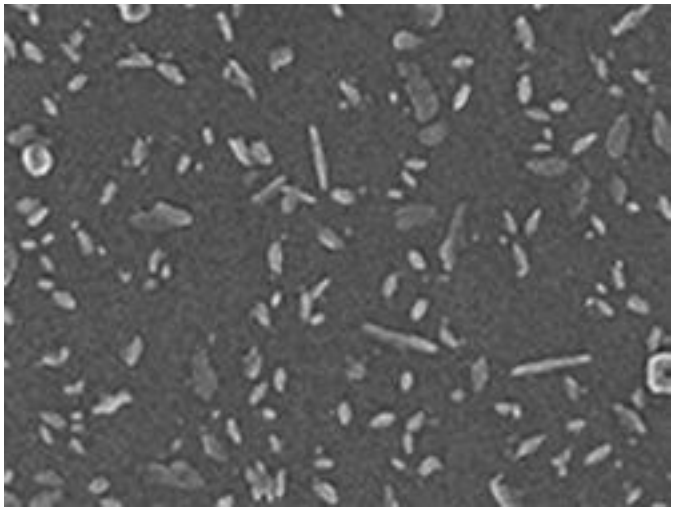
Ultra sectioning :

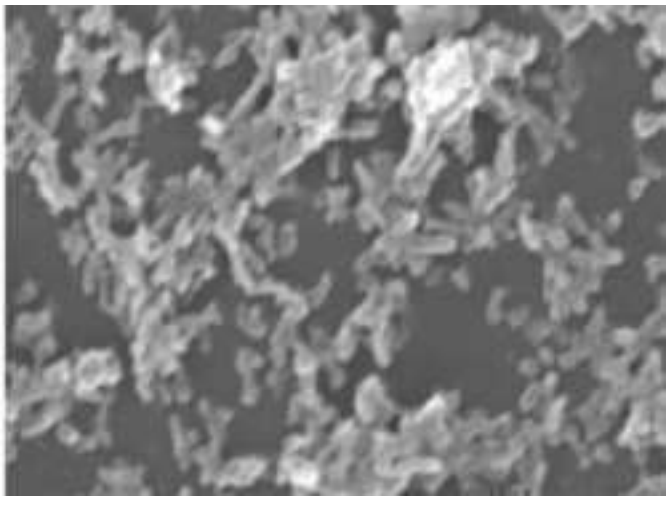
To obtain extremely thin sections from this plastic block, Ultra-microtomes with diamond knife or glass knives are used.

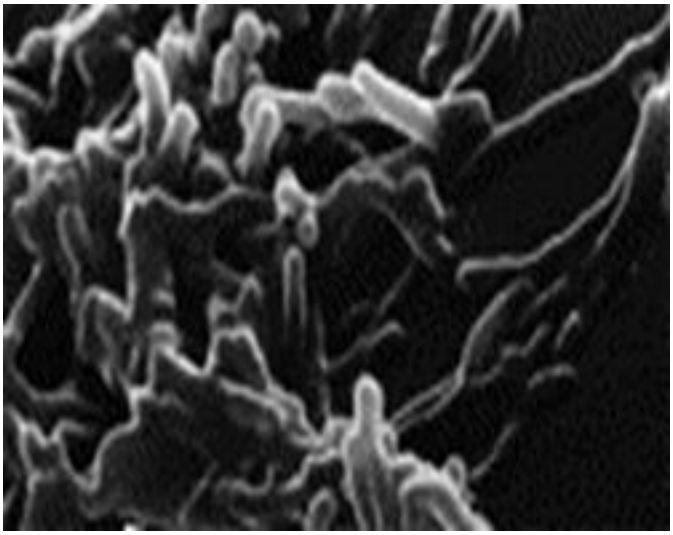
Staining :

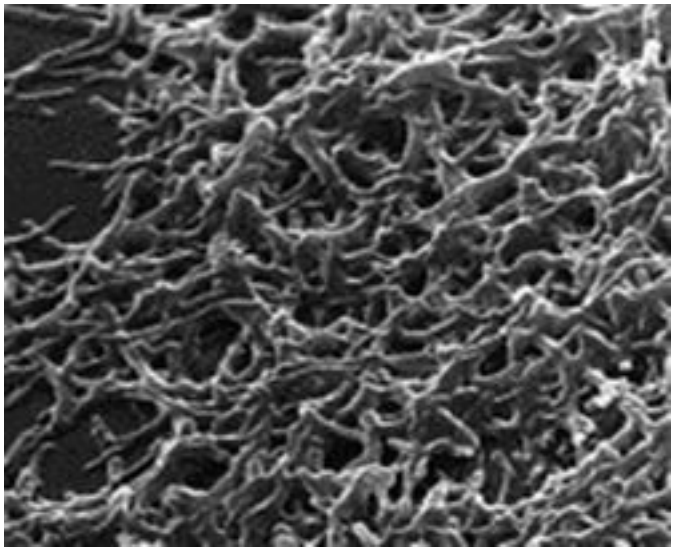
Specimens are stained with heavy metals such as lead, uranium, phosphotungstic acid. The thin sections soaked in solutions of heavy metals like lead citrate, uranyl acetate or osmium tetroxide is also used for staining.

However, electron microscopy emerging a power tool to investigate the detailed structure and configuration of the mycobacteria which may lead to an understanding the role of infections and in transmitting the diseases. Bacterial biofilms are often associated with infections specially with medical implants such as catheters and other medical devices. In the natural world more than 99% of bacteria exist as biofilms and according to NIH report more then 75% of all human infections are associated with biofilms formation. Biofilms are slimy, glue-like substance that excreted by bacteria and aggregate on living surface. Biofilms are formed to protect the bacteria from host defences, antibiotics and from harsh environmental conditions. Biofilms are found almost everywhere in nature, including soil, water pipes, and even inside the human body. Attachment of mycobacteria involved in biofilm formation in the liquid air interface is a complex process, with many variables such as pH, nutrient levels, iron, oxygen, ionic strength and temperature, affecting the outcome.





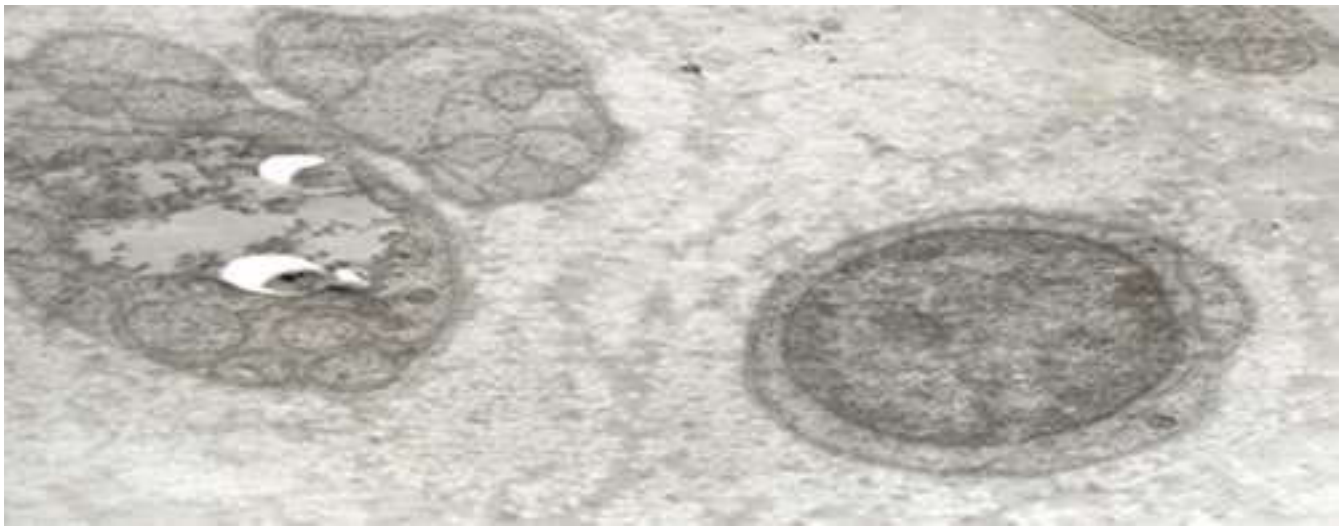




Figures : *Showing various forms of Biofilm developed by Mycobacteria*

However, many mycobacterial species are known to form biofilms, little is known about either the genetic requirements, patterns of gene expression. In micro array hybridisation we have found that six genes were expressed in *M.avium.* In *M. tuberculosis* MDR isolates seven genes were expressed and two genes Rv0359 and Rv3526 were homologus as earlier reported in *P. areuginosa* and *M. avium* which might be responsible for biofilm formation.

On the other hand electron microscopy used to investigate the detailed structure and configuration of the mycobacteria in the Schwann cells (SC) and endothelial cells in leprosy patients. In multibacillary patients, the ultrastructural study showed significant changes in peripheral nerves and endoneurial blood vessels. It was revealed that besides the SC the endothelial cells of endoneurial blood vessels also frequently harbor bacilli. In possibacillary patients showed the degenerative changes of SC and hypertrophy of endothelial cells leading to narrowing or complete occlusion of lumen of endoneurial blood vessels. The endothelial cells of endoneurial blood vessels were found to be loaded with bacilli and this bacillary loaded endothelial cell was found to release bacilli into the lumen through its ruptured membrane. Therefore, we have concluded that SC is not only targeted cells of *M.leprae* infection but endothelial cells are equally responsible for harboring bacilli and transmission of *M.leprae* in various parts of the body.



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