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Analytical Techniques for Biomedical Nanotechnology

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Chapter 12

Transmission electron microscopy for biomedical nanotechnology

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The field of nanotechnology is a multidisciplinary fieled that combines knowledge from various disciplines of science, i.e., materials science, applied physics, molecular biology, polymer science, and engineering etc. Nanomaterials measured on nanoscale form the foundation of nanotechnology and, based on their specific characteristics, i.e., size, distribution, composition and morphology, exhibit superior chemical, physical, magnetic, electronic and biological properties. Currently, the nanomaterials exist in a number of potential forms, i.e., nanoparticles, nanofibers, nanorods, nanowires etc making them suitable for various biomedical applications involving tissues and cells. TEM not only provides detailed topological, morphological, compositional information about the materials at nanoscale but also helps in the visualization of biological structures at a higher resolution than that offered by light microscopy. This chapter reviews the latest developments in TEM used for physico-chemical characterization of nanomaterials as well as the progress of nanoparticles of various kinds in the biomedical field.

12.1 Introduction

During the last few years, transmission electron microscopy (TEM) has emerged as an essential tool in the growing field of biomedical research and is widely used for the physical and chemical characterization of materials at nanoscale. The human eye has its own limitations and can reach its limit within a particular range of dimensions. The eye is not even able to distinguish or resolve between closely related points after a certain distance, it is unable to resolve the embedded green splendor of forests at a distance, and the neighboring planets are thus out of reach of the human eye. In order to achieve greater precision, sharpness, and resolution, humans invented hand lenses–the ordinary microscopes, microscopes, telescopes,

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and are still in the process of upgrading and updating these tools to achieve the highest magnification, resolution, sharpness etc. To observe the celestial bodies we built conical telescopes, and to see microscopic living and physical entities, we built microscopes. The functions of these two are essentially the same: resolution, sharpness, and magnification. While a telescope makes large and far away objects appear nearer and smaller, a microscope makes smaller objects appear larger. Microscopes and telescopes are able to achieve their objectives as they possess a set or series of lenses arranged in a particular pattern which are able to converge and bend the incident light until it forms an image on the retina of the naked eye and, which passes through the other end. However, this chapter is related to the microscope and, in particular, it concerns a type of microscope that converges and distorts not beams of light, but electrons, and is thus known as an electron microscope. A microscope is used to observe objects or structures that are not visible to the naked eye. Normally, our eyes cannot see objects smaller than 0.1 mm, so a microscope is helpful in observing smaller objects than this. The applications of a microscope are found in all spheres of research and result in the broad interdisciplinary perspectives in unraveling the scientific myths and marvels associated with life science, physical science, and chemical science to name just a few. With the help of this we are able to directly study the biological ultra-structure, particle analysis or material characterization, molecular nature and mechanism of disease, determining the structure of bacteria, viruses etc.

12.1.1 Nanotechnology

Nanotechnology or nanoscience, in the field of applied science, is the set of all techniques and related sciences used and studied in the 1–100 nano (i.e., 10^{-9} m) scale. In nanotechnology, fabrication within this range is widely used in interdisciplinary fields, such as applied physics, materials science, semiconductor physics, large molecular chemistry (which focuses on the non-covalent effect of molecules in the field of chemistry), autonomous machines and robotics, chemical engineering, mechanical engineering and electrical engineering. ‘Nano’ is a Greek word, which literally means very small or minute. Every particle whose size is 100 nm or smaller is considered a ‘nanoparticle’. The fineness of a nanoparticle can be estimated from the fact that the diameter of a human hair is 60 000 nm. The term nanotechnology was first used by Norio Taniguchi in the year 1974. Since the beginning of life on Earth, with the continuous changes in nature—constructioon of various nano-particles has been occurring. Nano means substances that are made up of elements of very fine size (billionths of a meter). Nanotechnology is the engineering of molecules and atoms, which integrates disciplines such as physics, chemistry, bioinformatics, and biotechnology. Nanotechnology is growing rapidly in medical and bio-engineering due to its small size, superior capacity, and durability. With the use of nanotechnology, there is less friction in engines, which increases the life of machines. Also fuel consumption is less (Fulden *et al* 2021, Rachel *et al* 2020, Bayda *et al* 2019, Li *et al* 2004).

The 21st century is going to become the nano century. Today there is competition to make the size of objects smaller and stronger. Large scale research is taking place across the world to develop nanotechnology in various fields. Nanotechnology has immense potential in many fields such as electronics, medicine, auto, bioscience, petroleum, forensics, and defense, due to its microscopic size, unmatched strength, and durability. The extremely small size changes the chemical and physical properties of the nanoparticles. For example, when zinc metal nanoparticles are formed, they become transparent (Jadoun *et al* 2021). Nanoparticles are being used in everything from consumer products to medical devices, cosmetics, chemicals, electronics and optics, environment, food and packaging, fuels, energy, textiles and paints, new generation medicines, and plastics etc (Mohanpuria *et al* 2008, Rajan *et al* 2015, Joginder *et al* 2017). Nanotechnology is expanding existing science to the nanoscale, or reshaping existing science into a new modern term. In agriculture, nanoparticles are being used in nano-fertilizers, nano-pesticides/weeds, storage, preservation, product quality improvement, and flavoring, etc (Harpreet *et al* 2021, Kah *et al* 2019, Nazia *et al* 2019). Broadly speaking, nanoparticles can be divided into organic and inorganic substances. Inorganic nanoparticle metals (silver, aluminum, tin, gold, cobalt, copper, iron, molybdenum, nickel, titanium) and their metal oxides are being used extensively. For example, silver nanoparticles are used in refrigerators, clothing, cosmetics, toothbrushes, water filters, etc. Similarly, zinc oxide nanoparticles are used in cosmetic creams. The glass in tall buildings or vehicles can be easily cleaned with the coating of gold nanoparticles. Copper nanoparticles are being used in medicine as fungicides and bactericides. They have the ability to destroy bacteria and fungi (Lucia *et al* 2021, Saba *et al* 2020, Fernando and Sung 2009).

12.1.2 Origin of nanotechnology

The first use of the principles of nanotechnology (but before the name was coined) was Richard Feynman's lecture, 'There's plenty of room at the bottom' during the American Physical Association meeting at Caltech, December 29, 1959. Feynman mentioned a method that suggests the creation of microscopic devices for the quantification of single molecules and granules. He noted the decreasing effect of gravity during this period and the increasing prominence of surface tension and Van der Waals attraction. Professor Norio Taniguchi of the Tokyo University of Science is credited with coining the term nanotechnology. Its definition was described in depth in the 1980s by Eric Drexler, who popularized the science and instruments of the nanoscale with his lectures and in his books. Nanotechnology and nanoscience in the 1980s resulted from two inventions: cluster science and the observational tunneling microscope (STM). With their help, fullerenes were developed in 1986 and carbon nanotubes, and a few years later researchers synthesized semiconductor nanocrystals, which led to the invention of many nanotubes (Sattler 2010).

12.2 Electron microscopy

The first electromagnetic lens was invented by Hans Busch in the year 1926 and he filed a patent for an electron microscope (EM) in 1928, however he did not build the microscope. The first EM was built by Ernst Ruska in the year 1931, a physicist at the University of Berlin, and Max Knoll, an electrical engineer and in the same year, Reinhold Rudenberg, the scientific director of Siemens-Schuckertwerke, acquired the EM patent.

12.2.1 Invention of electron microscope

In 1933, Ernst Ruska further developed an EM developed on the original model that was capable of producing a higher resolution image than optical microscopy (OM). In 1937, Manfred von Arden developed the first scanning electron microscope. In the year 1938, the first commercial EM was released by Siemens-Shakertwerke and from there transmission electron microscopes became more readily available in other regions of the world. The EM was further developed during the Second World War, when the first electromagnetic lens was developed. Its development has led to considerable success in increasing the differentiation potential (Nellist 2008) and since then it has acted as a powerful tool for science and industry.

12.2.2 EM—working principle

The discovery of the EM is based on the fact that the circular electromagnetic field acts on a beam of electrons. It is very similar to the action of a beam of photons on a glass lens. Electron microscopes make use of the magnetic properties of electrons, with Louis de Broglie hypothesized that electrons possess wave properties, taking magnification to a whole new level. Here in case of EM the circular electromagnetic field acts like a lens which is made of several thousand coils of wire enclosed in an iron case. With the passage of a current through the coil, a magnetic field is generated which gives a direction to the movement of electrons froming a beam of electrons having the properties of an electromagnetic wave of very short wavelength. Accelerated by an electric field is the wavelength (λ), 1 V accelerating voltage. This 100 kV accelerating voltage yields a wavelength of 0.04 nm, which is 10 000× less than visible light which increases the resolving power and more useful magnification is obtained (Nellist 2008). An EM can magnify an object up to 5000–10 000 000 times more than that of a light microscope.

12.2.3 Magnification of electron microscope

The magnification of an image produced on a photographic plate depends on two factors: the magnification of a microscope is equal to the product of the magnifications of its eyepiece and objective lenses, namely

Magnification of microscope = Magnification of the eyepiece × Magnification of the objective

Let the magnification of the objective of a compound microscope be 10× and the magnification of the eyepiece is 5×, then the overall magnification of the compound microscope will be 10 × 5 = 50× (50 times).

12.2.4 Resolving power

The typical electron beam has a wavelength of about 0.005 nm, which is generated by 50 000 V of electricity. Its resolving power is 0.002 11 nm.

Due to the difficulties in making and using magnetic lenses, only 0.1–0.2 nm resolution power is now available.

12.2.5 Uses of electron microscope

(i) Magnification from 20 000 to 100 000 times is possible by electron microscope. Therefore, the cellular organelle can be studied at the molecular level.
(ii) Various bacteria and viruses can be studied in detail with this microscope.
(iii) The distribution of enzymes present in the cell can be studied with an electron microscope.
(iv) The macromolecular level compositions of the cell can be studied.

There are two types of electron microscopes:
SEM—scanning electron microscope
TEM—transmission electron microscope

The images formed by both the SEM and TEM types of microscope and their working principles are completely different from each other.

12.3 Transmission electron microscopy

The field of nanotechnology inovolves life science, material science, physical and biochemical sciences, engineering, and technology of materials in which out of three at least one dimension should be in the range of less than 100 nm (Sattler 2010). Interestingly, the modern concept of nanotechnology was introduced by physicist Richard P Feynman in the 20th century in which nanometer size clusters were proposed as 'computer bits', Williams and Carter 2009, Nellist 2011). The first TEM was created by Max Knoll and Ernst Ruska in 1931 which is a microscopy technique in which a beam of electrons passes through and interacts with a very thin specimen/sample and the interaction of the electrons with the sample results in forming an image which is magnified and focused on the imaging device such as a fluorescent screen or a photographic film. Subsequently, the sensor of a CCD camera detects this and produces an image. Interestingly, TEM was the most elaborate and powerful technique that was available for the imaging of materials but due to inadequate spatial resolution, the TEM instruments were unable to meet the challenges of imaging 1 nm size atom-clusters and that led to improvements in the spatial resolution of TEM instruments (Williams and Carter 2009).

12.3.1 Advancement in transmission electron microscope

TEM is a very rapidly developing field and spontaneously the scope, applications, its breadth of information in terms of applicability or output, and power of the various types of using TEM techniques to unravel and expand the breadth of knowledge in the field of material science is expanding with advancing time. Interestingly, a modern TEM constitutes a complex characterization facility which is able to collect the diffraction patterns from volumes ranging from a few cubic nanometers in size and imaging samples to the atomic scales. In addition, when the TEM technique is amalgated with tomography, a technique which is able to derive a three-dimensional (3D) information from two-dimensional (2D) images, it becomes possible to know the structure and shape of nanostructures in 3D, even with atomic resolution. Moreover, TEM also allows spectroscopy to be peformed that is capable of analyzing not only the composition of the sample but also the bonding, optical, and electronic structure properties in 2D as well as 3D. Thus, a large number of studies can now be carried out *in situ* in the TEM with simultaneous characterization (Nellist 2011).

12.3.2 Instrumentation of TEM

TEM works on three basic or essential systems:

(a) An electron gun which produces the electron beam and the condenser system which focuses the beam onto the object. Filament or cathode is the main source of electrons and a narrow beam of electrons is emitted from the cathode through the cathode ray tube and the electrons pass through this tube and reach the condenser lens. This lens is a magnetic coil, which condenses electrons into the plane of the object.

(b) The image-producing system consists of the objective lens and a magnetic coil, which produces the first magnified image of the object. This image is of intermediate type, has a movable specimen stage, intermediate and projector lenses, a magnetic coil which magnifies the image produced by the objective lens and which focus the electrons passing through the specimen to form a real, highly magnified image.

(c) The image-recording system, which converts the electron image into an image which can be perceived by the human eye. The photographic plate receives the final image of the object. The object is always to be placed between the condenser lens and the objective lens. The image-recording system usually consists of a fluorescent screen for viewing and focusing the image and a digital camera for a permanent record. Apart from this, a vacuum is also maintained in the path of the electron because transport of electrons is possible only in vacuum (figure 12.1) (Dalip *et al* 2018, Audrey and Peter 2014).

TEMs are capable of producing much higher resolution images than light microscopes, due to the short de Broglie wavelength of electrons. This property

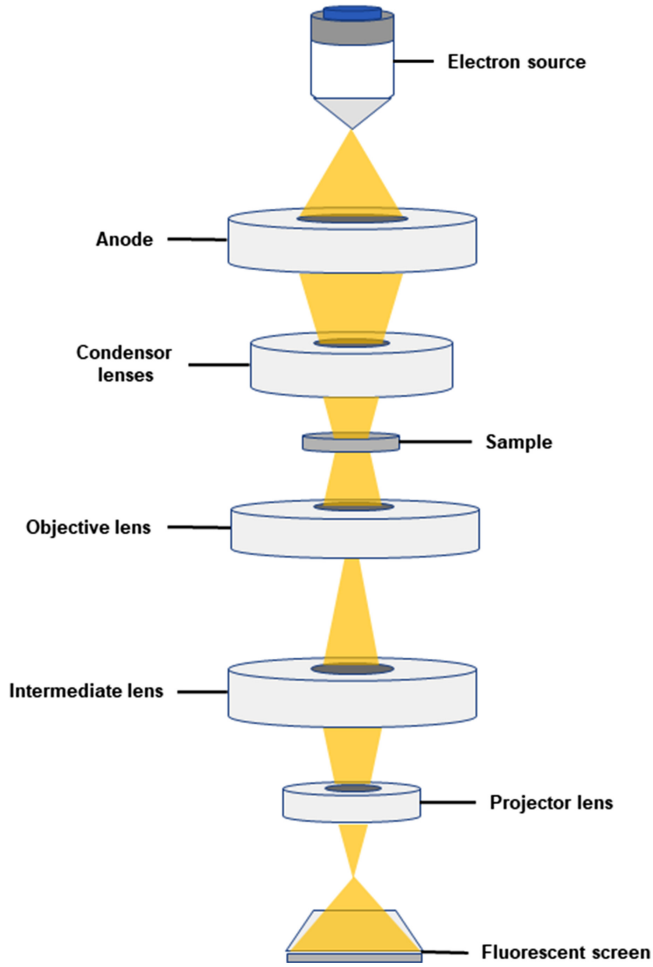

Figure 12.1. Instrumental components of transmission electron microscope.

enables the instrument to make very fine resolutions, even to the depth of a single column of atoms, which is thousands of times less than even the smallest object differentiated by a light microscope. TEM thus plays an important role in the major analysis in both physical and biological sciences, with areas such as cancer research, materials science, virology, pollution, and semiconductor research being important.

12.4 Working principle of transmission electron microscopy

The TEM technique is based upon the principle of electron transmittance and can provide detailed information about shapes, sizes, defects, and structures of nanoparticles through diffraction pattern. It helps in micro-structural examination of materials through high-resolution imaging. Optical and electron microscopes are very similar in producing the image of a specimen, but, the resolving power of the

EM is very large as compared to the OM due to the use of an electron beam of about 10^6 times shorter wavelength in EM than that of a light photon in an OM. Electrons are emitted through the filament or cathode. This electron beam is incident on the object by the condenser lens. These electrons collide with the atomic nuclei of the molecules present in the object. Atomic nuclei of higher molecular weight scatter the electron beam more than atomic nuclei of low molecular weight. The formation of an image in an EM depends on the scattering of electrons. Therefore, atomic nuclei with high atomic mass contribute more to the formation of the image. The electron beam follows a vertical path through the microscope, which is held within a vacuum. The magnetic field generated by the objective and projector lenses focused the electron beam on the photographic plate. The electrons which are scattered by atomic nuclei in the object and flow out through the aperture of the objective lens. The intermediate image of the object is formed by the objective lens. The projector lens further magnifies this image. The magnified image of the object on the photographic plate is due to the electrons scattered by the atomic nuclei of the object (Williams and Carter 2009).

TEM is a commonly used electron microscope. In this microscope an electric field pushes electrons from the negatively charged electrode. Electromagnets generate a magnetic field that focuses electrons on the stained tissue. Many electrons are released directly by penetrating the tissue, but some electrons are absorbed or dispersed by the atoms of metallic stains. Those electrons, which pass through the tissue, are focused by electromagnets on a screen fitted with a phosphorescent material. The electrons hitting the screen excite the phosphorescent material to emit visible light. Electrons that are not transmitted by the tissue leave dark marks on the visible plate. Thus, dark and bright regions in the image formed by TEM show more or less electron density. Electrons can also be focused on the photographic plate (Audrey and Peter 2014).

12.5 Specimen preparation for TEM

Sample preparation of a biological sample. i.e., cells or tissue, in TEM is a multistep process in which each step if not properly followed can virtually degrade the quality of the final electron micrograph. The specimen preparation in TEM consists of the following steps (figure 12.2):

I. **Slicing and cleaning** The first step is to thoroughly clean the surface of the specimen with a suitable buffered solution (physiological pH) so as to get rid of unwanted deposits if any. For cleansing one can also make use of ultrasonic baths but caution is required in order to prevent the sample from being damaged.
II. **Primary fixation** The primary fixation is the most significant and crucial step in the preparation of a biological sample. The fixation steps stabilize and protect the cell structure from changes or damage during subsequent treatments and irradiation. Primary fixation can be carried out using two methods, i.e., chemical fixation and cryofixation.

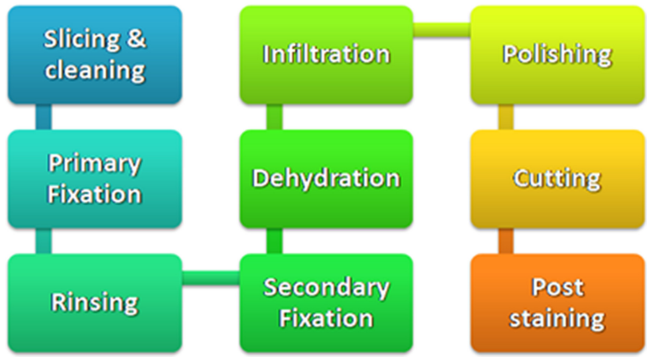

Figure 12.2. Basic steps for specimen preparation in TEM.

Chemical fixation This method is mainly used for stabilization of biological samples and makes use of chemical substances, i.e., glutaraldehyde, paraformaldehyde, acrolein, osmium tetra oxide, etc for irreversible/reversible cross linking of protein molecules with molecules in close proximity.

Cryofixation The modern approach used to immobilize and preserve the cell structure. This method involves rapid freezing of the sample in liquid helium or liquid nitrogen without crystal formation. The water content in the sample thus gets converted into a vitreous ice form.

III. **Rinsing** The fixation step may result in an increase in acidity of the specimen. Therefore, to avoid such a state and to maintain the pH, the specimen should be rinsed thoroughly using a buffer solution such as 0.1 M PBS/cacodylate buffer (pH 7.3).

IV. **Secondary fixation** After chemical fixation, post fixation or secondary fixation of the sample is carried out using osmium tetra oxide to further enhance the quality of the fixation and for increasing the contrast of the microscopic structures inside the sample. In osmium tetra oxide, osmium is a heavy metal that fixes the unsaturated lipids. This process ensures that the phospholipids forming membranes are preserved and are not eliminated during the dehydration step. Secondary fixation is essential to stain the specimen and to protect the specimen during other steps employed such as infiltration and cutting.

V. **Dehydration** Afterwards dehydration or freeze drying of the sample is carried out using an organic solvent (ethanol, acetone, propylene oxide, etc) to replace the water content of the sample with organic solvent having lower surface tension and to embed the sample in an epoxy-type resin that is immiscible with water.

VI. **Infiltration** In this step, epoxy resin is embedded within the cell, to make it hard so that it can bear the pressure of cutting or sectioning. The specimen is first placed in a mold containing epoxy resin and later cured into a hard block using heat or UV light.

VII. **Polishing** After infiltration, some materials undergo polishing to improve the quality of the image. Use of ultrafine abrasives in polishing gives the specimen a mirror-like finish.

VIII. **Cutting** For study under an electron microscope, the specimen should be extremely thin or semi-transparent in nature so that it allows the electron beam to pass through it. Therefore, the sample needs to be cut into semi-thin sections with a glass or diamond knife, using an ultra-microtome. The process of cutting of the specimens into semi-thin and ultrathin sections is also known as ultra-microtomy. These semi-thin and ultrathin sections are then mounted on specimen grids to be viewed under the microscope. The size of each section needs to be within the range 30–60 nm to obtain high resolution.

IX. **Post-staining** For biological specimens staining is usually performed twice, first before the dehydration step and later after sectioning of the sample. In post-staining, heavy metals such as lead citrate, uranyl acetate etc are used to increase the contrast between various structures in the specimen, and to scatter the electron beams (Malatesta 2016).

12.6 Applications of TEM in biomedical nanotechnology

During the last two decades, nanobiotechnology researchers have dramatically upgraded themselves and, in the last few years, the direction of research has progressed in biomedical applications, developing a new field named nanomedicine. TEM is a valuable technique not only for the physico-chemical characterization of newly developed nanoparticles, but also can explore the influences of nanocomposites on biological samples, providing information important for the establishment of efficient diagnostic and therapeutic applications (Malatesta 2016). A superfluity of nanoparticles has been established for different approaches: *in vivo* bio-imaging, drug delivery systems, antimicrobial research, scaffold components for tissue engineering, sensors application, and many other novel uses (Shi *et al* 2010, Nan-Yao *et al* 2019, Liang *et al* 2020). The greatest advantage of nonmaterial in biomedicine is their size (1–100 nm) facilitating their crosstalk with the biomolecular components; in addition, they can assume various conformational shapes (such as shells, rods, dishes, spheres etc), making them suitable for wider applications e.g. surface functionalization can be done for targeting specific cell types. Brief description of recent application of TEM in biomedical nanotechnology researches are provided in below descriptions.

12.6.1 Bio-imaging application

Nanomaterials generally express unique optical, magnetic, and acoustic properties, which manifest as an identical tool in biomedical research such as bio-analytics, biomedical imaging and bio-imaging based therapy (figure 12.3). Light-responsive nanoparticles such as quantum dots, metal nanoparticles, have an edge over others due to their better optical properties, i.e., brighter luminescence, small size, good biocompatibility, easy surface modifications, high sensitivity and higher photo

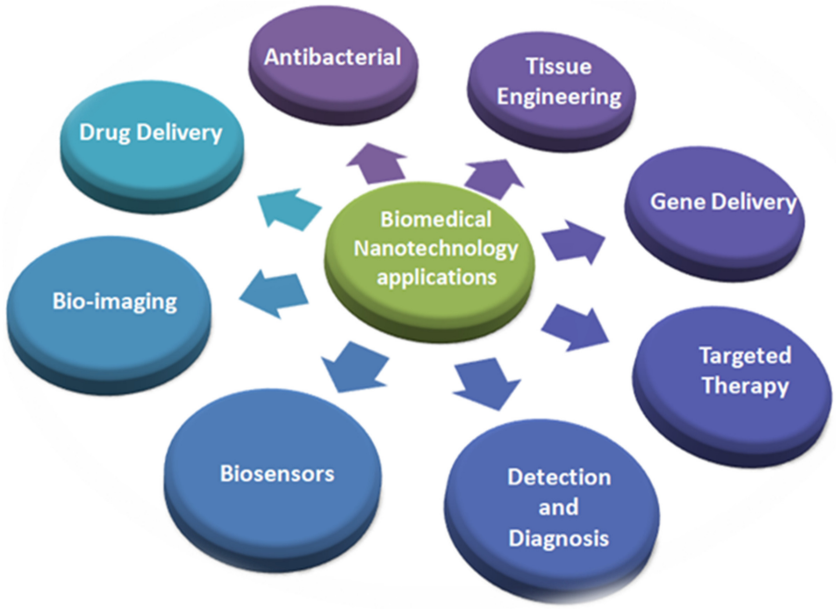

Figure 12.3. Application of nanotechnology in biomedical research.

stability and these characteristic features have thus enabled the sensitive detection of biological samples and the labeling of reporter molecules. TEM images of such nanoparticles are able to demonstrate the nanostructure as well as the size in nanometer scale.

Liang *et al* designed a novel NIR photo-activated photo-sensitizer based on TiO_2 coated UCNP core/shell nanocomposites (UCNPs@TiO_2 NCs) by using water phase transfer to synthesize UCNPs. $NaYF_4$: Yb^{3+}, Tm^{3+} @ $NaGdF_4$: Yb^{3+} core/shell UCNPs can effectively convert NIR light into UV emission, which is matched with the absorption of the TiO_2 shell (Liang *et al* 2020) (figure 12.4). This team had shown the nanostructure and size through TEM imaging.

Das *et al* in a current study, described the bio-imaging property of wire shaped copper nanostructures synthesized via microwave irradiation with single step doping of carbon nanodots (CDs) (Das *et al* 2020). The nanostructure of the CuCs was observed using TEM. The results suggest the wire-like structure of CuNPs in which CDs were observed to be grafted forming CuCs. The selected area electron diffraction (SAED) pattern matches with copper where two major planes (2 0 0) and (2 2 0) were observed to be present in the SAED pattern.

12.6.2 Drug delivery application

The application of nanotechnology in the field of medicine, known as nanomedicine, has the potential to make a useful impact on human health for clinical practice and research. Nanotechnology-based materials offer a variety of new approaches for clinical practices ranging from prevention to treatment and diagnosis of many

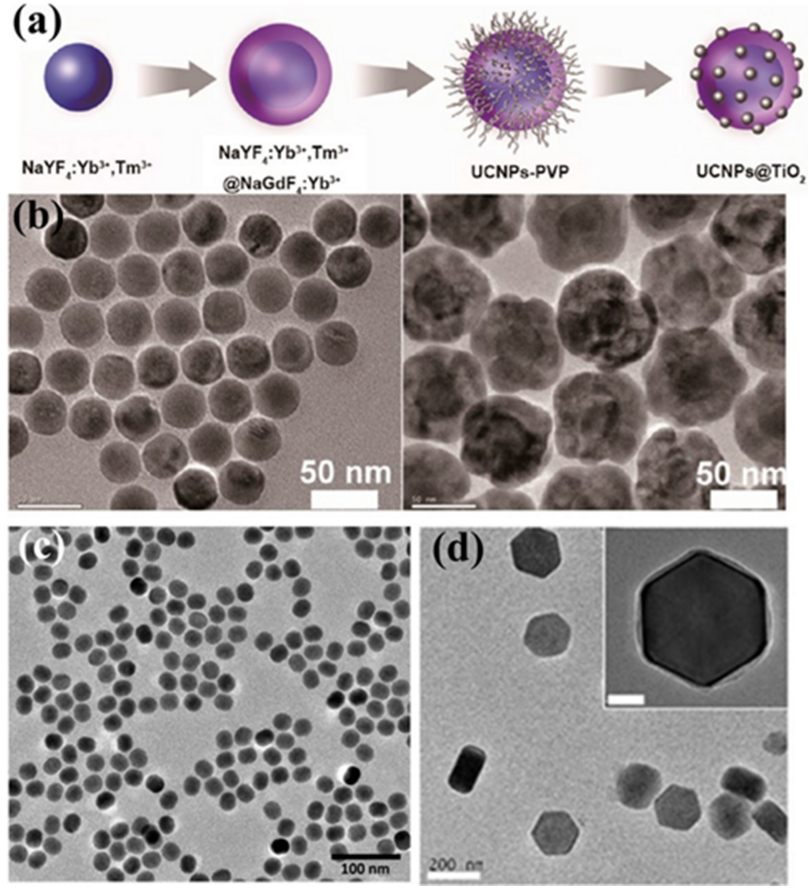

Figure 12.4. (a) Schematic illustration of the hydrothermal preparation synthetic procedure of UCNPs@TiO_2 NCs. (b) TEM images of the original $NaYF_4$:Yb^{3+}, Tm^{3+} cores (scale bar=50 nm). (c) Sol–gel method for synthesized 2-aminoethyl dihydrogen phosphate-stabilized $NaYF_4$:Yb^{3+}, Er^{3+} nanoparticles (scale bar: 100 nm). (d) UPP@ovalbumin was prepared by microemulsion synthesis. Reproduced from Liang *et al* (2020). CC BY 4.0.

diseases and conditions. Herein, we present TEM analysis on nanoparticles focused on drug delivery systems that has been performed in our laboratory.

In the case of radiolabeled nanoparticles, besides nanoparticle characterization, the TEM technique was used to confirm the stability of the nanoparticles after radio labeling. During the last decades a big family of nanostructures that have a surface-acting action, such as nanoparticles (NPs), lipid nanocarriers, and many more, have been developed to be used as drug delivery systems (DDSs) (Petrushevska *et al* 2019). Magnetically assembled drug-loaded nano chains are one of the recent findings in nanocarrier-based delivery technology. The key to controlling the length of the synthesized nano chains is the duration of the reaction mixture's exposure to the magnetic field. The chain length can be optimized depending on the biochemical environment of the target molecule. Through TEM the cluster number and length of these chains can be measured accurately (figures 12.5(a)–(h)) (Kralj and Makovec 2015).

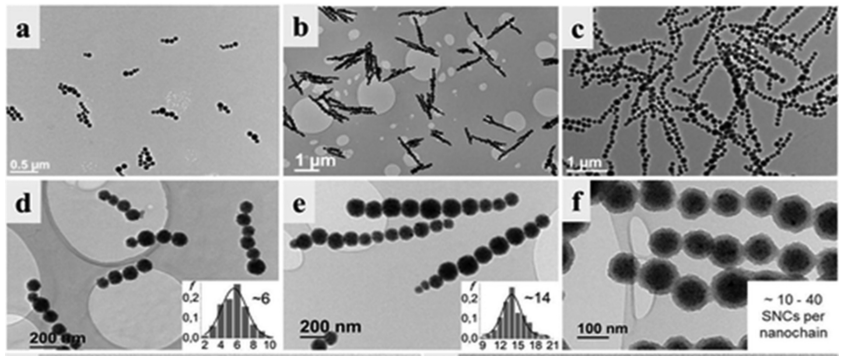

Figure 12.5. (a) and (d) TEM images of the CHAINS 6 composed of around six clusters, (b) and (e) TEM images of the CHAINS 14 composed of approximately 14 clusters, and (c) and (f) TEM images of CHAINS L collected of up to 40 clusters per nanochain. The insets in images (d), (e), and (f) show the length distributions of the nano chains determined by the manual counting of the clusters assembled into individual nano chains. Reprinted with permission from Kralj S and Makovec D (2015). Copyright (2015), American Chemical Society.)

Classical TEM and SEM techniques frequently have to be adapted for an accurate analysis of formulation morphology, especially in the case of hydrated colloidal systems (Malatesta 2016).

12.6.3 Biosensor application

Nanotechnological developments have facilitated the development of nanostructures such as nano optical fibers, nanoparticles, nanowires, and nanotubes, and research on applying these to devices are proving quite successful because these devices can be used to manufacture high-speed, high-density, subminiature sensors, thus enabling the detailed analysis of very small numbers of specimens. Biosensors improved using nanotechnology can prove to be beneficial for minimally invasive diagnosis, point of care, and early disease diagnosis. Such nano biosensors can detect various types of biomolecules such as enzymes, antibodies, antigens, acceptors, and DNA. However, biosensors should also be capable of measuring slight changes at the molecular level from the bond between biomolecules and a specific molecule.

Srijampa *et al* used gold nanoparticles (AuNP) as biosensors to understand the effects of charges on monocyte behavior. TEM images confirmed the morphology of both negatively and positively charged AuNP (AuNP−ve and AuNP+ve), which show a uniform and nearly spherical shape (Srijampa *et al* 2020, Farzadfard *et al* 2020). The average diameters of AuNP−ve and AuNP+ve were found to be 15.7 ± 2.6 nm and 14.8 ± 3.2 nm, respectively (figures 12.6(a) and (b)). Farzadfard *et al* used an electrochemical label-free biosensor designed for the detection of glycated albumin (GA) using reduced graphene oxide/Au nanoparticles (RGO/AuNPs) modified by anti-GA aptamer. TEM images of graphene oxide (GO) and rGO/AuNPs shows the uniform distribution of AuNPs on the surface of graphene sheets, demonstrating the suitable procedure for preparation of rGO/AuNPs with HEPES

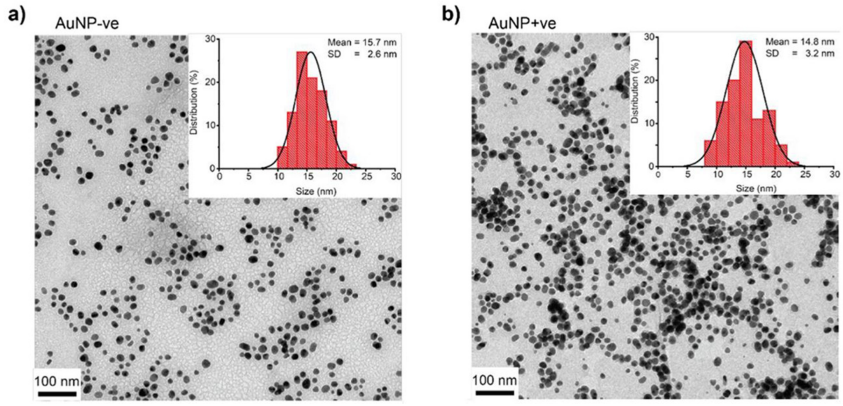

Figure 12.6. TEM images of (a) AuNP–ve and (b) AuNP+ve. Reprinted with permission from Srijampa *et al* (2020) Copyright 2020 American Chemical Society.

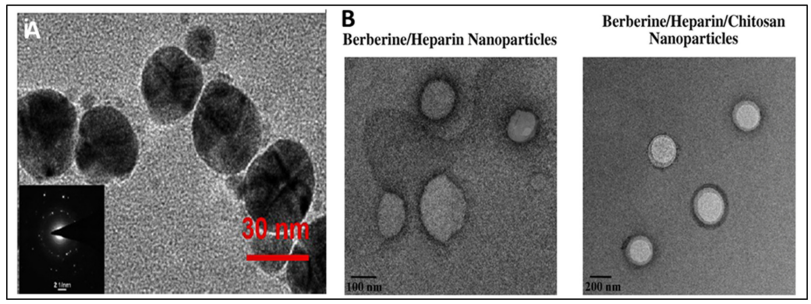

Figure 12.7. (A) The morphology of Ag-NP studied using TEM analysis. Reprinted with permission from Ray *et al* Copyright (2019) American Chemical Society. (B) TEM images of the berberine/heparin nanoparticles and berberine/heparin/chitosan nanoparticles. Reproduced from Patra *et al* (2018) CC BY 4.0.

as a reduce agent. The average diameter of AuNPs decorated on the surface of rGO sheets was 20 nm (Farzadfard *et al* 2020, Ray *et al* 2019).

12.6.4 Tissue engineering application

Tissue engineering is one of the most prominent examples of an interdisciplinary field, where scientists with different backgrounds work together to boost the quality of life by addressing critical health issues. Tissue engineering, in particular, has benefited immensely from nano- and microtechnology. For example, porous nanomaterials have led to highly efficient drug delivery systems because of their high affinity for target cells and tissues (Shafiee and Atala 2017). Ray *et al* demonstrated a facile and unique route to fabricate a hierarchical nanobiocomposite with effective loading of ZnO/silver nanoparticles (AgNPs) in order to attain excellent bactericidal efficacy with good and sustainable release profile (figure 12.7(A)) (Ray *et al* 2019).

Newman *et al* demonstrated that following intravenous administration, 2D graphene oxide (GO) nanosheets were largely excreted via the kidneys; however, a small but significant portion of the material was sequestered in the spleen (Newman *et al* 2020). Herein, they also interrogate the potential consequences of this accumulation and the fate of the spleen-residing GO. Through analyses using both bright-field TEM coupled with electron diffraction and Raman spectroscopy, they have revealed direct evidence of *in vivo* intracellular biodegradation of GO sheets with ultra-structural precision (Newman *et al* 2020). The alkaloid extracted from the barberry plant is named berberine. Researchers have developed a heparin/berberine nanocomposite, suppressing the growth of *Helicobacter pylori* and as well as reducing the cytotoxic effects in infected cells (figure 12.7(B)) (Patra *et al* 2018).

12.6.5 Antimicrobial application

Currently hybrid nanomaterials, consisting of both organic and inorganic components, are implemented for drug delivery, gene therapy, and phototherapy in addition to tissue regeneration, antibacterial, biomolecules detection, and imaging probes. This hybrid nanomaterial not only retains the properties of inorganic and organic constituents but also helps in tuning the properties of hybrid material through the combination of functional components (Goeun *et al* 2021, Xinmin *et al* 2021, Yawei *et al* 2020, Manoswini *et al* 2021).

Manoswini *et al* reported antibacterial and cytotoxic activities for hybrid AgNPs. From TEM images successful loading of Ag nanoparticles within the microgel was confirmed. High contrast AgNPs were uniformly dispersed in low contrast microgels. Also, the amount of encapsulation of AgNPs on the surface of microgels was comparatively higher in comparison to the core of microgels. In order to understand the interaction of gold nanoparticles with bacterial colony, Verma *et al* analyzed the TEM images captured for different bacteria–nanoparticle combinations (figure 12.8). They also described, the complete coverage of *Staphylococcus aureus* and *Enterococcus faecalis* with nanospheres, nanostars, and nanocubes. Furthermore, having a higher degree of polyanionic teichoic acids *S. aureus* has shown multilayer deposition as compared to *E. faecalis*. The cationic nanoparticles mostly aggregated around gram-negative bacterias due to the presence of the lipopolysaccharides and phospholipids (Verma *et al* 2015).

12.7 Challenges and future perspective of TEM

An electron scattering event can be either elastic or inelastic. In a TEM, when the incident high energy electrons interact with the thin TEM specimen, the scattered electrons carry abundant information of the targeting specimen, leading to a variety of operating modes by collecting different signals with different energy or momentum and bringing in comprehensive information about the phase, atomic structure, and chemical composition of the specimen. The correction of aberrations in the EM removes the major barrier to resolution that has existed since its invention.

The greatly improved sensitivity and signal to noise ratio is expected to allow single atom detection not only in imaging but also in spectroscopy, perhaps even in

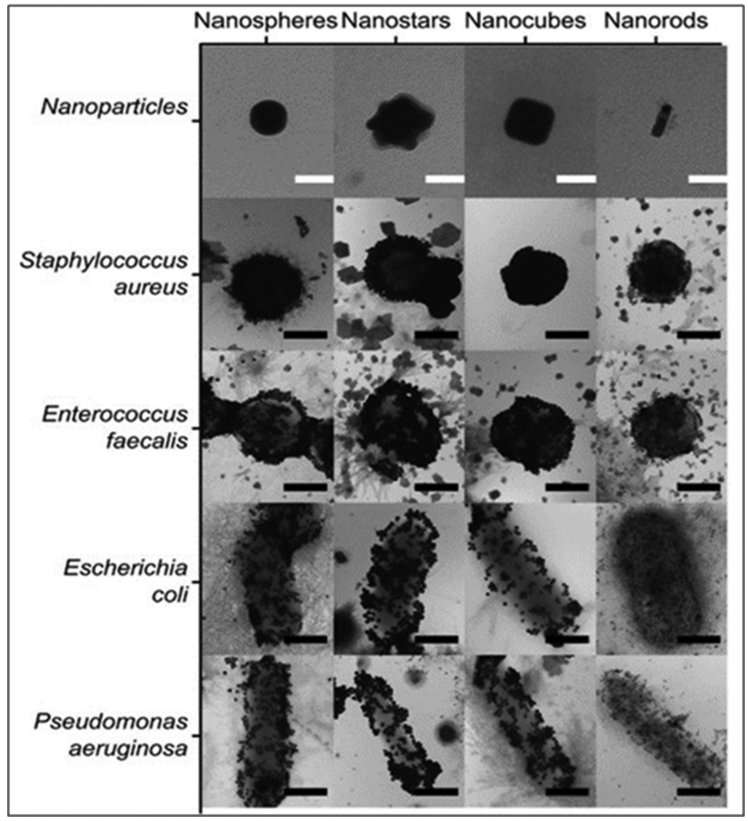

Figure 12.8. TEM images of each of the different shapes of nanoparticles aggregating around various Gram-positive and Gram-negative bacteria. Reproduced from M S Verma *et al* (2015) CC BY 4.0.

three dimensions. Despite its ability to produce highly magnified images of the object, it has some drawbacks of its own. Since electron microscopes are sensitive to vibration and electromagnetic fields they therefore need to be housed in an area that isolates them from possible exposure. Also, as a complete vacuum is required to study an object, it is difficult to study living specimens (Pennycook *et al* 2003). Additionally, sample analysis is limited to materials that are electron transparent in nature.

It is clear that the world of probing matter with electrons will experience revolutionary growth in capabilities in the next decade from ultra-small to ultra-fast and to multi-dimensions. Probing materials' functionalities *in situ* with high spatial- and temporal-resolution will continue to be a main theme. Although enabling instrumentation is often specific for scientific opportunities, there are cross-cutting areas include developing bright electron sources, fast detectors, and dedicated spectrometers. As modern science moves from the understanding of structure and ground states to dynamics and functionalities, such developments would result in transformative advances within fields of science or engineering that allow us to better address our national and international needs.

12.8 Conclusion

Nanoparticle size characterization represents a key step in nanotechnology research and development and quality control. During the development phase especially in the pharmacy industry, electron microscopy is one of the most powerful tools for determining these critical performance defining attributes. This is in detail reflected by the US Food and Drug Administration's (FDA) recommendation of the technique in identifying and demonstrating the efficacy and safety of innovator and generic drug submissions. Transmission electron microscopy provides detailed morphological, topological, compositional information about the nanomaterials, thereby acting as an efficient tool for the characterization of nanomaterials. Additionally, the necessity for ultra-structural analysis demonstrates the potential of TEM in the field of nanomedicine. Hence, the long-established and well-known TEM has been only partially exploited and offers researchers very detailed images of specimens at microscopic and nanoscale.

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