Part I

NANO-FLOTILLAS TRAVERSING IN THE VEIN AS CARRIERS TO DELIVER THERANOSTICS

1

Diagnostic and Therapeutic Systems Using Nanomaterials

There is no standard therapeutic process, since there are so many different schools of therapy.

David D. Burns

1.1 Introduction

Both this book and chapter deal with therapy for various human disorders. Therapy is a word that is used for medical- or health-related treatments intended to relieve disorders. Hence, treatment and therapy can be considered synonyms Therapeutic remediation is associated with or depends on the diagnosis of health problems. The word "theranostic" is used to describe the combined efforts of diagnosis and therapy. Diagnostic and therapeutic devices or methods are often developed together. Useful devices are developed by working closely with the medical scientists who give input into a medical situation. For example, for oncotherapy, developing a therapy that is image guided so as to give localized treatment, especially for tumors and cancer; or image-guided catheters to inject chemotherapy directly into the blood vessels that feed a tumor, reducing the need for systemic chemotherapy.

In this chapter we will be discussing nanotherapy, which is a branch of nanotechnology that uses biocompatible nanoparticles as follows:

(i) To deliver a drug to a given target location in the body so as to treat the disease through a process known as targeted drug delivery [1];
(ii) For photodynamic chemotherapy [2];

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(iii) Antibody-conjugated nanoparticles as a theranostic agent for ultrasound contrast imaging [3];
(iv) Targeted near-infrared (NIR) nanoparticles for photothermal therapy [4]; and
(v) Anticancer or oncotherapy by facilitating cancer cell apoptosis [5].

Drug delivery using nanoparticles and nanoconjugates as well as developing diagnostic devices using nanodiagnostic agents are the major areas of research related to the nanotherapeutic system.

Nanotechnology is already being used for early disease detection and diagnosis, treatment and prevention of disease, and precise and effective therapy [6]. Nanotechnology and various nanoparticles have exhibited potential for detecting disease indicators and markers of early precancerous cells, viruses, specific proteins and antibodies.

Nanodiagnostics involves molecular diagnosis, which helps in developing personalized cancer therapy. A nanodiagnostic device is based on pharmacogenetics, pharmacogenomics, and pharmacoproteomic information as well as environmental factors, which influence response to therapy. In the future, with the advancement in nanodiagnostic technologies, it will be a faster way of doing complete health checkups. Moreover, it will help in tailoring the required medication specifically to the individual based on their genetic makeup, which will prevent unwanted side effects.

1.2 Nanodiagnostic Agents

Nanodiagnostics involves molecular diagnosis, which is mostly used for personalized cancer therapy. This field is currently under development and being researched the world over. It involves the use of nanoparticles (carbon nanotubes, nanoshells, gold, and other metallic nanoparticles, nanopores, graphene, and cantilevers), quantum dots (semiconductor nanocrystals, exhibiting strong light absorbance property that can be used as fluorescent labels for biomolecules), etc., for rapid diagnostic tests and nanorobots as tools to make repairs at the cellular level. Personalized medicine for cancer therapy needs the desired biomarkers. Hence, another very important input of nanodiagnostic technologies that is being envisaged is to refine the discovery of biomarkers using nanoparticles because they have high surface area to volume ratio (SVR) and multifunctionality. nanodiagnostic approach will be future point-of-care (POC) diagnostics and monitoring

technologies with the help of nanobiosensors and microarrays of biosensors-based biochip systems and microfluidic platforms for rapid diagnostic tests and rapid detection of various diseases or pathogen-specific biomolecules/markers, such as DNA, proteins, whole cells (e.g., circulating tumor cells), etc. The nanotools will offer the fabrication of small-scale portable devices.

The nanodiagnostic agents that are being researched are discussed in the following subsections for their applicability.

1.2.1 Bio-Barcode Assay (BCA)

Bio-barcode assay is already being used as a tool for rapid detection of protein-specific antigen (PSA), which is a marker for prostate and breast cancers [7]. It offers increased sensitivity and safety. For BCA assay, two different probes are used: (i) a magnetic microparticle conjugated to a PSA monoclonal antibody, and (ii) a gold nanoparticle to which a PSA polyclonal antibody and "barcode" oligonucleotides are attached. During the assay, PSA gets conjugated to gold nanoparticles (second probe) and becomes sandwiched between the two antibodies. Then, using magnet microparticle the antigen-containing complex is separated from the rest of the mixture. Here, the magnetic field draws the unbound magnetic microparticles to the walls of the container. The remaining particles that are left behind are involved in the detection. The separated complex is washed so as to de-hybridize the barcode oligonucleotides from the nanoparticle. The free oligonucleotides enable the detection of PSA. Using conventional DNA detection methods, barcode is detected. It is also detected by silver amplification method [8]. By this method, as low as 30 attomole/L in a 10 μL sample has been detected [7]. The sensitivity of this assay can be increased by manipulating the equilibrium of the reaction by changing the concentration of the magnetic microparticle probe (Figure 1.1).

1.2.2 Cantilever Beam

Cantilevers are a nanomechanical tool used in diagnostics. A cantilever beam is a beam which is anchored or fixed at only one end and the other end is free (Figure 1.2). The fixed end entirely resists the moments and shear generated by the loads acting on the beam. Beam that is fixed at one end cannot rotate or translate in the direction that load is applied. Whereas, the other end is free to rotate and translate in the direction of the applied load. Micromachined silicon cantilever beams are similar to those used in

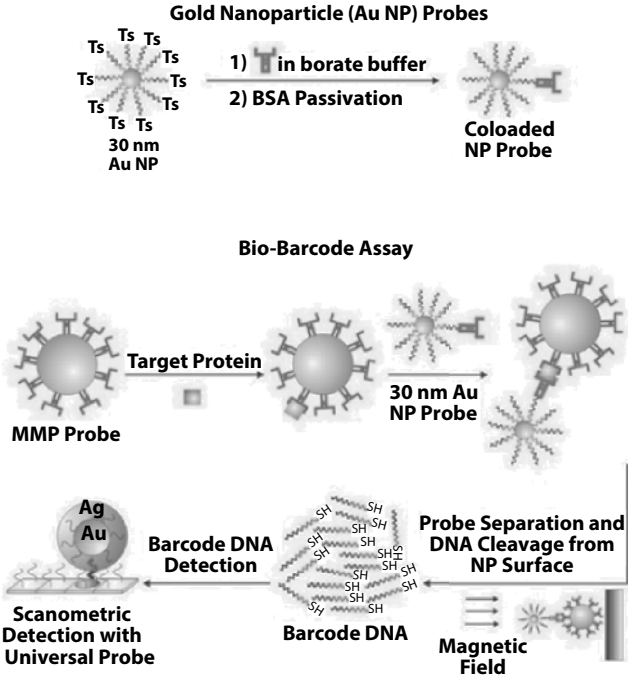

Figure 1.1 Barcode assay of PSA using nanoparticles. A schematic representation of the PSA AuNP probes (upper); and the PSA bio-barcode assay (lower).(Upper) Barcode DNA-functionalized AuNPs (30 nm) are conjugated to PSA-specific antibodies through barcode terminal tosyl (Ts) modification to generate the coloaded PSA AuNP probes. In a second step, the PSA AuNP probes are passivated with BSA. (Lower) The bio-barcode assay is a sandwich immunoassay. First, MMPs surface-functionalized with monoclonal antibodies to PSA are mixed with the PSA target protein. The MMP-PSA hybrid structures are washed free of excess serum components and resuspended in buffer. Next, PSA AuNP probes are added to sandwich the MMP-bound PSA. Again, after magnetic separation and wash steps, the PSA-specific DNA barcodes are released into solution and detected using the scanometric assay, which takes advantage of AuNP catalyzed silver enhancement. Approximately ½ of the barcode DNA sequence (green) is complementary to the "universal" scanometric AuNP probe DNA, and the other ½ (purple) is complementary to a chip-surface-immobilized DNA sequence that is responsible for sorting and binding barcodes complementary to the PSA barcode sequence. (Source: Scheme 1 from C. Shad Thaxton *et al.*, Nanoparticle-based bio-barcode assay redefines "undetectable" PSA and biochemical recurrence after radical prostatectomy. *PNAS*; 106(44): 18437–19442, 2009. Open access article ©2009 National Academy of Science).

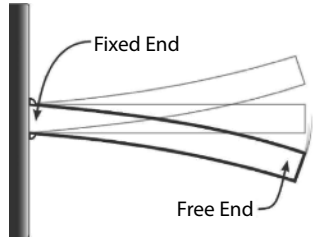

Figure 1.2 A cantilever beam.

atomic force microscopy, function by use of nanomechanical deflections. Because the beam is anchored only on the one end, thermal expansion and ground movement are fairly simple to sustain.

At present, cantilevers are one of the most promising technologies for clinical diagnosis. The micromachined silicon cantilevers are now being used for protein and DNA detection and quantification. The advantage of using a cantilever beam is that there is no toxicity concern. A standard cantilever biosensor can detect multiple target molecules from small biological samples especially for the detection of cancer at an early stage. Once a tumor becomes malignant, it is too late to treat the cancer with a maximum success rate. Since cancers spread through blood and particularly the lymphatic system in the body, it becomes important to measure multiple parameters of biological molecules. In a cantilever, biosensor biomolecules are adsorbed onto one side of the surface of the cantilever, causing a decrease in the surface free energy and generating a differential surface stress between either side of the cantilever beam as a result of adsorption of biomolecules occurring at one side of the cantilever. For DNA detection, the cantilever surface holds a particular DNA sequence capable of binding to a specific target. For DNA detection, at one side of the cantilever layer hybridization occurs between the target probes, changing the intermolecular interactions within a monolayer, which induces surface stress that bends the cantilever beam and initiates a motion (Figure 1.2). The deflection of the cantilever caused by surface stress change, which is in the range of several nanometers, is measured using a piezoelectric readout.

Based on the same principle, specific DNA strands are detected. Single-stranded DNA (ssDNA) is isolated using a cantilever sensor by coating one side of the cantilever surface with gold and applying a thiol linking agent at one end of the DNA (Figure 1.3). A variation of 3050 mN/m in surface stress occurs as a result of ssDNA adsorption. The process of surface stress is based on adsorption of sulfur atoms (sulfur atom acts as a receptor for a strand complementary to the DNA sequence) on the thiol agent attached

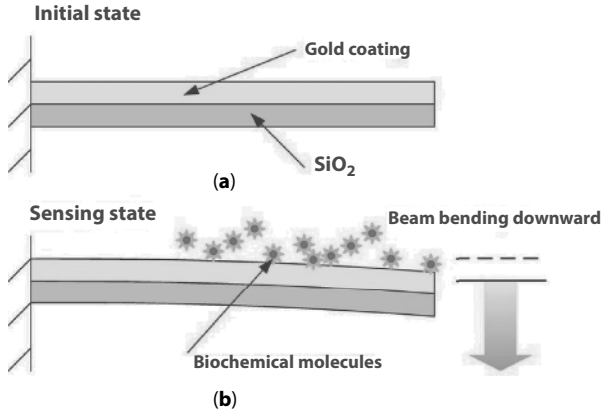

Figure 1.3 Cantilever beam response: (a) initial state and (b) sensing state.

to one end of the ssDNA that is also bound to the surface of the cantilever. The isolated DNA is then identified as noncomplementary strands that carry a mutation.

Carbon nanotube (CNT) has a cylindrical and hollow tube shape and a number of walls enclosing a nanotube, making it single-walled carbon nanotube (SWCNT) or multi-walled carbon nanotube (MWCNT); and an arrangement of carbon atoms with respect to tube axis (there are many kinds of chiral tubes) makes it a suitable material for preparing a cantilever. Mechanical (such as bending or bucking and high resilience to mechanical strains) and thermal (it shows thermal expansion under temperature) properties of CNT shows that cantilever can be fabricated from it and used for detecting cancer. Moreover, MWCNTs can adsorb light energy selectively depending on light polarity.

1.2.3 Carbon Dots/Carbon Quantum Dots

Quantum dots (QDs) are semiconductor crystals having their excitons or electrons and holes confined in all three dimensions of space. Consequently, QDs have electronic properties that are intermediate between those of bulk semiconductors and those of discrete molecules. They are very small particles (size ranges from 1–10 nm) that follow wave theory concept. Being very small in size, they possess larger band gap. This is because of the discreet energy level, which depends on the size and shape of the QD [1]. Because of their various optical and electronic properties like excitation of multiple fluorescence spectra, enhanced photostability, high quantum yield, and photocatalysis, QDs are used as a tool for biological imaging, diagnostics,

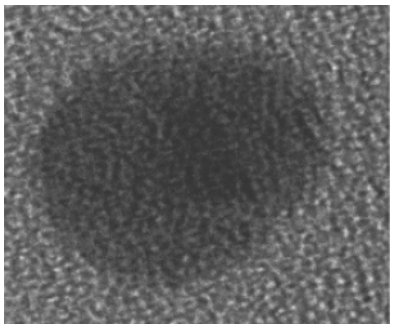

Figure 1.4 Transmission electron microscopy image of carbon dot.

and molecular histopathology. Though there are many known inorganic QDs, such as cadmium telluride (CdTe), cadmium selenide (CdSe), lead selenide (PbSe), gallium arsenide (GaAs), gallium nitride (GaN), indium phosphide (InP), indium arsenide (InAs), etc., due to their cytotoxicity and insolubility in water they are not suitable for biological applications.

Carbon dots, a new form of fluorescent carbon nanostructure (Figure 1.4), were discovered in 2004 by Xu *et al.* [9] and were termed as carbon quantum dots (CQDs) or carbon dots (CD), as they exhibited QD-like properties and characteristics such as they are. However, CDs are nontoxic, biocompatible, resistant to photobleaching, water soluble, and comparatively inexpensive to synthesize. For suitable application of CD as a theranostic agent it is often conjugated with other biogenic, organic or inorganic molecules [1] so as to get desired particle size and size distribution for the *in-vivo* distribution, stability, drug loading and drug release ability, uptake by cells, biological fate, toxicity; and the targeting ability of these delivery systems, availability to a range of cellular and intracellular targets, easy mobility and capacity to cross the blood-brain barrier to treat neurogenerative disorders. CDs are used as a tool for multiplexed diagnostics; Immunohistochemical assays; neurotransmitter detection; and cellular imaging. For this, CDs are used in electronic devices given below.

1.2.3.1 CD as Bioimaging Agent

Bioimaging is a noninvasive method to visualize biological processes in real time without interfering with the living processes, giving information on the 3D structure of the specimen from the outside, without physical interference. It has been used for imaging various cancer cells, bacteria, viruses, drosophila, etc.

1.2.3.2 CD as Sensor

Carbon dots exhibit photoluminescence (PL) emission in the near-infrared (NIR) spectral region under NIR light excitation. This property is useful as a sensor because of the transparency of body tissues in the NIR "water window." The PL from CDs is efficiently quenched by both electron acceptor or electron donor molecules in solution, because photoexcited CDs are excellent electron donors and electron acceptors. This makes the CD-based sensor/detector very sensitive and selective. The quenching refers to decreases in the fluorescence intensity of CD due to fluorescence resonance energy transfer (FRET). The CDs are easily internalized in the cells and are stable, highly sensitive, show specificity and accuracy; and are versatile material for designing multifunctional biosensors to detect: (a) *Metal ions* – fluorescence "turn-off" assays have been used for the detection of metal ions (Hg^{2+}, Cu^{2+}, Pb^{2+}, $Fe3^{+}$, Ag^{+}, and Cr^{6+}, etc). (b) *Small molecules* for detection of small molecules (Nitric Oxide, Phosphate, Reactive Oxygen Species. H_2S, Trinitrotoluene or TNT, Hydroquinone, Surfactant), instead of using fluorescence quenching mechanism, detection is done by restoring fluorescence of the quenched CDs. (c) *Biological pH* or *Physiological pH* are the pH value in living cells and tissues at which metabolic activities take place. It is determined by CDs by measuring the fluorescence intensity of the CD as it is highly sensitive to pH. CDs exhibit a strong emission at pH 7.0; whereas, under strongly alkaline or acidic conditions, the PL is nearly completely quenched [4]. Both *in-vivo* and *in-vitro* pH have been detected using CD as a tool [10]. The precursor for synthesis of CD plays an important role in its use for pH detection. For example, CD from ascorbic acid that exhibited a linear dependence on the pH of the solution in the range of 4.0 to 8.0 had potential application in colorimetric and fluorescent probes for pH measurement, generating a good linear calibration curve in the pH range from 6.0 to 8.0; whereas CD from citric acid displayed a good linear relationship with the solution pH value in the range of 2.55–5.19 and an excellent reversibility between pH 5 and pH 9 [11]. Another precursor, threonine-derived CD [12], has also shown that fluorescence of CDs was sensitive to pH in a broad range of 2.13–9.34. (d) *Nucleic acid/DNA detection* is done using different methods, one of them using methylene blue to quench the fluorescence of CDs; hence, ctDNA is joined in solution; the fluorescence of the solution was found to be restored. Based on this, a fluorescent sensor for DNA detection was designed; the detection limit was 1.0 × 10−6 mol/L with a linear range of 3.0×10^{-6} mol/L to 8.0×10^{-5} mol/L [13]. Another method of DNA detection is using $KMnO_4$ to trigger chemiluminescence of CDs. The DNA detection was performed in a linear

range from 10−18 to 10−14 mol/L with the low limit of detection being 8.56 × 10−19 mol/L [14]. There are several such methods being researched the world over. (e) *Vitamin detection* – so far riboflavin and vitamin B12 have been successfully detected by surface-functionalized CDs. It is done by CDs-based ratiometric sensor with high sensitivity of 1.9 nM for riboflavin. (f) *Protein detection* – Many different protein and enzyme detection sensors have used CDs such as an aptamer-functionalized CD [15]; a novel CDs-dsDNA sensor has also been developed to detect protein and enzymes [16], where PL is quenched in the presence of dsDNA to detect histone; addition of histone turns on the luminescence through unwinding dsDNA from CDs due to the strong affinity between histone and dsDNA. With the binding affinity between DNA and proteins, the quantitative detection of protein can be calculated from fluorescence restoration. Another turn-on, CDs-based nanosensor, is composed of CDs and gold nanoparticles for detection of cysteine with multiple signals. It has excellent selectivity and sensitivity [17]. (f) *Sugar detection* – CDs is used to detect glucose through peroxidase-like catalytic activity of CDs, which is achieved by assembling porphyrins on the surface of CDs by π-π stacking and electrostatic interactions [18]. The CDs catalyze the appropriated peroxidase substrate, producing a blue color in the presence of H_2O_2. However, this catalytic activity of modified CDs is temperature and pH (optimal pH was found to be 4.0) dependent but H_2O_2 concentrations do not affect it. The glucose oxidase immobilized CDs can detect glucose in the linear range from 20 μmol/L to 1 mmol/L with a detection limit of 7 μmol/L [19]. Therefore, CDs can be used for enzyme immobilization for several biomedical and technological applications.

1.2.4 Carbon Nanotubes (CNTs)

Since their discovery in 1992, carbon nanotubes have generated tremendous interest and efforts by scientists to look for their applications, resulting in applications being found in almost every branch of science. CNTs are allotropes of carbon having a mixture of sp^3 and sp^2 carbons with tubular graphenic structure that imparts semiconductor properties, the diverse geometries of which afford a spectrum of unique chemical, electrical, magnetic, and optical properties. Thus, a plethora of applications, which can be divided into two categories: (i) Applications of CNT in areaz other than biological systems; and (ii) Applications of CNT in biological systems.

Functionalization of CNT: For most of the applications, the CNT needs to be functionalized to increase its reactivity. CNTs are insoluble in water, polymer resins, and most solvents. Thus, they are difficult to evenly

disperse in a liquid matrix. However, CNTs can be made soluble in both organic solvents and in aqueous solutions by organic functionalization. To utilize the CNTs' novel physical properties in the manufacture of composite materials, as well as in other applications, it is necessary to physically or chemically attach certain molecules, or functional groups, to their sidewalls without significantly changing their desired properties. This process is called functionalization. Commonly, functionalization of CNT involves oxidation, fluorination, amidation, etc., either by attachment of organic moieties to carboxylic groups (that are formed by oxidation of CNT with strong acid) or by direct bonding to the surface double bonds. CNTs contain five-membered rings at the end of the tubes. These pentagonal carbons are oxidized by chemical treatments with suitable agents. Refluxing CNTs in a H_2SO_4/HNO_3 mixture results in a clear, colorless solution, which upon removal of solvent and excess acid gives a white solid containing functionalized CNTs. Oxidized carbon atoms can act as specific sites for adsorption of metal ions (Figure 1.5). Treating CNT with suitable agents helps in attaching amid, amine, -COOH, and -OH groups, which are interesting materials for many biological applications.

The properties of CNTs, which have influenced its application in biosystems, includes their surface morphology, which makes them a suitable material for adsorption, absorption of gases and liquids, data storage, and template for the various biological reactions. Apart from surface morphology, electrical and electronic properties, and conducting and semiconducting properties make CNTs different from the other materials. The band gap of CNTs as well as their photochemical properties have found wide application in purification of water or degradation of organic and inorganic

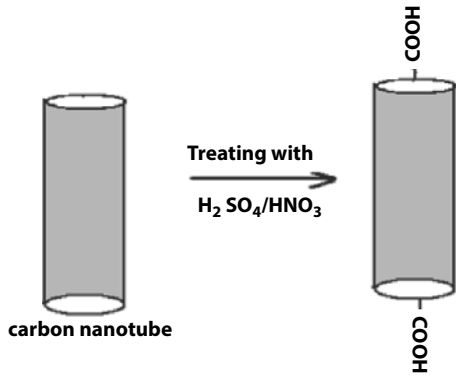

Figure 1.5 A schematic diagram showing functionalization of CNT (having both ends opened) with –COOH.

materials. Some of the successful applications of CNTs in diagnostics are discussed below.

1.2.4.1 Diagnostic Equipment Using CNT

Diagnostic equipment that uses CNTs as tools are: (i) Chemical sensors, (ii) Biosensors, (iii) Nanoprobes, (iv) DNA sensors, (v) Nanorobots, and (vi) X-ray devices.

1.2.4.1.1 Chemical Sensors Using CNT

Chemical sensors are those substances which change their surface conductivity when some specific materials get adsorbed at their surface. Surface conductivity depends on the ease with which electrons can migrate from cathode to anode (Figure 1.6).

The surface of any material contains either positively or negatively charged dangling orbitals. These sites are known as surface states. When electron migrates from cathode to anode, it encounters these surface states which hinder its transport to the anode. The conductivity of the surface depends on the surface states. When metal is exposed to other materials, adsorption of the materials occurs, which alters the percentage of dangling bonds present on the surface of metal. Hence, electrons moving on the surface alters the surface conductivity of the metal. The change in conductivity is related to the amount of material used for the adsorption; thus, one can find the concentration of the same material. The efficiency of this method depends upon the magnitude of change in conductivity with concentration.

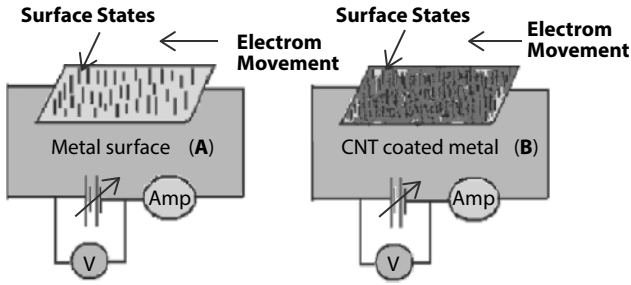

Figure 1.6 (A) Dangling orbital shown by vertical lines present over a smooth metal surface; (B) Metal surface coated with CNTs. Each CNT possesses several dangling orbitals, thus increasing the concentration of surface states.

Moreover, there are single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT). The MWCNTs have been found to be very suitable electrode material for electrochemical sensors, as they have a wide electrical potential window, fast electron transfer rate, and large surface area; and are low cost and easy to use.

However, there have been reports of using SWCNT capacitor for chemical detection [20] because the capacitance of SWCNTs is highly sensitive to many chemical vapors and this transduction mechanism is used for a fast, low-power sorption-based chemical sensor. In the presence of a dilute chemical vapor, molecular adsorbates are polarized by the fringing electric fields radiating from the surface of a SWCNT electrode, which causes an increase in its capacitance. Hence, by thinly coating the SWCNTs with chemo-selective materials that provide a large, class-specific gain to the capacitance response, a high-performance, highly sensitive, fast and completely reversible gas sensor is constructed.

1.2.4.1.2 Biosensor Using CNT

As mentioned earlier, properties like its insolubility in water and possibility of cytotoxicity have been an obstacle during the course of investigation of biological uses of CNTs. As far as biological acceptability is concerned, there is a dispute: one group of scientists feels that it is toxic and another group advocates that it is not chemically toxic, but because of its small size it may be toxic; hence, depending upon the requirements, carbon can be used. To combat this problem, CNTs are functionalized by attaching biological molecules such as lipids, proteins, biotins, etc. Then they can usefully mimic biological functions, such as protein adsorption, and bind to DNA and drug molecules (Figure 1.7). This is expected to help in gene

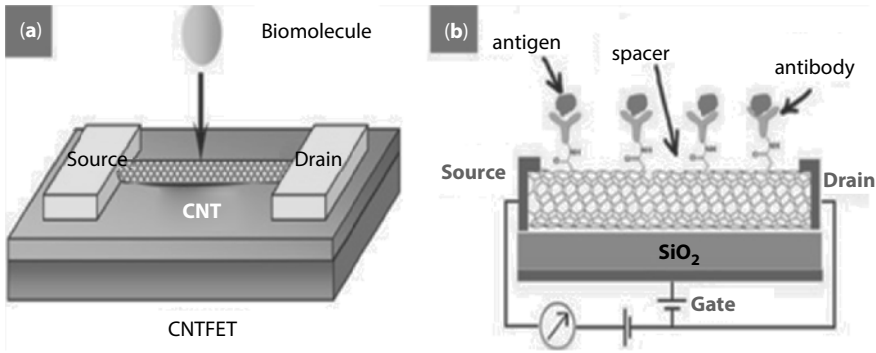

Figure 1.7 Schematic diagram of CNT-based biosensor.

therapy and drug delivery. For biosensors, molecules, such as carboxylic acid (COOH), poly m-amino benzoic sulfonic acid (PABS), polyimide, and polyvinyl alcohol (PVA), have been used to functionalize CNTs. Functionalized CNTs can become soluble in water and other highly polar, aqueous solvents. Biosensors are already been used for bio-stress detection and for glucose and DNA detection.

1.2.4.1.3 Nanoprobe Using CNT

Probes are devices for investigating and obtaining information on an unknown region. CNTs have been used for making probes [21, 22], e.g., AFM (atomic force microscope) probes. Conventional AFM tips are made of silicon or silicon nitride and are pyramidical in shape, having 5 nm radius of curvature. Moreover, nanoprobes made of CNTs have high resolution. As their cylindrical shape and small tube diameter enable imaging in narrow and deep cavities, they have mechanical robustness, which enhances the life of probes by minimizing sample damage during repeated hard crashes onto substrates and low buckling force; hence, they can be applied for imaging soft biological materials.

1.2.4.1.4 DNA Sensor Using CNT

For applications in diagnostics, vaccine, and drug delivery, or multi presentation of bioactive molecules, soluble CNTs are further derivatized by coupling with amino acids and bioactive peptides that are immobilized on the external walls of CNTs. Since CNT has very large surface to volume ratio, it contains large concentrations of "dangling bonds" which behave either like Lewis base or Lewis acid. The advantage of large dangling bonds is that chemicals can be temporarily loaded and unloaded from CNT, because dangling bonds help to form sort of a chemical bond which is not as strong as normal chemical bond (Figure 1.8). Therefore, CNTs are promising material for biomedical applications because one is able to tailor them for a specific purpose.

DNA is a coding molecule of living organisms that controls all the functions of the whole system. Therefore, since DNA can sense malfunctioning of the body parts, it can be used in nanosensors and CNT provides an excellent platform to attach DNA. Surface-confined MWCNTs have been shown useful in facilitating the adsorptive accumulation of guanine, a nucleobase which greatly enhances its oxidation signal. SsDNA/SWCNT-FET sensors have been found to be very efficient and fast and can detect a variety of odors within a fraction of second.

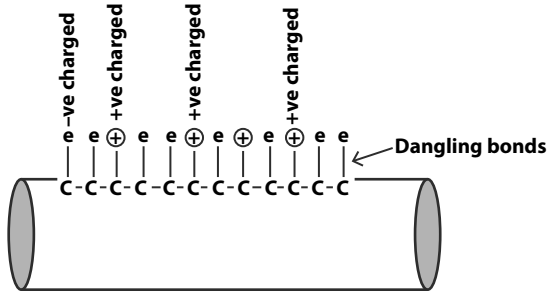

Figure 1.8 Schematic of a CNT showing presence of dangling bonds due to electronically unsatisfied carbon present on the surface. During the formation of the material, electron may get lost from the surface, creating a +ve charge, or it may contain unsatisfied electrons such that overall surface behaves like a neutral surface.

1.2.4.1.5 Nanorobots Using CNT

Feynman's talk, "There is Plenty of Room at the Bottom" indicated the possibility of a nanorobot. However, it is still in the R&D phase. Robotics is the use of technology to design and manufacture intelligent machines that can perform specifically programmed tasks. Nanorobots are nano size microscopic devices that work at the atomic, molecular, and cellular level. These devices are very sensitive to acoustic signals, hence can be programmed using sound waves to perform specific tasks. They are being researched for the purpose of maintaining and protecting the human body. When implanted into the bloodstream, these nanorobots can identify the cause of fever, the harmful cells and quarantine it, as well as provide the desired dose of medicine to the infected area; and can help in breaking up blood clots and kidney stones, and treat arteriosclerosis by breaking up plaque. Carbon in the form of diamond/fullerene is the principal element making up the bulk of a medical nanorobot. CNT is used for making smaller and faster components that will consume less energy and computerized parts will have high speed and high-capacity memory. Zhang *et al.* [23] have reported a carbon nanotube-based charge-controlled speed-regulating nanoclutch (CNT-CC-SRNC) composed of an inner carbon nanotube (CNT), an outer CNT, and the water confined between the two CNT walls, which proposes utilizing electrowetting-induced improvement of the friction at the interfaces between water and CNT walls. As the inner CNT is the driving axle, molecular dynamics simulation results demonstrate that CNT-CC-SRNC is in the disengaged state for the uncharged CNTs, whereas water confined in the two charged CNT walls can transmit the torque from the inner tube to the outer tube. Importantly, the proposed CNT-CC-SRNC

can perform stepless speed-regulating function through changing the magnitude of the charge assigned on CNT atoms. CNT-based actuators are used for high-technology applications such as humanoid robots, artificial and damaged hearts, artificial limbs, medical prosthetic devices, etc.

Another option is the use of molecular nanotechnology (MNT) or nanorobotics in surgery. In nanorobotics, surgeons move joystick handles to manipulate robot arms containing miniature surgical instruments at the ports. Another robot arm contains a miniature camera for a broad view of the surgical site. It results in less stress for surgeons and less pain for patients; at the same time, high precision and safety is achieved. MNT allows *in-vivo* surgery on individual human cells. Nanorobotics-based surgery can be used for gall bladder, cardiac, prostrate, bypass, colorectal, esophageal, and gynecological surgery. However, nanorobotic systems for performing surgery require the ability to build precise structures, actuators, and motors that operate at molecular level to enable manipulation and locomotion.

CNTs are also used as drug carriers for targeted drug delivery and for attaching to the body, preferentially similar to formation of bone tissue, etc. CNTs are also used for water and air filtration, hydrogen and energy storage.

1.2.4.1.6 X-Ray Devices Using CNT

Traditional X-ray is generated by heating a metallic filament (cathode) that emits accelerated electron, which is bombarded on a metal target (anode) to generate X-rays (Figure 1.9a). This X-ray generation method consumes high energy, has slow response time and has limited lifetime. Romero *et al.* [24] have reported that field emission is a better mechanism of extracting electrons than thermionic emission because electrons are emitted at room

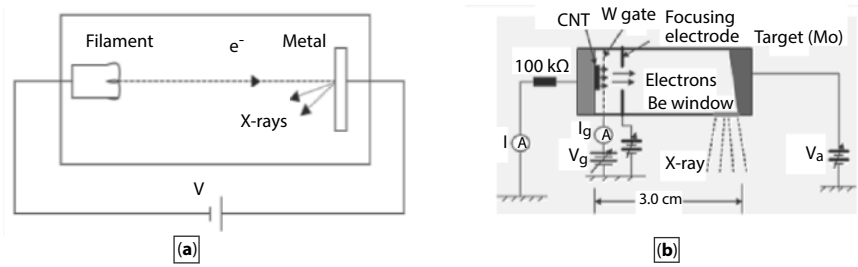

Figure 1.9 Schematic diagram of (a) Traditional method of generating X-rays; (b) CNT-based microfocus X-ray tube.

temperature, the output current is voltage controllable and the voltage necessary for electron emission is less. An optimal cathode material should have high melting point, low work function, and high thermal conductivity. CNTs meet all these requirements and hence are used as a cathode material for generating free-flowing electrons.

Electrons are readily emitted from their tips either due to oxidized tips or because of curvature when a potential is applied between a CNT surface and an anode. Continuous and pulsed X-rays can be generated using a CNT-based field emission cathode (Figure 1.9b) [25], which has fast response time, fine focal spot, low power consumption, can be miniaturized, has longer life, minimizes the need for cooling, and is low cost. Moreover, miniaturized X-ray devices can be inserted into the body by endoscopy to deliver precise X-ray doses directly at a target area only.

1.2.4.1.7 Surgical Supplements Using CNT

The surgical supplements or surgical aids that use CNTs as tools are: Nanomedicinal devices, bioactive nanomaterial in bone grafting, nanotweezers, etc. Surgical supplements are being designed to reduce the surgery using macro instruments that are cumbersome for both the surgeon and the patient, that may lead to surgical error due to the surgeon's fatigue; moreover, macro surgical instruments are not suitable for certain delicate surgeries such as surgeries related to the heart, brain, eyes, and ears. Using CNTs, R&D is on the way to investigate smart instruments such as forceps, scalpels, and grippers with embedded sensors to provide improved functionality and real-time information to aid surgeons. CNTs are expected to be useful for optically guiding surgery, leading to easy removal of tumors and other diseased sites.

1.2.4.1.8 Nanotweezers Using CNTs

Carbon nanotube-based nanotweezers have already been created for manipulation and modification of biological systems such as structures within a cell; moreover, they have the potential to be used in medical nanorobotics also. These nanotweezers can be used (i) for manipulation and modification of biological systems such as DNA and structures within a cell, (ii) as nanoprobes for assembling structures, (iii) to aid in increasing the value of measurement system, (iv) and, as demonstrated, nested CNTs can make exceptionally low-friction nanobearings [26]. There is a need to investigate the use of CNTs as nanoprobes for crossing into the tumor, but not crossing into healthy brain tissue.

1.2.4.1.9 CNT as Tool for Tissue Engineering (TE)

Tissue engineering involves replacement of anatomic structure of the damaged, injured or missing tissue or organs by agglomerating biomaterials, cells and biologically active molecules (Figure 1.10) [27, 28]. The 3D scaffold of biomaterials provides mechanical support to the growing cells or tissue that can then be transplanted into the system. The schematic approach shown in Figure 1.10 demonstrates how tissue engineering uses a biomaterial scaffold to directly grow the body's own cells in a lab and then implants the cells back into the body. The scaffold directs cell behavior, e.g., migration, proliferation, differentiation, maintenance of phenotype and apoptosis, by facilitating sensing and responding to the environment via cell matrix communications and cell-cell communications [29, 30].

Scaffold should have highly porous surfaces so as to allow seeding of cells at high densities as well as facilitating proper cell-cell interaction through a regulated molecular myriad [31]. Carbon nanotube has potential for multiple uses in tissue engineering [32]. Both SWCNT and MWCNT possess properties such as ordered structures with high aspect ratio, ultralightweight, high mechanical strength, high electrical and thermal conductivity, metallic or semi-metallic behavior, and high surface area. These properties have made CNTs a favorite material for nanofabrication of compatible

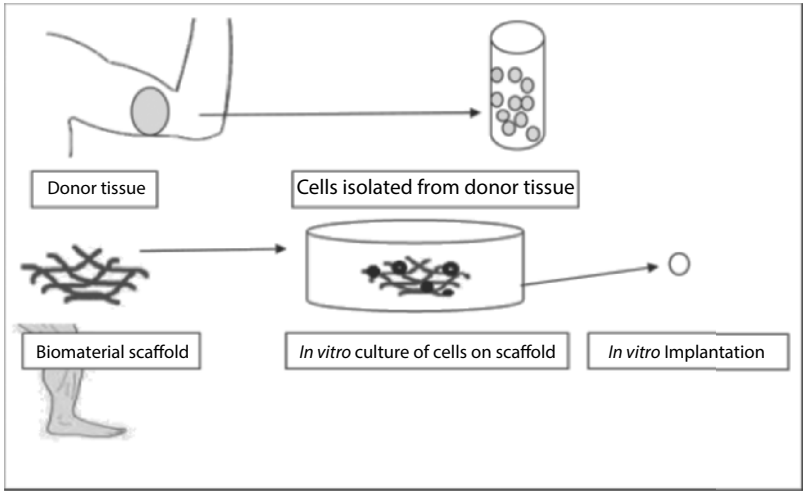

Figure 1.10 Schematic representation of use of tissue engineering on a scaffold and then implant it in the desired organ of the body. (*Source: Carbon Nanofibers, Ed. Sharon and Sharon 2020, Wiley-Scrivener*).

biomaterials which can act as scaffold for engineering tissues [33]. The areas where CNTs have significant relevance for tissue engineering are:

(i) Cell tracking and labeling; CNTs are feasible as imaging contrast agents for optical labeling, magnetic resonance, and radiotracer modality;
(ii) Monitoring cellular physiology;
(iii) Regulating cellular behavior; and
(iv) Structural support for tissue engineering.

1.2.4.1.10 Gene Delivery Using CNT

For gene delivery inside a cell or cell organelles, nano-sized devices that can pass through the delicate cell membrane without damaging it are needed. Since CNTs have needle-like geometry, high elasticity and strength, Gao *et al.* successfully used amino-functionalized MWCNTs for gene delivery [34], as it interacted with negatively charged DNA and the cell membrane and delivered the GFP (green fluorescent protein) gene into mammalian cells without any cytotoxicity. Simultaneously, Pantarotto *et al.* [35] also found that functionalized, positively charged, water-soluble CNTs can penetrate into cells and transport plasmid DNA by formation of noncovalent DNA-CNT complexes; giving the possibility that CNTs can be used as novel non-viral delivery systems for gene transfer. Chen and Rajewsky [36] have also shown that a CNT-based "nanoinjector" can penetrate a cell without damaging the membrane, even after repeated use. Later, Pan *et al.* [37] developed novel poly-amidoamine dendrimer-modified multi-walled carbon nanotubes (dMWCNTs) for gene delivery to improve the amount and delivery efficiency of genes taken by CNTs into human cancer cells. Compared with traditional gene-delivery carriers, poly-amidoamine dMWCNTs exhibit maximal transfection efficiencies and inhibition effects on tumor cells.

1.2.4.1.11 CNT as Drug Delivery Vehicle

A smart drug delivery system is being worked out for increased efficacy of a drug. The work is focused on developing a carrier that can deliver the drug to its target selectively with controlled release profile, so as to improve therapeutic efficacy and reduce the dose of the drug, thus minimizing unwanted toxic effects of the drugs. Nanoscale devices attached with antibodies and loaded with drugs can serve as a targeted drug delivery vehicle that can transport chemotherapeutics or therapeutic genes into diseased cells while sparing the loading of healthy cells with drugs. CNTs have

shown the possibility of being used for drug delivery by Sharon's group [38]. Diameters of SWCNTs are similar to the diameter of a molecule of DNA, hence can easily traverse through the cell. However, the length of CNTs vary according to the method of production and it is important that they are tailored to the right size for drug delivery. Since carbon nanomaterials (CNMs) are insoluble in both polar as well as non-polar solvent, they exhibit very low processability. Functionalization of CNT by $–NH_2$, –COOH, -OH, and > C=O groups overcomes the problem of processability. The outer shell of MWCNT very often contains discontinuous spots and imperfections. These local vacancies are closed by the functional groups mentioned above.

Since CNTs are prepared at very high temperature, i.e., around 750 °C onwards, which may be damaging to the drug molecules to be loaded, drug molecules cannot be loaded during synthesis of CNT. Drugs may be bound to the CNT by: (a) Incorporation into the interiors of a nanocapsule [39], (b) Covalent binding [40, 41] (c) Electrostatic binding [42], and (d) Surface adsorption [43].

Gately and Panhuis [44] attempted *in-situ* filling of open-ended SWCNTs that are synthesized by the electric arc process. However, because of the high speed of the transient phenomena and the restricted volume while SWCNT formation occurs in the plasma, a very low amount of filler entered the SWCNT before the closing of the tubule while it grows.

Sloan *et al.* (1998) [45] have filled SWCNTS with ruthenium by soaking SWCNT in a solvent at room temperature. Liquid phase filling of SWCNT with samarium oxide along with the molten filler (molten salts) has also been attempted by putting them together in a sealed ampoule.

Opening of MCWNTs in supercritical water (SCW) medium creates an alternative possibility for filling. So far, CNT has been filled with halides, oxides, heavy metals, gases like hydrogen, carbohydrates, DNA, enzymes, and other proteins. But none of the drug has yet been loaded or filled in CNT.

1.2.4.1.12 CNT for Pollution Control

Carbon nanotube is semi-conducting in nature, i.e., depending upon its internal diameter exhibit for photocatalytic killing capacity of microbes by disrupting the cell membrane [46]. Moreover, CNTs have also been able to disrupt the plant cell wall and membrane; hence, their various applications are being envisaged such as killing unwanted algae, as a sterilant, etc.

In the photocatalytic process, if it comes in contact with another material, particle of a semiconductor can form a depletion region like one forms

a p:n junction. When its interface is illuminated with a light of photon energy greater the band gap of the materials, formation of electron/hole pairs takes place in a similar fashion as one gets when a p:n junction is illuminated. These photon-generated electron/hole pairs are highly reactive and can initiate oxidation/reduction process with any organic material which comes in physical contact with them.

Carbon nanomaterial (CNM) is also being tried as a filtration tool. Due to its antimicrobial capacity, its use as a filter material can solve and improve water purification and filtration. It is being thought of as a better alternative than existing water purification and filtration technology. Along with this, the basic application of CNM for water purification can also be incorporated into bandages and sterilant to clean surfaces to make them bacteria proof.

1.2.4.1.13 Anticancer Activity of CNT

A vision to use CNT for detecting cancer as well as a new substitute for metallic macro syringes to treat cancerous cell by targeted drug delivery to cancer cells is now being realized by several research groups, who are also developing CNTs as targeted delivery vehicles for anticancer drugs. These CNTs can ferry and deliver anticancer drugs and proteins into cells and act as miniature thermal scalpels that can bake a cancer cell to death. Prevention of precancerous cells by imaging agents and diagnostics from becoming malignant at early stages is a hot field where scientists are working on developing a method to detect cancer at very early stages so that its cure could be achievable. A landmark achievement is the development of a highly sensitive immunodetection of cancer biomarkers by amplifying CNTs [47].

In an attempt to increase the sensitivity of cancer biomarker detection and to decrease the need for large samples from which to detect those molecules, a "forest" of single-walled carbon nanotubes is used to detect lower levels of the prostate-specific antigen (PSA), which is a protein produced by the cells of the prostate gland. When the prostate gland enlarges, PSA levels in the blood tend to rise. PSA levels can rise due to cancer or benign (not cancerous) conditions. As PSA is produced by the body and can be used to detect disease, it is sometimes called a biological marker or tumor marker. Moreover, this new system requires between 5 and 15 times less sample than the other commercial detecting systems do and can detect PSA at levels as low as 4 pg/ml in a sample size of 10 μl of serum. Whereas, the existing standard assay has a detection limit of 10–100 pg/m and requires as much as 50–150 μl of sample. Bundles of SWCNT having

chemically reactive groups on the ends of the SWCNTs to which enzymes or antibodies capable of reacting or binding to specific biomolecules are attached were used for this purpose. An antibody that binds to PSA, which is used in detecting prostrate as well as breast cancer, was used. When antibody-labeled CNTs is added to a serum sample, any PSA present in the serum sample gets bound to the antibody. Using electrical impulses, the number of PSA molecules bound to CNTs can be measured.

To kill cancerous cells and identify biological targets, an attempt is being made to develop nanotube-based therapeutics and imaging agents. Due to their excellent electrical conductivity, CNTs are poised to become the key component of ultrafast, miniaturized diagnostic gear that may soon be able to detect the earliest signs of cancer from a pinprick of blood immediately

Wang *et al.* [48] used electrodes modified with CNTs to create highly sensitive devices capable of detecting specific sequences of DNA, including those associated with the breast cancer gene BRCA1. On the contrary, Sirdeshmukh *et al.* [49] attached antibodies to the surfaces of carbon nanotubes that can bind molecules shed by tumors into the bloodstream. In both cases, the idea is to incorporate the tumor-sensing nanotubes into a small electrical circuit, which would signal the presence of the tumor marker with a change in electrical conductivity.

Nanoshell and SWCNT have been found to kill cancer cells by using infrared radiation or lasers with very high power (35 W/cm^2 for nanoshell and 1–4 W/cm^2 for SWCNT) by heating the nanoshell and SWCNT to 55–70 °C. However, the level of temperature increases in SWCNT to an extraordinary level to create nano-bombs that explode at low laser intensities. By exploding SWCNT that is co-localized with cancer cells, one can selectively destroy the SWCNT as well as the cancer cells. This approach may be the only way of using SWCNT for therapeutics without causing any toxicity problems. Nano-bombs are made by bundling the CNTs and exposing them to light, thus generating heat. With a single CNT, the heat generated by the light is dissipated by surrounding air. But in bundles of CNTs, the heat cannot dissipate as quickly and results in explosion on the nanoscale**.** Nano-bombs are created due to the optical thermal transitions in SWCNT and the thermal-energy confinement in SWCNT bundles and subsequent vaporization of liquids between SWCNT in bundles, creating pressure that causes the eventual explosion. This phenomenon works in a host of different liquids such as alcohol, de-ionized (DI) water, and phosphate-buffered saline solutions. By hydrating SWCNT in sheets as well as by co-localizing it to cells, potent nano-bombs can be created to explode cancer cells completely. This process is highly localized with minimum collateral damage to neighboring cells.

Efforts are being made to use CNM as therapeutic agent for cancer treatment because CNT can absorb near-infrared light waves, which can pass harmlessly through cells. When a beam of near-infrared light falls on a CNT, electrons in the CNT become excited and begin releasing excess energy in the form of heat (a solution of CNTs under a near-infrared laser beam heats up to about 70 °C in two minutes). Dai and Flesher [50] utilized this property of CNTs and used it as therapeutic agent. Nanotubes were placed inside cells and irradiated by the laser beam; the cells were quickly destroyed by the heat. However, cells without nanotubes showed no such effects when placed under near-infrared light.

1.2.4.1.14 CNT for Neurodegenerative Disorder Therapy

Neurodegenerative diseases are due to disorders of neurons. Neurons are the fundamental units of the central nervous system (CNS). They are made up of a cell body (soma), several dendrites, and a single axon (Figure 1.11). More than one billion neurons exist in the CNS brain and spinal cord. Neurons are interconnected with sound precision and control our thoughts, emotions, movements, and sensation. Some of the neurodegenerative diseases are Parkinson's disease, Friedreich's ataxia, amyotrophic lateral sclerosis, temporal lobe epilepsy, and Alzheimer's disease.

There is apprehension regarding the biocompatibility and cytotoxicity of CNTs used in living cells. Some scientists believe CNTs to be a biocompatible material and have shown interest in developing various biological applications, whereas other believe it to be toxic material and do not advocate for its biological application. Biocompatibility of CNT has been favorably tested [51–54] for neural application. Nevertheless, considering our body is mainly built of carbon, it is difficult to assume that carbon in inert form can be toxic to the human body. Still, it is necessary to use CNT

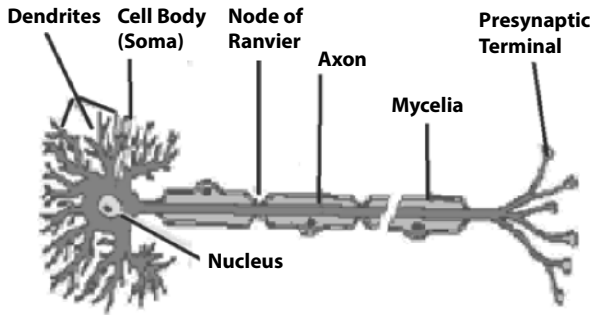

Figure 1.11 Schematic diagram of a nerve cell or neuron.

with caution for biological application. Since nanotechnology deals with materials of nanometric size, the possibility of its applications in medicine for treating neurodegenerative diseases are enormous. Nanomedicine has especially contributed to the understanding of many neurologic diseases and has played a critical role in their diagnosis and therapy. Some of the special properties of CNTs that make them a suitable material for neurodegenerative disorder therapy are:

(i) Their very high mechanical strength, high aspect ratio and stiffness as well as high flexibility; hence, CNTs can be used for building penetrating electrodes in neural prostheses.
(ii) Since they have good electrochemical stability, the possibility of damaging the electrodes and introducing abnormalities in neural function and cell structure is minimal.
(iii) CNTs of varied morphology (such as flat nanostructured continuous mats, sparse electrically conductive networks, localized three-dimensional nanoporous bushes, columnar closely packed forests, and spiked localized bundles or single fibers) can be grown on different surfaces. This allows tailoring of the neural interface morphology to better mimic the neural tissue microenvironment and to enhance electrical coupling.
(iv) Chemical functionalization of CNT allows designing of appropriate functional electrode coupling down to the subcellular nanoscale.
(v) Neuroprotective properties are exhibited by many hydroxy fullerenes and CNTs. A neurodegenerative capacity is also seen in CNTs acting as a conduit, which can then be mixed with stem cells to repair neurons. McKenzie *et al.* [53] have further investigated the effects of varying diameter (60–200 nm) and surface energy of carbon nanofiber (CNF) on astrocyte (the cells largely responsible for the scar tissue formation seen with current implantable neural devices) proliferation and function. It was found that astrocytes preferentially adhered to and proliferated on CNFs with larger diameters and higher surface energies. The authors concluded that CNFs with diameters smaller than 100 nm show potential for neural applications because of a speculated reduction in neural scar tissue formation.

MWCNTs that were chemically functionalized with carboxylic acid, ethylenediamine, or poly-m-aminobenzene sulfonic acid (each holding a different ionic charge at physiologic pH) were each observed to provide a substrate for neurite extension [52]. It was concluded that neurite extension was loosely based on the ionic charge, with positively charged ethylenediamine MWCNT producing the most neurite extension. Neither the CNT patterned surfaces nor the functionalized MWCNTs demonstrated cytotoxicity toward neuronal cells. Recently, Mazzatenta *et al.* [55] developed a SWCNT-neuron hybrid system and demonstrated that CNTs can directly stimulate brain circuit activity. Mattson *et al.* [56] used CNTs to serve as substrates for neuronal growth.

It can be concluded that since CNTs possess a high tensile strength, are ultralightweight, and have excellent chemical and electronic properties and thermal stability, plus also possess semi-metallic and metallic conductive properties and distinct electronic properties, they can be an excellent candidate that can be used as an artificial microdevice to replace the function of impaired nervous systems or sensory organs. Such neural interface implants could help to increase the independence of people with disabilities by allowing them to control various devices with their thoughts. Hybrid SWCNT neurons that can directly stimulate brain circuit activity are being investigated as a future neuroprosthetic device for patients suffering from neurodegenerative disorders.

1.2.5 Dendrimers

The word "dendrimer" comes from the Greek word "dendron" which means "tree." Dendrimers are also referred to as arborols/cascade molecules. They are composed of highly branched three-dimensional molecules of nano size. Dendrimers have an interior core, surrounded by an interior layer composed of repeated units radically attached to the core and exterior layer (terminal functionality) attached to interior generations (Figure 1.12). The properties of dendrimer that makes it highly suitable as a carrier for targeted drug delivery with controlled release of drug are: (i) Monodispersity, (ii) Nanoscale size and shape, (iii) Viscosity, (iv) High aqueous solubility, (v) High solubility in non-polar solutions, (vi) Non-crystallinity, (vii) Low glass temperature, and (viii) Low compressibility.

Dendrimers are synthesized by

(i) Divergent method, i.e., dendrimer is grown from core to periphery; here core molecules react with monomer molecules having two dormant and one reactive group.

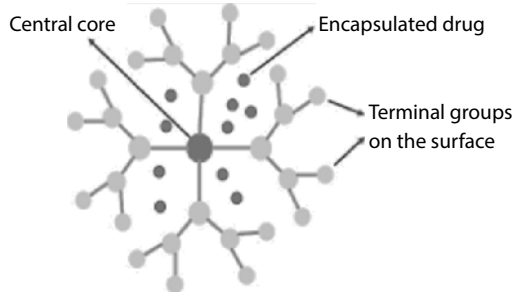

Figure 1.12 Schematic diagram of basic structural arrangement of dendrimer.

(ii) Convergent method, i.e., dendrimer grows starting from end group and progressing inwards. During the process the small molecules come together and reaction proceeds inward. Eventually the molecules become attached to the core.

1.2.5.1 Types of Dendrimers

The first polyamidamine (PAMAM) dendrimer synthesized in 1985 by divergent method was quite effective. Surface amine residues on PAMAM dendrimers bind to the phosphate backbone of nucleic acids through charged interactions are used in gene therapy. G6-7 PAMAM dendrimers are used for gene transfection; these dendrimers are typically 6–10 nm in length.

Another popular dendrimer is *Radially layered poly(amidoamine-organosilicon) (PAMAMOS) dendrimer* consisting of hydrophilic, nucleophilic polyamidoamine interiors and hydrophobic organosilicon exterior; and *Poly(propylene imine) (PPI) dendrimer* having primary amines as end groups and the dendrimer interior consisting of tertiary tris-propylene amines.

The main application of *Tecto-dendrimer* is in diseased cell recognition, diagnosis of disease, drug delivery, etc.

Simple PAMAM-drug complexes affect a broad spectrum of cells upon introduction to a living system; whereas PAMAM derivatized with folic acid is preferentially taken up by cancer cells, which are known to overexpress the folate receptor on their surfaces. Attaching additional treatment methods along with the folic acid, such as boron isotopes, cisplatin and methotrexate, have proven to be enhancing the navigating molecule.

Fréchet-type dendrimer is based on poly-benzyl ether hyperbranched skeleton. It has carboxylic acid groups as surface groups, which serve as good anchoring points for further surface functionalization. They also act as polar groups to increase the solubility of this hydrophobic dendrimer.

Amphiphilic dendrimers have a hydrophobic interior core and hydrophilic exterior. Hence, they are good carriers for drugs with poor solubility.

Drugs are loaded on the dendrimers either by simple encapsulation or by electrostatic interaction, i.e., the high density of functional groups like amine or carboxyl on surface of dendrimer have potential application in enhancing the solubility of hydrophobic drugs by electrostatic attractions. Another drug loading is done by covalent conjugation; here, the presence of large functional groups on the surface of dendrimers makes them suitable for covalent conjugation of numerous drugs with relevant functional groups.

Unloading or release of drug from dendrimers are (i) by *in-vivo* degradation of drug dendrimer, or (ii) due to change in physical environment such as change in temperature or pH.

1.2.5.2 Applications of Dendrimers

Dendrimers are used for various applications:

1. **As Blood Substitute:** The stearic bulk surrounding a hememimetic center significantly slows degradation compared to free heme. Santos *et al.* [57] have shown that polyglycerol dendrimers interact with human serum albumin (HAS) due to hydrophobic forces that play a major role in stabilizing the HSA–PGLyD complex.
2. **As Sensors:** Cadmium-sulfide/polypropylenimine tetrahexacontamine dendrimer composites are used as sensor to detect fluorescence signal quenching. Wang *et al.* [14] have developed a zwitterionic dendrimer encapsulated very small and highly stable gold nanoparticles (Au-G5MC NPs) in complex medium for highly sensitive and selective colorimetric detection of glucose, which showed peroxidase-like property that catalyzed oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of H_2O_2, producing a blue color product (oxTMB). This peroxidase-like reaction follows a typical Michaelis-Menten kinetics. The Km towards TMB exhibits lower value (0.194 mM) than that of horseradish peroxidase (HRP, 0.434 mM).

The linear concentration range of this method was from 14 μM to 166 μM with the detection limit down to 3.8 μM. More importantly, the detection was not interfered with by proteins due to the single zwitterionic layer on the Au-G5MC NPs surface.

3. **As Solubility Enhancers:** Dendrimers have hydrophobic core and hydrophilic outer surface, which enhances solubility of poorly soluble drugs by forming cascade and nonskid-chain-like synthesis of covalent and noncovalent complexes with drug molecules.
4. **For Gene Transfection:** Dendrimers are non-viral gene transfer agents, which enhance transfection by endocytosis.
5. **To Encapsulate Metal Nanoparticles:** Poly(amidoamine) dendrimers have tertiary amine group at the branching point. Metal ions are introduced into aqueous solution of dendrimer and metal ions form complex with the lone pair of electrons present at the tertiary amines. The ions are then reduced to zerovalent state to form nanoparticles that are encapsulated within the dendrimer.
6. **As Nanodrugs:** Poly(lysine) dendrimers with sulfonated naphthyl group are antiviral. PPI dendrimers with tertiary alkyl ammonium groups attached to the surface are antibacterial and Chitosan-Dendrimer hybrids act as antibacterial agents.
7. **As Hydrogel for Ocular Drug Delivery:** Crosslinked networks in dendrimers increase in volume in aqueous solution. Adding PEG groups to dendrimers extends their application to cartilage tissue production and seals ophthalmic injuries. Drug attached to the dendrimers efficiently delivers the drug to the eyes.
8. **For Pulmonary Drug (Enoxaparin) Delivery:** Dendrimers have been used for pulmonary delivery of enoxaparin. G2 and G3 positively charged PAMAM dendrimers form complex with enoxaparin and increases bioavailability of drug.
9. **For Transdermal Drug (Indomethacin) Delivery:** PAMAM dendrimers complex with NSAID'S improves drug permeation through the skin as penetration enhancers.
10. **For Active/Passive Targeted Drug Delivery:** Dendrimers are used for both active and passive targeted drug delivery to cancer cells.

11. **To Mimic Structure of Endostatin:** Dendrimers which mimic structure of endostatin exhibit antiangiogenic activity. Angiogenesis is an important process for tumor growth initiated by angiogenic factors. These factors bind to receptors on endothelial cells with dependence on heparin. Endostatin binds to heparin and prevents angiogenesis.
12. **As Carriers/Scaffolds for Diagnosis and Therapy:** For example, 5-nm size dendrimers are used for MRI contrast agents; highly branched dendrimers are used for tissue engineering applications, crosslinking agents, modulators of surface charge and surface chemistry and in scaffolds that mimic natural extracellular matrices.
13. **For Boron Neutron Capture Therapy:** Dendrimers are applied in boron neutron capture therapy for cancer patient. It is injected with boron attached to dendrimer, which migrates to cancerous cells, and then after irradiation with neutral beam of low-energy neutrons it generates alpha particles which destroy tumor cells.
14. **To Develop Vaccine:** Efforts are underway to develop vaccine by using dendrimers as carriers for small antigens, making it possible to prepare multimeric antigenic conjugates.

Among the nanoparticulate carriers, dendrimers have tremendous potential in the applications involving multifunctional nanoparticulate systems combining targeting, imaging, diagnostics, and therapy. Thus, this multifunctional, unique nanoparticulate carrier has the potential to detect diseases, deliver medications, and monitor the ability to change the current scenario of cancer research and diagnosis in real time.

Already there are products on the market that are based on dendrimer that have been approved by the FDA. For example, (a) Stratus® CS Acute Care™ (Dade Behring) for cardiac diagnostic testing, (b) SuperFect™ (Qiagen) gene transfection agent for a broad range of cell lines, (c) VivaGel® microbicide and anti-HIV is also currently in Phase 3 clinical trial, and (d) Poly(propylene imine) dendrimers are available under the name Astramol™.

1.2.6 DNA

Limitations in parasitological diagnosis, such as the presence of some morphologically indistinguishable parasites or parasites hidden in the host tissues, and also low sensitivity, led to the need to develop more sensitive

and specific new devices. Thus, evolved three molecular methods, i.e., biochemical immunological using antibodies and DNA (deoxyribonucleic acid) based. DNA has been used for fabrication and construction of nanostructures and devices. Because DNA is a genetic material with a molecule that is well programmed for self-assembly, it has high physicochemical stability. The high mechanical rigidity of short double helixes allows them to behave like a rigid rod spacer between two tethered functional molecular components on both sides. Attention is being paid to the use of DNA as a nanoscale tool for various applications such as biosensing, labeling, targeted imaging, cellular delivery, diagnostics, therapeutics, theranostics, bioelectronics, and biocomputing. DNA is now being used for the fabrication of nanostructures, i.e., for (i) the fabrication of artificial networks consisting of native DNA, (ii) the attachment or integration of DNA onto solid state surfaces, and (iii) the formation of metal or semiconductor nanoparticle assemblies along DNA.

The self-assembly property of DNA is being utilized for DNA-based metal nanoparticle organization by (i) electrostatic binding of positively charged colloids to the negatively charged DNA, (ii) specific binding of colloids to DNA via chemical bonds, which is achieved either by DNA and/or colloids being functionalized with thiol groups, with biotin-streptavidin complexes or colloids carrying oligonucleotides that can be hybridized with single-stranded parts of DNA, (ii) direct growth of nanoparticles along the DNA molecules by the formation of nucleation sites along DNA molecules followed by metal or semiconductor deposition by chemical reduction of the corresponding salt.

DNA is used for assembling metals or semiconducting particles to construct metallic nanowires and functionalized nanotubes. DNA-based molecular diagnostics are PCR (polymerase chain reaction) and DNA probes.

For parasite diagnosis, PCR is used to amplify target sequences and increase sensitivity. The ribosomal DNA/RNA and random primer amplification (RADP) PCR are highly sensitive but not good for identifying closely related species. Whereas, specific sequences of genomic DNA are highly specific for single species but not sensitive enough.

The genomic DNA constant has unique parasites and hosts DNA sequences. They are very sensitive and need small biopsy. Genomic DNA can be used to design probes with high specificity to detect single parasite species; or less specific to detect a group of parasites. One of the disadvantages of DNA probes is that they do not distinguish between dead and living parasites.

A potential DNA barcode for animal species diagnosis is the cytochrome c oxidase subunit I gene (COI) potential barcode. Mitochondrial DNA has signature for animal species, Chloroplast DNA has signature for plant species; whereas, for flowering plants, the nuclear internal transcribed spacer region and the plastid trnH-psbA intergenic spacer are potential DNA barcode. rRNA genes are the ideal markers for microbial identification. For fungal diagnostics, Microsatellite DNA sequences are the marker. However, the major challenge is in developing a method that does not rely on polymerase chain reaction or other target amplification systems that require additional instrumentation and reagents.

By conjugating the unique physical properties of QDs or wires with the remarkable recognition capabilities of DNA a very miniaturized device can be made for biological, electronics and optical applications, including biosensors and probes (Figure 1.13). Conjugates of self-assembled DNA-streptavidin have also been used as nanobiosensors. DNA-based nanocarriers are also prepared for gene delivery, which is vital for gene therapy. DNA aptamers are also being researched for providing specific recognition capabilities against many non-nucleic-acid targets (from ions to full protein and cells). Moreover, for appending DNA to inorganic or polymeric nanoparticles, purely DNA-based nanoparticles have been found to be an excellent assembly platform and have applications in sensing, imaging, and immunotherapy.

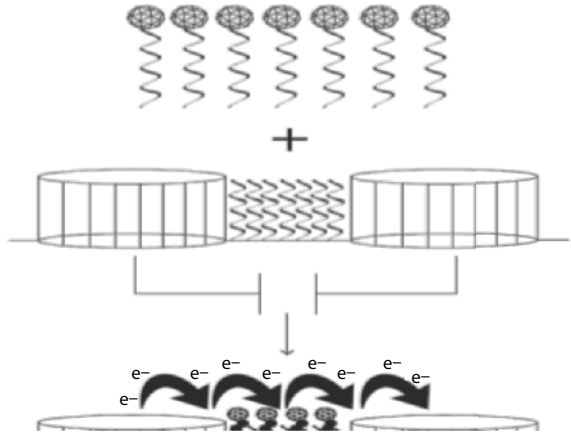

Figure 1.13 Schematic illustration of detecting DNA hybridization signal by integrating the electrical conductivity of nanoparticles using an electrical detection system. (Source: Khalid M. Abu-Salah *et al.; Journal of Biomedicine and Biotechnology* Vol. 2010, Article ID 715295) [Permission request sent to the author].

1.2.7 Nanocrystals/Quantum Dots (QDs)

Nanocrystals are particles having at least one dimension < 100 nm, with single or polycrystalline arrangement. Nanocrystals of 2–10 nm diameter exhibit properties like quantum dots (QDs). QDs are semiconductor nanocrystals exhibiting properties that are intermediate between the properties of bulk semiconductors and discrete molecules. For example, they display quantized energy levels and size-dependent fluorescent properties. The fluorescent properties of QDs make them uniquely advantageous for *in-vivo* targeted fluorescence imaging, traceable delivery, and therapy. The QDs that are being used for these purposes are of group II–VI (e.g., CdSe); but CdSe being toxic heavy metals, they have limited *in-vivo* applications. Carbon QDs, Graphene QDs and Silicon QDs are biocompatible semiconductors and are mostly being used in healthcare.

With the advancement in nanocrystal/QD-based biocompatible material, a new field has evolved, i.e., *Drug Nanocrystals*. They are nanometer size pure solid drug particles and are different from polymeric nanoparticles, which consist of a polymeric matrix and an incorporated drug; but drug nanocrystals do not consist of any matrix material. The advantage of producing powder of drug-nanocrystals is that since it increases solubility of poorly soluble drugs without using any surfactants, the drug powder can be incorporated into a variety of dosage forms and is suitable for oral drug delivery. Moreover, since it is a cost-effective process and stable because it uses GRAS (*Generally Recognized As Safe material accepted by the US FDA*) stabilizing agents against agglomeration, such as lecithin, Tween 80, poloxamer 188, sodium glycocholate or low-molecular-weight polyvinylpyrrolidone (PVP), it also makes the nanocrystals less susceptible to electrolytes in the body. Drug nanocrystals are synthesized by:

1. Bottom-up approach using precipitation method. Here, the drug is dissolved in a solvent which is then added to a water with continuous stirring to precipitate the nanocrystals. The drug-nanocrystals are then removed by filtration and air-dried.
2. Another bottom-up approach uses the cryo-vacuum method. In this method an aqueous, organic, or aqueous–organic cosolvent solution; aqueous–organic emulsion; or drug suspension, is atomized into a cryogenic liquid nitrogen at -196 °C to produce frozen nanocrystals which are then lyophilized at -22 °C to obtain free-flowing, very pure

nanocrystals powder, without using surfactants or harmful reagents.

3. Top-down approach using the wet milling method at < 40 °C in milling media, (dispersion medium "normally water," stabilizer, and the drug). Either tumbling ball mill or a stirred media mill is used for milling from hours to days depending on the drug's hardness.

   Top-down approach using high-pressure homogenization in either water (DissoCubes®) or in non-aqueous media such as liquid PEG or oils (NanoPure®). The particles are reduced by cavitation and shear forces. The size of the particles obtained by this method depends on the nature of the drug, the pressure applied and the number of homogenization cycles. The advantages of using drug-nanocrystals are that they have enhanced oral bioavailability, rapid onset of action, improved dose proportionality, and there is reduction in fed/fasted effects and side-effect.

1.2.7.1 Applications of Nanocrystals/Quantum Dots (QDs)

Inorganic fluorescent semiconductor nanoparticles, such as QDs or nanophosphors, are being investigated for their use in increasing the sensitivity, specificity, biological labeling, biological diagnosis, and the possible analysis of multiple analytes. For *in-vitro* applications, they are used as markers for biomolecules because of their optical properties for several diagnostic assays, such as PCR, for the construction of biochips or for multiplexing, conversely to the traditional dyes used in everyday clinical practice.

Erogbogbo *et al.* [58] demonstrated the use of encapsulated biocompatible SiQDs in multiple cancer-related *in-vivo* applications, such as tumor vasculature targeting, sentinel lymph node mapping, and multi-color NIR imaging.

In another attempt. Murata and Tabata [59] prepared gelatin nanospheres incorporating quantum dots and iron oxide nanoparticles for multimodal cell imaging. This multimodal probe can simultaneously visualize cells by optical and magnetic resonance (MR) imaging modalities. Gelatin nanospheres incorporating QD and IONP were treated with octa-arginine (R8) of a cell-penetrating peptide. When incubated with normal human articular chondrocytes, it was efficiently internalized into the cells. This QD complex exhibited cytotoxicity at the R8 concentration of 320 μM.

1.2.8 Nanoparticles as Diagnostic Tool

Nanoparticles have found entry into healthcare (diagnostics, drug delivery, therapy, tissue engineering, biosensors, imaging, measuring, manipulating matter at the nanoscale, helping in detection, prevention, and treatment) as they possess some unique properties, viz. (i) higher chemical reactivity, (ii) increased mechanical strength, (iii) faster electrical and magnetic responses owing to their surface area to volume ration, (iv) ability to attach to biomolecules, allowing detection of disease biomarkers at very early stage, and (v) the ability to readily interact with biomolecules because of their very small size, hence gaining access to many areas of the body by passing through intracellular spaces. Different types of nanoparticles have been used as a tool for diagnostics, which include inorganic nanoparticles such as SPIONs, gold nanoparticles, polymeric nanoparticles, etc.

1.2.8.1 Inorganic/Metal Nanoparticles

By virtue of the fact that transition metals, such as gold, silver, and copper, have been known to possess many intriguing properties for ages, they continue to be exploited for their magnificent and lustrous attributes, leading to an avalanche of applications. Metals at bulk scale possess intriguing properties such as ductility, malleability, metallic luster, specific heat, magnetism, electrical conductivity, etc. These properties are simply due to the band structure formed from one mole of atoms (6×10^{23}) stuffed into a crystal lattice space. When such bulk metals are reduced to nanoscale size in such a way that the band structures are transformed into discrete energy levels, then certain remarkable properties become accentuated. This reduction in size leads to overwhelming response in the quantum size-dependent properties of nanoparticles such as:

(i) **Melting Point:** As the size of the particle decreases, there is decrement in the melting point of nanoparticles (Figure 1.14).

(ii) **Color:** The classical definition of color is partial absorption of light by electrons in matter leading to the visibility of complementary part of light. Light is totally reflected by the high density of electrons on smooth surfaces of metals, resulting in a silvery appearance and not showing any color; whereas, finely dispersed metals have very large surfaces at which light is totally absorbed through repeated reflections. Nanometals exhibit a size-dependent color.

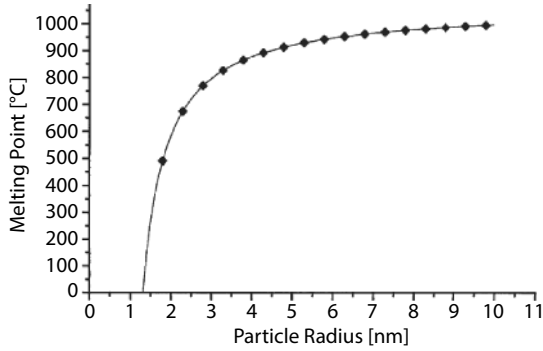

Figure 1.14 Relation between the size of gold nanoparticles and their melting point.

(iii) **Magnetic Properties:** The magnetic properties of nanometals are distinctly different from that of bulk material. With reduction in size of ferromagnetic nanoparticles, they become unstable below a certain size, because there is an increase in the surface energy, which provides sufficient energy for the domains to spontaneously switch polarization directions and become paramagnetic. But this transformed paramagnetism behaves differently from conventional paramagnetism and is referred to as superparamagnetism. In other words, ferromagnetism of the bulk materials disappears and gets transferred to superparamagnetism in the nanoscale due to high surface energy. Quantum effects begin to dominate the behavior of matter at the nanoscale—particularly at the lower end—and affect the magnetic behavior of materials.

(iv) **Electrical Properties:** The electrical conductance for the agglomerated metallic particles or the deposited nanosized thin films depends on the filling factor of the connected and empty spaces. The electrical conductivity of nanomaterials can be manipulated to have desired applications.

(v) **Thermal Properties:** Plasmonic nanoparticles, like gold nanoparticles, can convert the absorbed light into heat via a series of nonradiative processes. This property is also size- or shape-dependent.

Based on the abovementioned properties, the metal nanoparticles are used for various applications, such as diagnostics, biomedicine, targeted

delivery of drugs, tissue engineering, and imaging labels, by overcoming the many biological, biophysical, and biomedical barriers.

Functional nanomaterials have recently attracted strong interest as potential drug delivery vehicles or diagnostic tools, but also as optical nanomaterials. Many nanoparticles and nanoconjugates are already in the pipeline for therapeutics for medical applications (Table 1.1).

Table 1.1 Some nanoparticles-based therapeutics for medical applications.

Study phase	Product	Description	Use	Manufacture
Preclinical	MRX-2	Nanoparticle preparation Camptothecin	Malignant tumors	ImaRx Therapeutics
Preclinical	Targeted Nanothera-peutic	NIT with polymer coated iron oxide magnetic particle	Solid tumors	Triton BioSystems
Preclinical	AuroLase™	Gold nanoshell	Head & neck cancer	Nanospectra Biosciences Inc.
Phase1	INGN 401	Nanoparticle formulation of tumor suppression gene	Lung cancer	Introgen Therapeutics
Phase2	VivaGel	Dendrimer-based microbicide gel	HSV prevention	Starpharma Pty Ltd
Phase2	MRX-815	Nanobubble technology	Treatment of intravas-cular clot	ImaRx Therapeutics
Phase 1 & 2	Cyclosert-Campto-thecin	β-cyclodextrin polymer	Solid tumor	Calando Pharmace-uticals

1.2.8.1.1 Magnetic Nanoparticles

Nanoparticles of magnetic metals and oxides have attracted great interest in recent years because of their unique physical and chemical properties, especially maghemite (γ-Fe_2O_3). They are being used for magnetic resonance imaging contrast agent, information storage, and macroscopic quantum tunneling associated with size quantization and electronic quantum confinement effects.

In magnetism, the north pole and south pole of a magnet are fictitious. The simplest structure in magnetism is a magnetic dipole, i.e., a north pole and south pole that cannot be separated from each other. On the basis of the Domain theory, Faraday classified different substances into three groups: Dimagnetism, Paramagnetism, and Ferromagnetism (Figures 1.15 and 1.16).

Dimagnetism is an unmagnetized state with zero magnetic dipole moments. In diamagnetic substances, the magnetic moments of the electrons in an atom cancel each other out; hence, the resultant magnetic moment of the atom is zero. In a magnetic field, acquired dipole moment

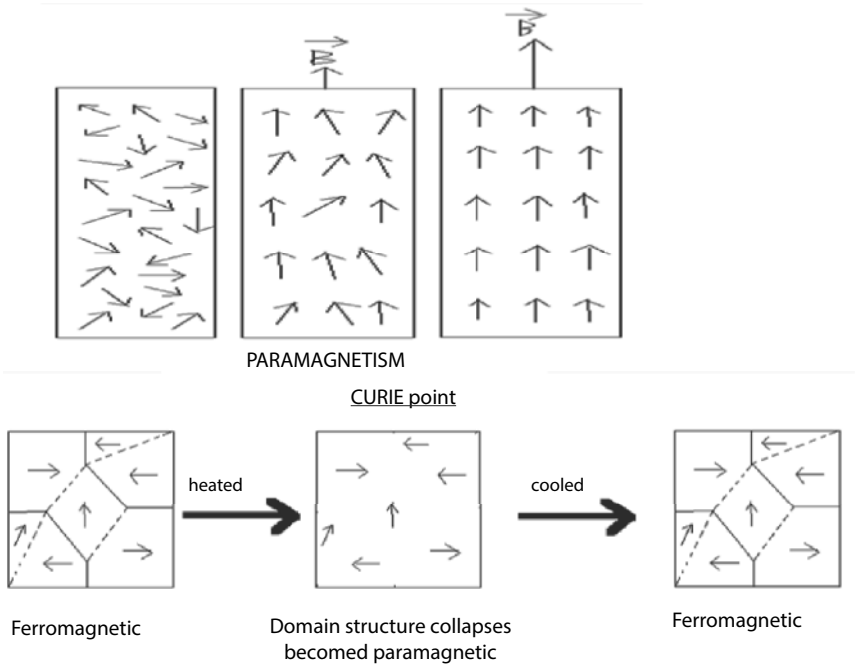

Figure 1.15 Curie point.

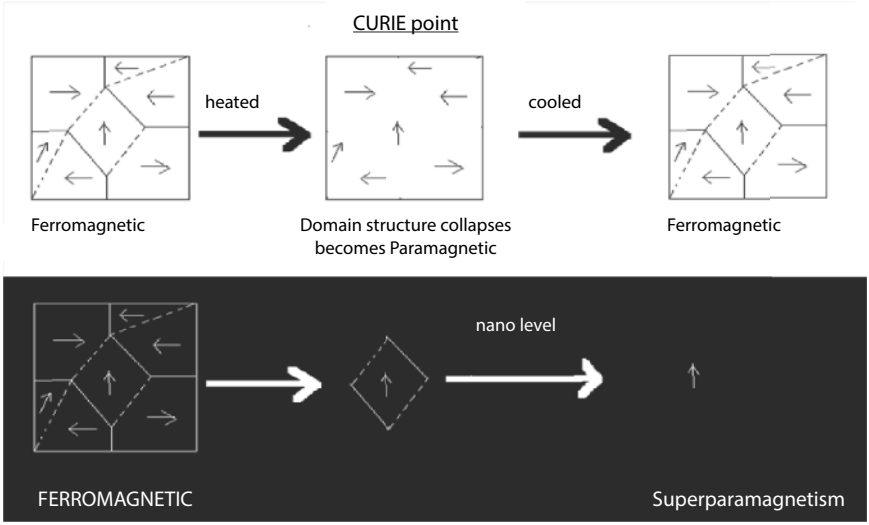

Figure 1.16 Superparamagnetism.

is at right angles to the direction of the magnetic field, e.g., copper, water, alcohol, hydrogen, etc.

Paramagnetism is also an unmagnetized state, but it has weak magnetic dipole moments. In paramagnetic substances, the magnetic moments of the electrons in an atom do not all cancel out, so that the atom, as a whole, has a weak magnetic moment. In a magnetic field, they align themselves in the direction of the magnetic field, e.g., aluminum, platinum, titanium, oxygen, etc.

Ferromagnetism is an unmagnetized state with magnetic dipole moments. In a magnetic field domains align themselves in the direction of the magnetic field; already aligned domains that grow in size are iron, cobalt, gadolinium, nickel, etc.

Superparamagnetism is when a ferromagnet or ferrimagnet is sufficiently small, and acts like a single magnetic spin that is subject to Brownian motion. Its response to a magnetic field is qualitatively similar to the response of a paramagnet, but much larger.

Different types of magnetic nanoparticles (MNPs) are promising nanomaterial for targeted therapy by conjugating peptides or antibodies on the surface of MNP and also for imaging of malignant brain tumors. They are a versatile diagnostic tool as they are manipulated using external magnetic field. This "action at a distance" phenomenon combined with intrinsic penetrability of magnetic field into human tissue enables their detection

in vivo using MRI. Many MNPs are used as contrast agent for MRI, such as magnetic iron oxide nanoparticles (MPIOs) composed of Fe_2O_3, γ- Fe_2O_3 (maghemite) or magnetite crystals < 20 nm diameter.

Superparamagnetic iron oxide nanoparticles (SPIONs), or Fe_3O_4, are extensively used to enhance contrast in MRT monitors anatomical, physiological, and molecular changes during the evolution of a disease or treatment. SPION has also found application in targeted destruction of tumor tissue through hyperthermia, targeted and controlled delivery of drugs, drug monitoring, and for magnetic resonance imaging (MRI).

SPIONs (Figure 1.17) are useful for cell tracking and calcium sensing. A family of calcium indicators for MRI is formed by combining a powerful SPION-based contrast medium with the calmodulin (a calcium-sensing protein) and its target. To reveal small and otherwise undetectable lymph node metastases, 2–5 nm SPION is used in conjunction with MRI. Ultrasmall SPION enhances MRI for imaging cerebral ischaemic lesions. A dextran-coated iron oxide nanoparticle enhances MRI visualization of intracranial tumors for more than 24 hours. SPIONs used in diagnostics are made of an iron oxide core and coated by either inorganic materials like silica or organic materials such as phospholipids, or natural polymers such

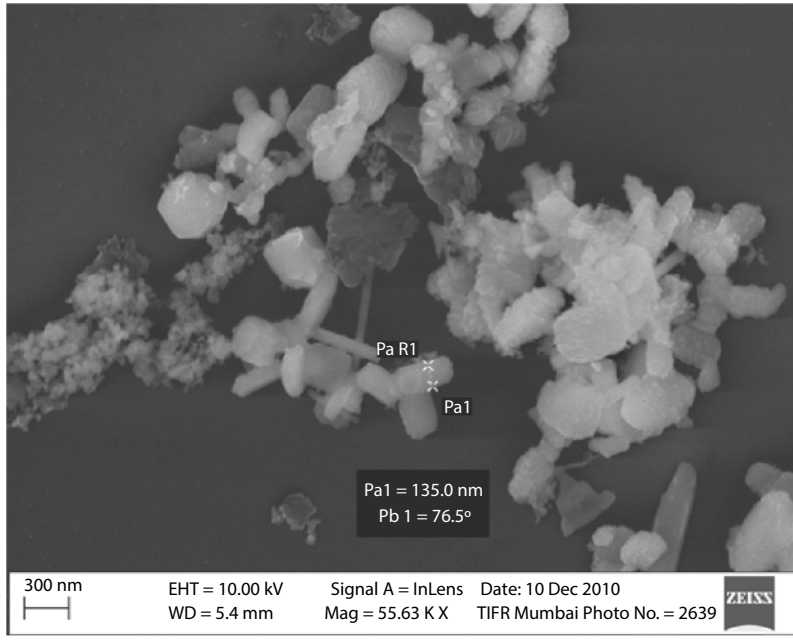

Figure 1.17 SEM image of SPION.

as dextran or chitosan. SPIONs are a versatile agent for early diagnosis of cancer, atherosclerosis and other diseases. In a nutshell, iron oxide NP improves MRI images of cancer tumors. The nanoparticle is coated with a peptide that binds to a cancer tumor, once the nanoparticles are attached to the tumor the magnetic property of the iron oxide enhances the images from the MRI scan.

Huang *et al.* [60] have shown that manganese oxide (MnO) nanoparticles coated with human serum albumin (HSA) is a prominent MRI T1 contrast agent.

1.2.8.1.2 Gold Nanoparticles

The inception of metal nanotechnology, particularly gold colloids, can be traced back to at least the 5th century BC for its use in making ruby glass and providing a reddish tinge to ceramics. Similar types of colloidal gold suspensions were even prepared by ancient alchemists by the reduction of gold salts with all kinds of organic substances, including urine! Similar mixtures were used by the Hindu chemists for Ayurveda, the ancient system of Indian medicine [61]. Gold nanoparticles have emerged as attractive nanomaterial for biological and biomedical applications because of their physical and chemical properties. The gold core is inert and essentially non-toxic to cells. The particles absorb and resonantly scatter visible and near-infrared light upon excitation of their surface plasmon oscillation. The plasmon resonance band can be tuned over a wide spectral range by changing intrinsic parameters such as the material (bi-metallic or hybrid particles), the size, or the shape (sphere, rod, cube, triangle, cage, etc.).

There is widespread interest in the optical properties of precious metal nanoparticles of unusual shape such as nanorods, nanotriangles, nanoshells, and nanocaps. Gold nanoparticles of these shapes might have applications in various fields for detection and treatment. Unlike solid gold nanospheres (which manifest only a transverse plasmon resonance at ~525 nm), these new shapes exhibit additional localized surface plasmon resonances (SPR) whose sensitivity depends on their geometry.

Gold nanoparticles having an anisotropic shape; for example, rods are of interest due to their unique properties. These particles have two distinct plasmon bands; one as a result of light being absorbed transversely (short axis), while the other due it being absorbed along the long axis, i.e., longitudinally. As the rod length increases, so does the plasmon frequency. This means that nanorods may be modified to have strong absorption, sparing a wide range of wavelength, i.e., visible, near infrared region. Simply by changing the rod length it also possesses different chemical affinities

for different crystallographic facets, making possible controlled assembly. Gold nanorods are shown to link fairly end to end under suitable conditions. This is owing to the differences in gold atom packing on the end faces when added to side face packing, direct plasmonic coupling resulted in controlled frequency shift.

Gold nanoparticles and gold nanoshells can be used with very high sensitivity for the detection of DNA and proteins [62, 63] to label DNA or protein molecules (including antibodies), so as to bind them to respective targets.

Small sections of DNA can be attached to gold particles smaller than 13 nm in diameter. These attach onto a sensor surface only in the presence of a complementary target. If a patterned sensor surface of multiple DNA strands is used, the technique can detect millions of different DNA sequences simultaneously. Gold nanoparticles are especially effective labels for sensors because of a variety of analytical techniques that can be used to detect them. Green *et al.* [64] proposed that by using SPR as an optical technique the refractive index of very thin layers of material adsorbed on a metal can be measured; hence, offering real-time *in-situ* analysis of dynamic surface events. Therefore, it can define rates of adsorption and desorption for surface interactions. The interaction of locally adjacent gold nanoparticle labels that have bonded to a target produces changes in optical properties, such as change in color of the colloidal gold, that can be used for detection.

1.2.8.1.3 Silver Nanoparticles

Stability of metal nanoparticles, such as gold and silver, plays a pivotal role in their clinical and para-clinical applications, viz. drug delivery, hyperthermia or antimicrobial therapy. In a solution of colloidal dispersion, stability is governed by a plethora of factors. Silver nanoparticles exhibit striking colors from light yellow to brown. The colors are size-dependent.

Taton *et al.* and Cao *et al.* [8, 65] have shown that using the Raman spectroscopy detection method, silver can be involved in the visualization process. Gold nanoparticles coated with silver shells and silver-coated gold particles (40–100 nm in size) exhibit strong light-scattering properties that can be detected by standard dark-field microscopy with white light illumination [66, 67].

1.2.1.8.4 Other Metal Nanoparticles

There are some other metal nanoparticles that are being research for their use in diagnostics such as:

- *Cadmium:* Cadmium sulfide and cadmium selenide quantum dots have revolutionized the facile band-gap engineering of materials. Biological systems, especially bacteria, are trained to transform toxic cadmium ions into insoluble nontoxic cadmium sulfide nanoparticles.
- *ZnS:* Zinc sulfide nanocrystals synthesized by yeast *Candida glabrata* and *Schizosaccharomyces pombe* find their application in the treatment of specific dermatological conditions. These nanomaterials produce free radicals that can destroy the psoriatic cells upon UV irradiation *in situ.* Further damage to the normal skin could then be prevented by the free radical scavengers such as glutathione (GSH), which has been proved to be a very important ingredient in the engineering of size-controlled production of nanocrystallites, and is shown to be the biomolecules that cap ZnS nanocrystals synthesized by yeast *C. glabrata* and *S. pombe*.

1.2.8.2 Polymeric Nanoparticles (PNPs)

Polymeric nanoparticles are solid colloidal particles ranging in size from 10–1000 nm (1 μm). They are extensively used for drug delivery by dissolving, entrapping, encapsulating or attaching to a nanoparticle matrix. Because these systems have very high surface areas, drugs may also be adsorbed on their surface. Their nanometer size promotes effective permeation through cell membranes and stability in the bloodstream. They are prepared either as nanospheres or nanocapsules. Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery such as tissue engineering. Polymers used for synthesis of polymeric nanoparticles are:

1. *Natural hydrophilic Polymers* are proteins (gelatin, albumin, lectin, legumin, vicilin) and polysaccharides (alginate, dextrin, chitosan, agarose, pullulan);
2. *Synthetic Hydrophobic Polymers* are pre-polymerized (poly(ε-caprolactone), PLA, PLGA, polystyrene) and polymerized in the process (poly (isobutyl cyanoacrylate), PBCA, PHCA, poly(methyl methacrylate) (PMMA)). The desired characteristics of a polymeric nanoparticle is:
   - Particle size and size distribution,
   - Surface area,
   - Surface chemistry,

- Surface coating and porosity,
- Hydrophilicity and surface charge density,
- Purity and quality,
- Stability,
- % Entrapment efficiency, and
- % PNP yield.

Polymers are gaining importance in developing a functional nanosystem as an intelligent tool for diagnostics, prognostics, and controlled and sustained delivery of protein, peptide, pDNA, and other therapeutic agents to specific targets (tissue, cell, and intracellular). This is done by incorporating adsorption or covalent coupling of various polymers, natural polymers like carbohydrates, endogenous substances/ligands, peptides, proteins, nucleic acids, and polysaccharides to the surface of nanoparticles. Functional polymeric nanosystems that are being extensively explored are polymeric micelles and liposomes. Polymeric nanoparticles offer advantages due to their biocompatibility, non-immunogenicity, non-toxicity, biodegradability, simple preparation methods, high physical stability, possibility of sustained drug release, and higher probability for surface functionalization. Polymeric nanoparticles can be grouped as (i) stealth, (ii) polysaccharide-decorated biomimetic, (iii) bioadhesive, and (iv) ligand-anchored functional polymeric nanoparticles (*f*-PNPs). Polymeric nanoparticles used in optical diagnostics for diagnostic imaging of cancer, biomarker analysis, and immunoassays, due to the progress achieved in the tailoring of their physicochemical properties. Some of the commonly used polymeric nanoparticles are biodegradable poly-€-caprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA) phospholipids; and natural *polymers* such as dextran or chitosan.

Stimuli-responsive polymeric nano-micelles are another addition to application of polymeric nanoparticles in diagnostics, such as mPEG-Hyd-PCL-CIN [(hydrazine bond, -Hyd-); cinnamic acid (CIN); P], mPEG-SS-PCL-CIN and MeTTMN-loaded nano-micelles that are used for photodynamic therapy (PDT) that is driven by cytotoxic reactive oxygen species (ROS) generated by photosensitizer (PS) upon light irradiation. This type of therapeutic has minimal invasiveness, *in-situ* workability, and high spatiotemporal precision. Some polymeric nanoparticle-based products that are now on the market are presented in Table 1.2.

Table 1.2 Polymeric nanoparticle (NP)-based products in the pipeline.

Company	Technology	API	Route of administration
Novavax, USA	Micellar NP	**Testosterone**	**S.C.**
BioAUiance, France	Poly butyl Cyanoacrylate	**Doxorubicin**	**I.V.**
American BioSciences, USA	Albumin-drug NP	**Paclitaxel**	**I.V.**
Wyeth Pharmaceuticals, USA	Drug NP	**Rapamycin**	**Oral**
BioSante, USA	Calcium Phosphate NP	**Insulin**	**Oral**

1.2.9 Nanorobotics

What is a robot? A robot is a mechanical or virtual artificial agent that is usually an electromechanical machine that is guided by computer program or electronic circuitry.

Types of robots are: (i) Mobile robots, (ii) Rolling robots, (iii) Walking robots, (iv) Stationary robots, (v) Autonomous robots, (vi) Beam robots, (vii) Virtual robots, and (viii) Remote control robots.

What is robotics? Robotics is the branch of technology that deals with the design, construction, operation, structural disposition, manufacture, and application of robots and computer systems for their control, sensory feedback, and information processing. Robotics encompasses the sciences of engineering, electronics, mechanics, and software. These technologies deal with automated intelligent machines that can take the place of humans in hazardous or manufacturing processes. Robotics is the technology used in designing intelligent machines built for a specific purpose and programmed to perform specific tasks.

What is a nanorobotic? The possibility of nanorobots was first proposed by Richard Feynman in 1959 in his talk, "There's Plenty of Room at the Bottom." The inventor of nanorobotics is Adriano Cavalcanti. His nanorobots were integrated as a model based on nano-bioelectronics for applications in environmental monitoring, brain aneurysm, diabetes, cancer, and cardiology. His advanced prototype provided a suitable integrated circuit approach using an effective wireless platform. Nanorobots are nanodevices that will be used for the purpose of maintaining and protecting the human body against pathogens. Nanorobots are microscopic devices on the scale of a nanometer. They are expected to work at the atomic, molecular and cellular level to perform both medical and other industrial tasks. These are very sensitive to the acoustic signal, hence, can be programmed using the sound waves to perform the specified tasks. These nanorobots can identify a particular harmful cell and try to quarantine it. They have rapid and high operational speed, fast and precise diagnosis, and can remain operational for years, decades or centuries.

Nanorobots are also known as nonoids, nanites, nanomachines and nanomites. Components of a nanorobot are: sensor, electrode, capacitor, membrane, actuator, translator, and catalysts. Designing involves constructing interior (vacuum environment) and exterior space (not affected by chemical liquids of our body). Techniques used are bottom-up, assembling structures atom by atom or molecule by molecule, deriving designs from biological models in which an electric motor is attached for its movement inside the blood vessels.

However, being a new developing field, there is a lack of knowledge; hence, dark-side and environmental hazards have to be taken care of. When these tiny nanorobots are implanted into our bloodstream to detect the cause of a fever; they can travel or swim through the vascular and even digestive system to the appropriate system and provide a dose of medication directly to the infected area. The energy required for swimming in the blood vessels or digestive system comes from using blood sugar as fuel.

Carbon (probably in the form of diamond or diamondoid nanocomposites) is one of the main elements comprising the bulk of a medical nanorobot because of their strength and chemical inertness. Other light elements that are being used in nanorobots are hydrogen, sulfur, oxygen, nitrogen, fluorine, silicon, etc., depending on the special purposes in nanoscale gears and other components. Swimming robots are made of polystyrene microspheres or microbeads. The method used in the microrobot is the bacteria propulsion method, in which the bacteria is propelled by rotating their flagella with high speed, and then the bacteria adhere to the microbeads. By rotating their flagella, the attached bacteria push forward by using different

chemicals that control the on/off motions of microrobots. These chemicals bind to the flagellar motor and restrict their movement. The chemicals used for this purpose are EDTA and copper ions. The Cu ions are used to stop their motion and EDTA (ethylene diamine tetra acetic acid) used to restart their motion. For propulsion of the microbeads by bacteria, the attachment takes place in two steps: (i) *Reversible*: In reversible attachment the bacteria adhere to the microbeads with van der Walls, acid-based interactions and electrostatic forces. These are weaker bonds; hence, this attachment is reversible. (ii) *Irreversible*: In irreversible attachment the bacteria are irreversibly attached to microbeads by forming strong bonds. Moreover, there are also speed controlling techniques using biochip and nubots.

Various applications of nanorobots that has been envisaged so far are*:*

- Breaking up blood clots;
- Fighting cancer by cancer detection and treatment;
- Removing parasites;
- Breaking up kidney stones;
- Treating arteriosclerosis (deposition of plaque along the walls of arteries) by breaking up the plaque in the arteries by releasing chemicals at the site;
- Nanorobots can be programmed to activate sensors and regularly measure the sugar/glucose levels in desired intervals of time as well as controlling it;
- Nanorobots are also programmed to emit a signal at specified times;
- Nanorobots can perform therapeutic and diagnostic functions such as ultrasound, biopsy, ans laser; and produce heat by retractable arm.

1.2.10 Nanoshells

Nanoshells are exceptionally biocompatible, as their behavior is almost identical to that of gold colloids. Their optical properties can be tuned by changing the size of the nanoparticles, which allows them to be used for development of immunoassays capable of simultaneous analysis of multiple antigens [68]. Nanoshells consist of concentric spherical nanoparticles with a dielectric core, usually consisting of gold sulfide or silica, surrounded by a thin gold shell. The relative thicknesses of the core and outer shell decides the optical resonance of gold to go into the mid-infrared region. Gold nanoshells could allow direct, rapid, and economically feasible analysis of whole blood samples by further manipulation of

the surface properties to expand the absorption wavelength range to the near-infrared, just above the absorption of hemoglobin and below that of water, thus avoiding the interference from hemoglobin and allowing direct analysis of whole blood [69]. Gold nanoshells also provide great sensitivity for the detection of DNA and proteins [70].

1.2.11 Nanowires

Nanowires are ultrafine wires or linear arrays of dots formed by self-assembly. They can be made from a wide range of materials. Semiconductor nanowires made of silicon, gallium nitride, and indium phosphide have demonstrated remarkable optical, electronic and magnetic characteristics (for example, silica nanowires can bend light around very tight corners). Nanowires have potential applications in high-density data storage, either as magnetic read heads or as patterned storage media, and electronic and opto-electronic nanodevices for metallic interconnects of quantum devices and nanodevices. The preparation of these nanowires relies on sophisticated growth techniques, which include self-assembly processes, where atoms arrange themselves naturally on stepped surfaces, chemical vapor deposition (CVD) onto patterned substrates, and electroplating or molecular beam epitaxy (MBE). The "molecular beams" are typically from thermally evaporated elemental sources.

1.2.12 Optical Tweezers

Optical tweezers are a revolution in micromanipulation. Optical tweezer and Nanotweezer are two different devices. Optical tweezers use light to trap, manipulate and position micron-size objects. Optical tweezer is an instrument which uses a focused laser beam to provide an attractive or repulsive force, depending on the index mismatch (typically on the order of pico-newtons) to physically hold and move microscopic dielectric objects. These tightly focused beams of light can trap micron-sized objects (Figure 1.18). It has been successfully used in studying a variety of biological systems. Dielectric objects are attracted to the center of the beam. The force applied on the object depends linearly on its displacement from the trap center. Optical tweezers can investigate pico-newton to femto-newton forces exerted on microscopic objects.

For biological specimens, Nd:YAG laser (1064 nm wavelength) is used because biological specimens are most transparent to laser wavelengths around 1000 nm. This assures very low absorption coefficient, thus minimizing damage to the specimen. Arthur Ashkin was awarded the Nobel

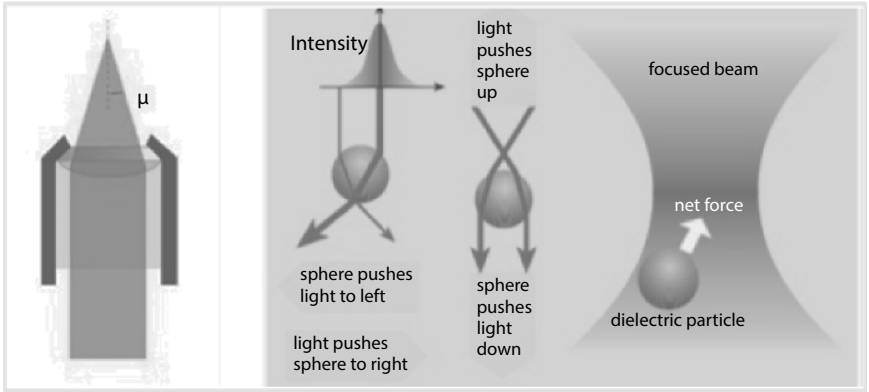

Figure 1.18 Trapping a particle with light.

Prize in Physics in 2018 at the age of 96 for his invention of Optical Tweezer. It is also known as single-beam gradient force trap. The use of a highly focused laser beam provides an attractive or repulsive force on the order of pico-newtons; these forces are used to physically hold and move microscopic objects in a manner similar to tweezers. An optical tweezer is composed of tight focusing beam; therefore, it requires high numerical aperture (NA) and magnification of 1000x.

$$N.A. = nSin\,(\mu)$$

n is refractive index of the medium between the sample and objective lens

$$\text{Using oil immersion } n \sim 1.3$$

$$\text{so N.A. } >1 \text{ is possible.}$$

Application of optical tweezer is the area where physics meets biology. The advantage of using optical tweezer is its non-contact and non-invasive working. Optical tweezer is based on the principle that light can exert forces on small dielectric objects. For this reason, it is often used to manipulate and study single molecules by interacting with a bead that has been attached to that molecule. DNA and the proteins and enzymes that interact with it are commonly studied in this way. This technique can be used in the differential diagnosis of tissue growths, calculi, cysts, abscesses, etc.

Guo and Li [71] have shown applications of single trap, dual-trap and multi-trap optical tweezers used for force management to study biophysical

problems such as mechanical deformation of cell membrane and binding energy between plant microtubule and microtubule associated protein. Moreover, possible application of the optical tweezer technique for tapping, transporting and patterning of metallic nanoparticles, which can be harnessed to the plasmon resonance property of these nanoparticles is also reviewed.

1.2.13 Serum Albumin

Albumin is the main protein (with a concentration of 3.5–5.0 g/dl) present in human blood plasma, also known as serum albumin because it is component of blood serum (Figure 1.19). It is water soluble, especially in water half saturated with salts, as it binds water, cations (such as Ca^{2+}, Na^{+} and K^{+}), fatty acids, hormones, bilirubin, thyroxine (T4), and pharmaceuticals (including barbiturates); its main function is to regulate the oncotic pressure of blood. It is also present in extracellular spaces. Albumin is also found in milk (α-lactalbumin) and egg white (ovalbumin, conalbumin/ovatransferrin glycoprotein. Albumin is also present in chicken, fish, yogurt, meat, tofu, potatoes, peanuts, cheese, castor bean, etc. Albumin is an attractive macromolecular tool/carrier as it is available in pure form, is biodegradable, nontoxic, non- immunogenic, and has a half-life of 20 days.

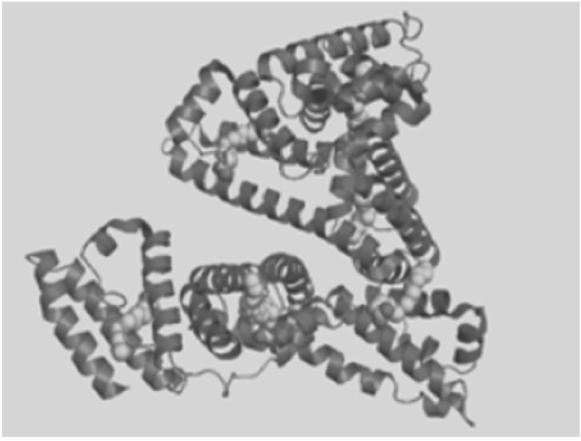

Figure 1.19 Serum albumin - C2936H4624N786O889S41, Mol. Wt. 66,500 Dalton. It has a single polypeptide chain of 585 amino acid containing 17 Di-sulfide bonds 2. About 55% α-helix and the remaining 45% is the β-structure. The three domains appear to be arranged in an elliptical pattern. This protein molecule can bend and twist in such a way as to achieve maximum stability or lowest energy state. (*Source: Wikipedia*).

Albumin has may applications such as (i) as a stabilizing agent in formulations containing proteins and enzymes; (ii) for the preparation of microspheres and microcapsules for experimental drug delivery system; (iii) as an antibacterial agent; and (iv) as an antioxidant. Albumin hydropersulfide is used as a reactive oxygen species scavenger in oxidative stress condition such as chronic kidney disease. There are trials to use albumin as a tool to prepare nanoparticles of curcumin with albumin for *in-vitro* and *in-vivo* evaluation; as a universal carrier protein for various biomolecules and nutrients such as long-chain fatty acids and heavy metal ions; and to target antitumor (cabazitaxel) drugs against prostate cancer in inflamed or malignant tissues.

1.3 Summary

This chapter dealt with the possibilities of various nano-flotilla that can be used as diagnostic tools with enhanced functioning wherever they are applied. The importance of functionalization and surface orchestration of nanodiagnostic tools was stressed as it increases the reactivity and applicability in non-biological as well as biological systems. Moreover, limitations and drawbacks in their use were also briefly discussed.

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