Chapter 11
Nanomicrobiology

Introduction

Microbiology is the study of microorganisms such as bacteria and viruses as well as the diseases caused by them. Application of nanobiotechnology in microbiology can be termed as nanomicrobiology. It includes some of the diagnostic procedures already mentioned in Chapter 3. Role of microorganisms in disease and their management using nanobiotechnology will be discussed in this chapter.

Nanobiotechnology and Virology

Study of Interaction of Nanoparticles with Viruses

Scanning surface confocal microscopy, simultaneous recording of high-resolution topography and cell surface fluorescence in a single scan enables imaging of individual fluorescent particles in the nanometer range on fixed or live cells. This technique has been used to record the interaction of single virus-like particles with the cell surface and demonstrated that single particles sink into the membrane in invaginations reminiscent of pinocytic vesicles (Gorelik et al 2002). This method provides a technique for elucidating the interaction of individual viruses and other nanoparticles, such as gene therapy vectors, with target cells.

Silver nanoparticles undergo a size-dependent interaction with HIV-1 and particles in the range of 1–10 nm attached to the virus (Elechiguerra et al 2005). The regular spatial arrangement of the attached nanoparticles, the center-to-center distance between nanoparticles, and the fact that the exposed sulfur-bearing residues of the glycoprotein knobs would be attractive sites for nanoparticle interaction suggest that silver nanoparticles interact with the HIV-1 virus via preferential binding to the gp120 glycoprotein knobs. Due to this interaction, silver nanoparticles inhibit the virus from binding to host cells, as demonstrated in vitro.
Study of Pathomechanism of Viral Diseases

Nanobiotechnology-based diagnosis of viral infections described in Chapter 3. Research in nanobiotechnology may be helpful in understanding the pathomechanism of viral diseases and devising strategies for treatment. An example is the neurotropic herpes simplex virus (HSV), which infects mucosal epithelia and enters nerve terminals, from where it travels in axons to dorsal root ganglia neurons and delivers its genome into the nucleus of the cell body. In the nucleus, the genome may give rise to infectious progeny or become latent with little gene expression. The silenced genome can be reactivated upon stress and establish a productive infection in the peripheral nervous system and, later, also in the mucosal periphery. To achieve this, a virus must elude host restrictions at multiple levels, including entry, cytoplasmic transport, replication, innate, and adaptive immune recognition, and egress from the infected cell.

Research on virus nanoparticles has provided cues to the regulation of cytoplasmic transport. Viruses that replicate their genomes in the nucleus make use of the microtubule and the actin cytoskeleton as molecular motors for trafficking toward the nuclear membrane during entry and the periphery during egress after replication. Analyzing the underlying principles of viral cytosolic transport will be helpful in the design of viral vectors to be used in research as well as human gene therapy, and in the identification of new antiviral target molecules (Dohner and Sodeik 2005).

Nanofiltration to Remove Viruses from Plasma Transfusion Products

One of the complications of blood transfusion is transmission of viral infections. Nanofiltration, use of nanotechnology in viral removal filtration systems, is an important safety step in the manufacture of plasma-derived coagulation factor concentrates and other biopharmaceutical products from human blood. Nanofiltration of plasma products has already been carried out since the early 1990s to improve margin of viral safety, as a complement to the viral reduction treatments, such as solvent-detergent and heat treatments, which are applied for the inactivation of HIV, hepatitis B, and hepatitis C viruses. The main reason for the introduction of nanofiltration was the need to improve product safety against nonenveloped viruses and to provide a possible safeguard against new infectious agents potentially entering the human plasma pool. Nanofiltration has gained quick acceptance as it is a relatively simple manufacturing step that consists of filtering protein solution through membranes of a very small pore size (typically 15–40 nm) under conditions that retain viruses by a mechanism largely based on size exclusion. Recent large-scale experience throughout the world has now established that nanofiltration is a robust and reliable viral reduction technique that can be applied to essentially all plasma products. Many of the licensed plasma products are currently nanofiltered.
The technology has major advantages as it is flexible and it may combine efficient and largely predictable removal of more than 4–6 logs of a wide range of viruses, with an absence of denaturing effect on plasma proteins. Compared with other viral reduction means, nanofiltration may be the only method to date permitting efficient removal of enveloped and nonenveloped viruses under conditions where 90–95% of protein activity is recovered. New data indicate that nanofiltration may also remove prions, opening new perspectives in the development and interest of this technique.

Role of Nanobacteria in Human Diseases

Nanobacteria are mineral-forming, sterile-filterable, slow-growing Gram-negative infectious agents (Wilk and Martirosian 2004). They are detected in bovine/human blood and urine. Nanobacteria-like particles have been detected in synovial fluids of arthritis patients and were shown to gradually increase in number and in size in culture (Tsurumoto et al 2006).

According to their 16S rDNA structure, nanobacteria belong to the alpha-2 Proteobacteria, subgroup, which includes the Brucella and Bartonella species. *Nanobacterium sanguineum* (nanobacteria) is the smallest self-replicating organism ever detected—at 50–500 billionths of a meter, 1/1,000th the size of the smallest previously known bacteria. Primordial proteins in nanobacteria, only recently identified in the atmosphere, could play a significant role in clouds, accelerating the formation of cloud droplets and interconnecting nanobacteria (and possibly nanobacteria and other microorganisms), thus enhancing their chances to eventually reach the Earth (Sommer and Wickramasinghe 2005).

Nanobacteria have been implicated in a variety of human diseases associated with pathological calcification. Their most remarkable characteristic is the formation of carbonate apatite crystals of neutral pH and at physiologic phosphate and calcium concentrations. The extracellular mineralization forms a hard protective shelter for these hardy microorganisms and enables them to survive conditions of physical stress that would be lethal to most other bacterial species. The Olavi Kajander group (Finland) suggests that the apatite produced by nanobacteria may play a key role in the formation of all kidney stones, by providing a central calcium phosphate deposit around which other crystalline components can collect. Nanobacteria seem to be causative agents of diseases related to biomineralization processes. Nanobacteria are also associated with calcified geological specimens, human kidney stones, and psammona bodies in ovarian cancer. Much research has focused attention on the potential role these particles may play in the development of urologic pathology, including polycystic kidney disease, renal calculi, and chronic prostatitis. Recent clinical research targeting these agents has proven effective in treating some patients with refractory category III prostatitis (Wood and Shoskes 2006).
**Nanobacteria and Kidney Stone Formation**

Approximately 12% of men and 5% of women develop kidney stones by the time they reach the age of 70 years but exactly how kidney stones form is not known. Kidney stones can be debilitating and recur in 50% of patients within 5 years. Kidney stone formation is considered to be a multifactorial disease in which the defense mechanisms and risk factors are imbalanced in favor of stone formation. One theory is that if nanoparticles accumulate in the kidney, they can form the focus of subsequent growth into larger stones over months to years. Other factors, such as physical chemistry and protein inhibitors of crystal growth, also play a role.

Mineral forming nanobacteria are active nidi that attach to, invade, and damage the urinary epithelium of collecting ducts and papilla forming the calcium phosphate center(s) found in most kidney stones. Scientists at NASA have used multiple techniques to determine that nanobacteria infection multiplies faster in space flight simulated conditions than on earth (Ciftcioglu et al 2005). Nanobacteria are considered to initiate kidney stone formation as they grow faster in a microgravity environment and may explain why astronauts get kidney stones on space missions. This discovery may prove to be critical for future exploratory missions to the moon and Mars. For further proof to this hypothesis, screening of the nanobacterial antigen and antibody level in flight crew before and after flight would be necessary. This concept also opens the door for new diagnostic and therapeutic techniques addressing nanobacterial infection in kidney stones.

Nanoparticles, isolated from renal stones obtained at the time of surgical resection, have been analyzed and propagated in standard cell culture medium (Kumar et al 2006). Nanoparticles were propagated from the majority of renal stones. Isolates were susceptible to selected metabolic inhibitors and antibiotics and contained conserved bacterial proteins and DNA. These results suggest that renal stone formation is unlikely to be driven solely by physical chemistry; rather, it is critically influenced by specific proteins and cellular responses, and understanding these events will provide clues toward novel therapeutic targets. Using high-spatial and energy resolution near-edge x-ray absorption fine structure at the 25-nm spatial scale, it is possible to define a biochemical signature for cultured calcified bacteria, including proteins, polysaccharides, nucleic acids, and hydroxyapatite (Benzerara et al 2006). These preliminary studies suggest that nanoparticles isolated from human samples share spectroscopic characteristics with calcified proteins.

**Nanobacteria in Cardiovascular Disease**

Scientists at the Mayo Clinic have examined surgical specimens from patients with cardiovascular pathology to evaluate human vascular tissue for the presence of similar nanometer-scale objects (Miller et al 2004). Analysis of areas with positive immunostaining identified spheres ranging in size from 30 to 100 nm with a spectral pattern of calcium and phosphorus (high-energy dispersive spectroscopy).
Nano-sized particles cultured from calcified but not from noncalcified aneurysms were recognized by a DNA-specific dye, incorporated radiolabeled uridine, and after decalcification, appeared via electron microscopy to contain cell walls. Therefore, nanometer-scale particles similar to those described as nanobacteria isolated from geological specimens and human kidney stones can be visualized in and cultured from human calcified cardiovascular tissue. In a further study nanoparticles were found near plaque-filled arteries in animal models. The study suggests that nanoparticles potentially represent a previously unrecognized factor in the development of arteriosclerosis and calcific arterial disease.

**Nanobiotechnology for Detection of Infectious Agents**

The rapid and sensitive detection of pathogenic bacteria is extremely important in medical diagnosis and measures against bioterrorism. Limitations of most of the conventional diagnostic methods are lack of ultrasensitivity or delay in getting results. Several nanotechnology-based methods have already been described in this chapter including ferrofluid magnetic nanoparticles, ceramic nanospheres, and nanowire sensors for viruses. A bioconjugated nanoparticle-based bioassay for in situ pathogen quantification can detect a single bacterium within 20 min (Zhao et al 2004). The nanoparticle provides an extremely high fluorescent signal for bioanalysis and can be easily incorporated in a biorecognition molecule such as an antibody. The antibody-conjugated nanoparticles can readily and specifically identify a variety of bacteria such as *Escherichia coli O157:H7* through antibody–antigen interaction and recognition. This method can be applied to multiple bacterial samples with high throughput by using a 384-well microplate format. It has a potential for application in ultrasensitive detection of disease markers and infectious agents.

Detection of single-molecule hybridization has been achieved by a hybridization detection method using multicolor oligonucleotide-functionalized quantum dots (QDs) as nanoprobe (Ho et al 2005). In the presence of various target sequences, combinatorial self-assembly of the nanoprobe via independent hybridization reactions leads to the generation of discernible sequence-specific spectral codings. This method can be used for genetic analysis of anthrax pathogenicity by simultaneous detection of multiple relevant sequences.

**Sensing of Phage-Triggered Ion Cascade for Detection of Bacteria**

Researchers at Texas A&M University (College Station, TX) have developed a novel nanotechnology to rapidly detect and identify bacteria (Dobozi-King et al 2005). The technique called SEnsing of Phage-Triggered Ion Cascade (SEPTIC) uses a nanowell device with two antenna-like electrodes to detect the electric field fluctuations that result when a bacteriophage infects a specific bacterium and then identifies the bacterium. The technology had a 100% success rate in detecting and
identifying strains of *E. coli* quickly and accurately. The technique works because only a specific phage can infect a specific bacterium. When a bacteriophage infects a bacterium, the phage injects its DNA into the bacterium and “reprograms” it to produce multiple copies of the phage, called virions. During the infection process, about 100 million ions escape from the host cell. This ion leakage causes fluctuations in the electric field around the bacterium, and the nanowell detects these fluctuations. The Texas A&M University System holds a provisional patent on the technology.

Rapid and sensitive identification of bacteria is extremely important in clinical, veterinary, and agricultural practice, as well as in applications to microbiological threat detection and reduction. It will also be useful in the current fight against bioterrorism. Eventually, every medic or soldier may be equipped with a cell phone-like wireless SEPTIC biolab. The researchers’ ultimate aim is to have a biochip where hundreds of nanowells and their preamplifiers are integrated. Each nanowell covers a different phage, and if a relevant bacterium is present, the corresponding nanowell will signal and identify the bacterium. This would be a pen-size biolab that would be able to identify hundreds of bacteria in 5 min.

**Viral Detection by Surface-Enhanced Raman Scattering**

Although surface-enhanced Raman scattering (SERS) is well known, previous attempts to use spectroscopy to diagnose viruses failed because the signal produced is inherently weak. A spectroscopic assay based on SERS using silver nanorods, which significantly amplify the signal, has been developed for rapid detection of trace levels of viruses with a high degree of sensitivity and specificity (Shanmukh et al 2006). The technique measures the change in frequency of a near-infrared laser as it scatters viral DNA or RNA. That change in frequency is as distinct as a fingerprint. This novel SERS assay can detect spectral differences between viruses, viral strains, and viruses with gene deletions in biological media. The method provides rapid diagnostics (60 s or less) for detection and characterization of viruses generating reproducible spectra without viral manipulation. It is also quite cheap and is very reproducible.

**Detection of Single Virus Particles**

Microfabrication and application of arrays of silicon cantilever beams as microresonator sensors with nanoscale thickness has been applied to detect the mass of individual virus particles (Gupta et al 2004). The dimensions of the fabricated cantilever beams were in the range of 4–5 μm in length, 1–2 μm in width, and 20–30 nm in thickness. The virus particles used in the study were vaccinia virus, which is a member of the Poxviridae family and forms the basis of the smallpox vaccine. The frequency spectra of the cantilever beams, due to thermal and ambient noise, were measured using a laser Doppler vibrometer under ambient conditions. The change in
resonant frequency as a function of the virus particle mass binding on the cantilever beam surface forms the basis of the detection scheme. This device can detect a single vaccinia virus particle with an average mass of 9.5 fg. Such devices can be very useful as components of biosensors for the detection of airborne virus particles. This technology has been refined as described under nanocantilever biosensors.

Rapid, selective, and sensitive detection of viruses is crucial for implementing an effective response to viral infection, such as through medication or quarantine. Established methods for viral analysis include plaque assays, immunological assays, transmission electron microscopy, and PCR-based testing of viral nucleic acids. These methods have not achieved rapid detection at a single virus level and often require a relatively high level of sample manipulation that is inconvenient for infectious materials.

Scientists at the Harvard University (Cambridge, MA) have reported direct, real-time electrical detection of single virus particles with high selectivity by using nanowire field effect transistors (Patolsky et al 2004). Measurements made with nanowire arrays modified with antibodies for influenza A showed discrete conductance changes characteristic of binding and unbinding in the presence of influenza A but not paramyxovirus or adenovirus. Simultaneous electrical and optical measurements using fluorescently labeled influenza A were used to demonstrate conclusively that the conductance changes correspond to binding/unbinding of single viruses at the surface of nanowire devices. pH-dependent studies further show that the detection mechanism is caused by a field effect and that the nanowire devices can be used to determine rapidly isoelectric points and variations in receptor–virus binding kinetics for different conditions. Larger arrays of reproducible nanowire devices might simultaneously screen for the presence of 100 or more different viruses. Finally, studies of nanowire devices modified with antibodies specific for either influenza or adenovirus show that multiple viruses can be selectively detected in parallel. The possibility of large-scale integration of these nanowire devices suggests potential for simultaneous detection of a large number of distinct viral threats at the single virus level.

**Fluorescent QD Probes for Detection of Respiratory Viral Infections**

Respiratory syncytial virus (RSV) causes about one million deaths annually worldwide. RSV mediates serious lower respiratory tract illness in infants and young children and is a significant pathogen of the elderly and immune compromised. Although it is only life-threatening in one case out of every 100, it infects virtually all children by the time they are 5 year old. Approximately 120,000 children are hospitalized with RSV in the United States each year. Few children in the United States die from RSV but it causes 17,000–18,000 deaths annually among the elderly.

Rapid and sensitive RSV diagnosis is important for infection control and efforts to develop antiviral drugs. Current RSV detection methods are limited by
sensitivity and/or time required for detection, which can take 2–6 days. This can delay effective treatment. Antibody-conjugated nanoparticles rapidly and sensitively detect RSV and estimate relative levels of surface protein expression (Agrawal et al 2005). A major development is use of dual-color QDs or fluorescence energy transfer nanobeads that can be simultaneously excited with a single light source.

A QD system can detect the presence of particles of the RSV in a matter of hours. It is also more sensitive, allowing it to detect the virus earlier in the course of an infection (Bentzen et al 2005). When an RSV virus infects lung cells, it leaves part of its coat containing F and G proteins on the cell’s surface. QDs have been linked to antibodies keyed to structures unique to RSV’s coat. As a result, when QDs come in contact with either viral particles or infected cells they stick to their surface. In addition, co-localization of these viral proteins was shown using confocal microscopy. The potential benefits for such an early detection system are that it can

1. Increase the proper use of antiviral medicines. Although such medicines have been developed for some respiratory viruses, they are not used often as therapy because they are only effective if given early in the course of infection. By the time current tests identify the virus, it is generally too late for them to work.

2. Reduce the inappropriate use of antibiotics. Currently, physicians often prescribe antibiotics for respiratory illnesses. However, antibiotics combat respiratory illness caused by bacteria and are ineffective on viral infections. An early virus detection method would reduce the frequency with which doctors prescribe antibiotics for viral infections inappropriately, thereby reducing unnecessary antibiotic side-effects and cutting down on the development of antibiotic resistance in bacteria.

3. Allow hospital personnel to isolate RSV patients. RSV is extremely infectious so early detection would allow hospital personnel to keep the RSV patients separate from other patients who are especially susceptible to infection, such as those undergoing bone-marrow transplants.

Currently, there are three diagnostic tests available for identifying respiratory viruses like RSV. The “gold standard” involves incubating an infected sample in a tissue culture for 5 days and then using a fluorescent dye to test for the presence of the virus. The main problem with this technique is that the virus is multiplying in the patient at the same time as it is growing in the culture. This has caused many hospitals to switch to real-time PCR, which is extremely sensitive but still takes 36–48 h because of the need for a technician well trained in molecular biology to conduct the test in a reference laboratory. The third method, the antigen test, takes only 30 min but it is not sensitive enough to detect the presence of the virus at the early stages of an infection. By comparison, the new QD method takes 1–2 h and is even more sensitive than real-time PCR. It can detect the presence of RSV within an hour after the virus is added to a culture. Another advantage of the QD method over detection systems that rely on fluorescent protein is that the protein “bleaches out” in minutes while QDs maintain their brightness for hours.
It is estimated that it will take only 2–3 years to develop and validate the QD test. All the components are available off-the-shelf and any one can put together one of these detection systems. The system should also be relatively inexpensive. The most costly ingredient is the QDs: a small bottle that contains enough of the material for about 200 tests costs $300. As a result, this could be one of the earliest medical applications of nanotechnology. The next step will be to develop a QD cocktail capable of simultaneously detecting the presence of at least five major respiratory viruses: influenza A and B, parainfluenza, and metapneumovirus, in addition to RSV. This should be possible because one can use different colors of QDs simultaneously. The colored QDs are attached to different “linker” molecules that bind to different RSV surface structures. QDs are available in a dozen different colors, and antibodies specific to the other four respiratory viruses have been identified and can be used as linker molecules. Such a test would be able to diagnose more than 90% of all the cases of viral respiratory infection. The existence of such a test could encourage the development of improved therapies for respiratory viruses. Without a good diagnostic test for a specific viral infection, pharmaceutical companies are not motivated to develop effective treatments because physicians are unlikely to prescribe them very often.

Nanotechnology-Based Microbicidal Agents

Nanoscale Bactericidal Powders

Certain formulations of nanoscale powders possess antimicrobial properties. These formulations are made of simple, nontoxic metal oxides such as magnesium oxide (MgO) and calcium oxide (CaO, lime) in nanocrystalline form, carrying active forms of halogens, e.g., MgO·Cl₂ and MgO·Br₂. When these ultrafine powders contact vegetative cells of *Escherichia coli*, *Bacillus cereus*, or *Bacillus globigii*, over 90% are killed within a few minutes. Likewise, spore forms of the Bacillus species are decontaminated within several hours. Dry contact with aflatoxins and contact with MS2 bacteriophage (surrogate of human enterovirus) in water also causes decontamination in minutes.

A nanopowder of MgO can scour contaminated rooms of anthrax spores (Stoimenov et al 2002). Unlike antibacterial gases and foams, which are messy, corrosive, and ruin electrical equipment, the powder could be sprayed into rooms and swept or vacuumed up. The chemical specks attract oppositely charged spores. The particles then cut open and chemically break down the spores’ tough outer shell. The scientists tested the powder by blowing spores into a stainless-steel room, then cleaning them up with a squirt of nanoparticles. Based on this technology, NanoScale Materials Inc plans to market a dry powder dubbed FAST ACT (First Applied Sorbent Treatment Against Chemical Threats) that decomposes toxic chemicals. The powder contains reactive nanoparticles that attract and then break down
at least 24 commonly transported toxic chemicals, including some acids. Unlike foams, the powder need not be wet to be effective and works on liquids and vapors.

**Nanotubes for Detection and Destruction of Bacteria**

University of Pittsburgh (Pittsburgh, PA) researchers have synthesized a simple molecule from a hydrocarbon and an ammonium compound to produce a unique nanotube structure with antimicrobial capability (Lee et al 2004). The quaternary ammonium compound is known for its ability to disrupt cell membranes and causes cell death whereas the hydrocarbon diacetylene can change colors when appropriately formulated; the resulting molecule would have the desired properties of both a biosensor and a biocide.

The self-assembled nanotubes are perfectly uniform and organize themselves into an expanse of upright clusters that when magnified a million times resemble the fibers of a shag rug leading to the name “nanocarpet.” The self-assembling nanotubes all had the same diameter (89 nm) and wall thickness (27 nm). The nanocarpet measures about 1 μm in height, approximately the same height as the free-form nanotubes. This alignment of nanotubes in the absence of a template is unprecedented and represents an important step toward rational design of bioactive nanostructures. In addition, because they form within hours under room-temperature conditions, the significant costs of synthesizing carbon nanotubes can be reduced. Normally a neutral color, when exposed to ultraviolet light the nanotubes changed to a permanent deep blue. The process also chemically altered the nanotubes so that they became polymerized, giving them a more firm structure. Polymerized, these nanotubes could change from blue to other colors, depending on its exposure to different materials. For instance, in tests with acids and detergents, they turned red or yellow.

Because they display sensitivity to different agents by changing color, these nanotubes can be trained to kill bacteria. In the presence of *E. coli*, some strains of which are food-borne pathogens, the nanotubes turned shades of red and pink. Moreover, with the aid of an electron microscope, the researchers observed the tubes piercing the membranes of the bacteria like a needle being inserted into the cell. Both the polymerized (those that can change color) and the unpolymerized nanotube structures were effective antimicrobials, completely killing all the *E. coli* within an hour’s time. The findings have implications for developing products that can simultaneously detect and kill biological weapons. The research, funded by the Department of Defense’s Army Research Office, has as its goal the development of a paint that in the event of biological or chemical agents being deployed would change color and simultaneously destroy the deadly substances.

A research team at the Scripps Research Institute (La Jolla, CA) has developed antibiotic agents based on self-assembling cyclic peptide nanotubes that attach to, and poke holes through, bacterial cell membranes, thus killing the cell. These
self-assembling peptide nanotubes cleared infections of antibiotic-resistant bacteria in mice, even when injected far from the site of infection (Fernandez-Lopez et al 2001). Another promising example is a vaccine consisting of self-assembling virus-like particles for the prevention of infection of the genital tract by human papilloma virus, which can cause cervical cancer. Such particles are now being developed by MedImmune (Gaithersburg, MD) and GlaxoSmithKline (Uxbridge, United Kingdom).

**Carbon Nanotubes for Protection Against Anthrax Attack**

There has been significant interest in the binding of anthrax spores by molecular species, but with only limited success. Proteins and more recently peptides were used. However, despite the known presence of carbohydrates on the spore surface, carbohydrate–carbohydrate interactions have hardly been explored likely because of the lack of required specific platform for synthetic carbohydrates. Scientists at Clemson University (Clemson, SC) have reported the successful use of single-walled carbon nanotubes as a truly unique scaffold for displaying multivalent monosaccharide ligands that bind effectively to anthrax spores with divalent cation mediation to cause significant spore aggregation (Wang et al 2006). The work should have far-reaching implications in development of technologies to counteract bioterrorism such as by use of anthrax. For anthrax to be effective, it has to be made into a fine powder that can easily enter the lungs when inhaled. That nanotechnology-based agent clings to the anthrax spores to make their inhalation into the lungs difficult. Similar approach using sugar-coated carbon nanotubes to stop the spread of *E. coli* bacteria has been tested successfully in 2004.

**Nanoemulsions as Microbicidal Agents**

The antimicrobial nanoemulsions (NanoBio) are emulsions that contain water and soya bean oil with uniformly sized droplets in the 200–400 nm range. These droplets are stabilized by surfactant and are responsible for the microbicidal activity. In concentrated form, the nanoemulsions appear as a white milky substance with a taste and consistency of cream. They can be formulated in a variety of carriers allowing for gels, creams, liquid products, etc. In most applications, the nanoemulsions become largely water-based, and in some cases such as a beverage preservative comprise 0.01% or less of the resultant mixture. Laboratory results indicate a shelf life of at least 2 years and virtually no toxicity. The NanoBio nanoemulsions destroy microbes effectively without toxicity or harmful residual effects (Hamouda et al 2001). The nanoparticles fuse with the membrane of the microbe and the surfactant disrupts the membrane, killing the microbe. The classes of microbes eradicated are virus (e.g., HIV, herpes), bacteria (e.g., *E. coli*, *Salmonella*), spores (e.g., anthrax), and fungi (e.g., *Candida albicans*, *Byssochlamys fulva*). Clinical trials have shown
efficacy in healing cold sores due to herpes simplex virus 1 and toenail fungus. The nanoemulsions also can be formulated to kill only one or two classes of microbes. Due in large part to the low toxicity profile, the nanoemulsions are a platform technology for any number of topical, oral, vaginal, cutaneous, preservative, decontamination, veterinary, and agricultural antimicrobial applications.

Since it is nontoxic and noncorrosive, nanoemulsion can be used to decontaminate personnel, equipment, terrain, structures, and water. Further, tests by DTRA (Defense Threat Reduction Agency), an agency of the US Department of Defense, have demonstrated that the nanoemulsion is a chemical decontaminating agent. The US Army tested the nanoemulsion and nine other biodecontamination technologies against an anthrax surrogate. The nanoemulsion was one of four technologies that proved effective.

**Silver Nanoparticle Coating as Prophylaxis Against Infection**

The Institute for New Materials (Saarbrucken, Germany), a research institute specializing in applied nanotechnology applications, has developed a silver nanoparticles surface coating that is deadly to fungi and bacteria. The researchers added the germicidal ability by sprinkling copious amounts of silver nanoparticles through the coating material (every square centimeter contains more than one billion of the invisible particles) and aligning them so that they release a tiny number of silver ions. These ions are the death knell for fungus and bacteria that might have succeeded in gathering on the surface despite its already dirt-repellent qualities. Applications include any surface where germs can gather and possibly endanger people’s health. That includes surfaces in hospitals, public buildings, factories, or in the home. The coating could be applied to almost any surface that people touch often such as metal, glass, or plastic and would remove the need for constant cleaning with liquid disinfectants, especially in areas where hygienic conditions are crucial. People who normally cannot use hearing aids that lie inside the ear because of the risk of infection of the auditory canal can safely wear nanocoated appliances.

Bio-Gate (Nürnberg, Germany) produces NanoSilver BG, a nanoporous silver powder with particle size ranging from 50 to 100 nm. It has a homogeneous distribution of nanoparticles in the material and antiinfective properties.

Silver nanoparticles have been incorporated in preparations for wound care to prevent infection. Acticoat bandages (Smith & Nephew) contain nanocrystal silver, which is highly toxic to pathogens in wounds.

AcryMed’s silver nanoparticle technology, SilvaGard, involves coating with silver nanoparticles with size range of 2 to 20 nm in a stable solution and antimicrobial treatment levels last for more than a year. With other technologies, nano-based silver coatings must be applied through vapor deposition, which coats only on one side, whereas AcryMed technology is a solution that provides a complete surface treatment rather than a coating.
Nanotechnology-Based Antiviral Agents

Nanocoating for Antiviral Effect

Laboratory testing of the permanent nanocoating developed by researchers at North Carolina State University College of Textiles and Emory University School of Medicine showed the coating kills 99.9% of influenza viruses and 99.99% of vaccinia viruses that cause rash, fever, head and body aches. The development may lead to being able to protect oneself from virtually all viruses and bacteria by simply exposing a surface to light. The technology has been licensed to LaamScience Inc.

In November 2006, Mass Transit Railway (MTR), the corporation that runs Hong Kong subway, announced that Nano Silver–Titanium Dioxide Coating (NSTDC, a nontoxic disinfectant) will be applied to surfaces that passengers commonly touch in order to enhance hygiene levels in MTR stations and trains. The coating is manufactured using nanotechnology, which maximizes coverage and effectiveness of NSTDC. Developed in Japan, NSTDC is certified to be effective in killing a wide range of bacteria, viruses, and mold including the H1N1 influenza virus A. It is used in hospitals, offices, and homes in Japan. NSTDC’s main component, titanium dioxide (TiO$_2$), has been approved for use in foods by the FDA and under the Public Health and Municipal Services Ordinance in Hong Kong.

Fullerenes as Antiviral Agents

A series of bis-fulleropyrrolidines bearing two ammonium groups have been synthesized and their activities against HIV-1 and HIV-2 have been evaluated (Marchesan et al 2005). Two trans isomers were found to have interesting antiviral properties, confirming the importance of the relative positions of the substituent on the C60 cage. None of the compounds showed any inhibitory activity against a variety of DNA and RNA viruses other than HIV.

Cationic, anionic, and amino acid-type fullerene derivatives have shown inhibitory effect against HIV-reverse transcriptase and HCV (Mashino et al 2005). Out of all derivatives of fullerenes, anionic fullerenes, were found to be the most active. All the tried fullerene derivatives were more active than the nonnucleoside analogue of HIV-RT inhibitor. The effect of long alkyl chains on fullerenes was not significant; rather it depressed the inhibition strength. The two important targets for anti-HIV characteristics are the HIV-protease and HIV-reverse transcriptase. The molecular modeling experimental designs exhibit that C60-core could penetrate into hydrophobic binding site of HIV protease. However, the mechanism of this anti-HIV activity is through HIV-protease inhibition, which has not been experimentally demonstrated.
NanoViricides

NanoViricides Inc is developing nanoviricides, which are nanomedicines that destroy viruses. A nanoviricide is a polymeric single chemical chain with covalently attached ligands that specify the virus target. The antiviral spectrum of the drug is determined by the specificity of the set of ligands attached to the chain, in addition to other functionally important aspects inherent in the chemistries. Nanoviricide is designed to seek a specific virus type, attach to the virus particle, engulf or coat the virus particle, thereby neutralizing the virus’s infectivity, destabilize and possibly dismantle the virus particle, and optionally it may also be made capable of attacking the viral genome thereby destroying the virus completely. They are different from any of the other micellar nanotechnologies as there are no metal particles attached and the micelles can penetrate the virus and bind to multiple sites for effective destruction of the virus. Active pharmaceutical ingredients are optional and can be hidden in the core of the nanoviricide missile.

Mechanism of Action of Nanoviricides

For a virus to infect a cell, it needs to bind to more than one site. For example, binding of HIV only to CD4 on T cells is insufficient to cause sustained disease; it needs HIV binding to at least two and possibly three different sites on the T cell and that too, at multiple points. For an antiviral to be effective, it should match the strategy to bind to more than one site on the virus. Ideally it should block all of these to prevent virus from infecting the cell and multiplying. Most of the current antiviral drugs have a single mechanism of action and block a single receptor. Drug combinations from different categories are required to increase the number of receptors blocked. Still this is not fully effective.

In contrast to other approaches, a NanoViricide™ micelle can recognize and bind to more than one type of binding site on the virus. The NanoViricide™ system enables design of a drug that binds to more than one type of site—currently as many as three different sites, on the virus—for a highly effective attack. NanoViricides Inc terms this as “multi-specific targeting.”

A NanoViricide™ drug goes much further than just blocking all of the binding sites of the virus. The base material of a NanoViricide™ is a specially designed polymeric micelle material. It has the ability to disassemble an HIV particle by itself. Thus, after coating the virus particle, the NanoViricide™ loosens the virus particle, and weakens it. Some virus particles will even fall apart (uncoat). This provides a further therapeutic benefit. NanoViricides plans to enhance the viral disassembly capabilities of the NanoViricide™ by attaching specially designed “molecular chisels” to the NanoViricide™. Once the NanoViricide™ micelles coat the virus particle, the attached “molecular chisels” will go to work. They literally insert themselves into the virus coat at specific vulnerable points and pry apart the coat proteins so that the virus particle falls apart readily. The mechanism of action of NanoViricide is depicted schematically in Fig. 11.1. This description is a simplification. There is no fully adequate explanation of the observed efficacy because the
Advantages of NanoViricides

NanoViricides have been compared to current approaches to viral diseases, which are seldom curative and some of the advantages include the following:

- Specific targeting of the virus with no metabolic adverse effects on the host.
- The biological efficacy of NanoViricides drugs may be several orders of magnitude better than that of usual chemical drugs. This in itself may limit the potential for mutant generation.
- There are also other key aspects of the design of NanoViricides that are expected to lead to minimizing mutant generation.
- Nanoviricides are safe because of their unique design and the fact that they are designed to be biodegradable within the body.
- The new technology enables rapid drug development against an emerging virus, which would be important for global biosecurity against natural as well as man-made (bioterrorism) situations. It is possible to develop a research drug against a
novel life-threatening viral disease within 3–6 weeks after the infection is found, i.e., as soon as an antibody from any animal source is available.

- It is possible to make a single NanoViricide drug that responds to a large number of viral threats by using targeting ligands against the desired set of viruses in the construction of the drug. It is possible to “tune” the specificity and range (spectrum) of a NanoViricide drug within a virus type, subtype, or strain, by appropriate choices of the targeting ligand(s).
- The safety of NanoViricide drugs is proven now as they specifically attack the virus and not the host.
- A variety of formulations, release profiles, and routes of administration are possible.
- Low cost of drug development, manufacture, distribution.

NanoViricide drug candidates are currently in preclinical studies. Clinical trials are planned. Initially injectable products are considered to be most effective but alternative routes of administrations such as nasal sprays and bronchial aerosols can also be developed. Various NanoViricide products will be described further along with relevant viral diseases.

Advantages of Nanoviricides over vaccines are as follows:

- Nanoviricides work where vaccines fail and are effective even when the immune system is impaired such as in AIDS.
- Nanoviricides work where effective vaccines are unavailable.
- Sufficient short-term protection for an individual outbreak cluster.
- Treatment can be started after infection.
- No need to vaccinate whole world population for control of a viral epidemic.

Advantages of Nanoviricides over immunoglobulin therapies are as follows:

- Fully chemical, room-temperature stable NanoViricides can be made against many diseases.
- Nanoviricides based on antibody fragment conjugates do not require humanized antibodies. Antibodies from virtually any source can be used for developing NanoViricides, thus significantly reducing time and cost of development.

Immunoglobulin therapies require the patient’s immune system (complement system) to function well, which is often not the case in advanced disease states. NanoViricides function completely independently of the human immune system while accomplishing the same goal of reduction in viremia.

**Nanotechnology-Based Vaccines**

Although a number of adjuvants are currently approved for use in veterinary species, only alum has been widely used in humans. While it induces strong antibody responses, cell-mediated responses are often low and inflammatory reactions at the site of injection are common. Immunological properties of a novel nanobead
Nanobead adjuvants have been investigated in a large-animal sheep model (Scheerlinck et al 2006). In contrast to alum, antigen covalently coupled to nanobeads induced substantial cell-mediated responses along with moderate humoral responses. No adverse reactions were seen at the site of immunization in the sheep. Thus, nanobead adjuvants in veterinary species may be useful for the induction of immunity to viral pathogens, where a cell-mediated response is required. These findings also highlight the potential usefulness of nanobead vaccines for intracellular pathogens in humans. Nanobeads measure 40 nm. Most adjuvants only stimulate antibodies against a particular disease. The nanobead technology gave the immune system a further boost, also producing T cells which are needed to eliminate viruses or cancer. The size of 40 nm is critical as it is similar in size to many viruses, where the nanobeads are taken up abundantly by the immune system and tricked into producing high levels of many types of T-cells.

**Nanofiltration of Blood in Viral Diseases**

Nanofiltration is the filtration of minute particles using a filter with nanopores. Scientists at the Queensland University of Technology (QUT) in Australia have developed specially designed ceramic membranes used as nanomesh for nanofiltration, which are so advanced they have the potential to remove viruses from water, air, and blood. Shortcomings of current membranes were that they often formed pin-holes and cracks during the fabrication process, resulting in wasted membranes.

QUT scientists introduced radical changes to the membrane texture because it is crucial for the separation efficiency of the material. Mesh structure, which is the most efficient form of filtration, has been successfully constructed on a nanoscale with ceramic fibers. This modification has increased the rates of flow that pass through the membranes by at least 10 times compared with current ceramic membranes, while maintaining the efficiency of capturing over 96% of the unwanted particles.

This technology could be used to filter airborne viruses such as the severe acute respiratory syndrome (SARS) and the avian flu virus. It may be possible to filter HIV from human blood to treat patients with AIDS.

**Nanoparticles to Combat Biological Warfare Agents**

Nanosilicate crystals are generated by a nontoxic electrochemical reaction when Bio-DECON™ (Sierra Pacific Research Company) is mixed with water. Although a chemical process creates the silicate crystals, it is a mechanical process that destroys the cellular wall of the spore, virus, or bacteria. Certain types of Gram-negative bacteria do not allow a chemical charge to penetrate the cellular wall. Bio-DECON mechanically penetrates the cellular wall of both Gram-negative as well as Gram-positive bacteria.
Bio-DECON was tested several years ago at the Battelle Memorial Institute as an antibacterial agent against *Bacillus anthracis* vegetative cells and spores. Results revealed that a laboratory-diluted 4% solution Bio-DECON (normal application is a 25% solution) had an immediate deleterious impact on the anthrax colony forming units, disabling 96% of the weapons grade form of *Ames anthrax* within 2 min of exposure. Continued exposure for 60 min increased the “kill ratio” to more than 99%. During a longer period of time, the effects of Bio-DECON continue to kill microorganisms for days, if not weeks.

Nanomaterials could play a role as an anthrax antibiotic. Chemists at Rice University have found that antibodies that latch on to dormant anthrax spores and drugs that destroy anthrax could be linked to the spherical carbon molecule fullerene to make an antibiotic. The drug would kick in when an inhaled spore germinates, killing the anthrax before it releases deadly amounts of toxin. This could be effective if one has just been exposed or the exposure is expected within 24 h following administration of the drug. The research group is in preliminary stages of investigating antibiotics such as vancomycin for anthrax infections.

Preventing the interaction of toxins with their cellular receptors CMG2 and ATR/TEM8 is an important goal for anthrax therapy. Scientists at the Scripps Research Institute (La Jolla, CA) have described novel nanotechnology approaches for the multivalent display of engineered receptor decoys, and their efficacy against anthrax lethal toxin in vitro and in vivo.