Nanodiagnostics in Microbiology and Dentistry

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21.1 INTRODUCTION

Nanotechnology is the study, design, creation, synthesis, manipulation, and application of materials, devices, and systems at the nanometer scale (one meter consists of 1 billion nanometers) [1]. Nanotechnology (sometimes shortened to "nanotech") is the study of manipulating matter on an atomic and molecular scale. Generally nanotechnology deals with structures sized between 1 and 100 nm in at least one dimension and involves developing materials or devices within that size. Much of the study of nanoscience relates to the phenomenon of self-assembly in which nanoscale building blocks self-assemble to form complex structures. Microbiology relates to nanoscience at a number of levels. Many bacterial entities are nanomachines in nature, including molecular motors like *flagella* and *pili*. Bacteria also form biofilms by the process of self-assembly (e.g., the formation of Curli-film by *Eschericia coli*). The formation of aerial hyphae by bacteria and fungi is also directed by the controlled and ordered assembly of building blocks. In addition, the formation of virus capsids is a classical process of molecular recognition and self-assembly at the nanoscale. Another completely different aspect is the use of nanoordered bacterial assemblies for nanotechnology. Nanoscience impacts several areas of microbiology. It allows for the study and visualization at the molecular assembly level of a process and facilitates identification of molecular recognition and self-assembly motifs and the assessment of these processes. These are relevant to many microbial processes as mentioned above.

21.2 **NANOMATERIALS**

Nanomaterials have been categorized as those materials which have structured components with at least one dimension less than 100 nm. Materials that have *one dimension in the nanoscale* (and are extended in the other two dimensions) are layers, such as thin films or surface coatings. Some of the features on computer chips come under this category. Materials that are *nanoscale in two dimensions* (and extended in one dimension) include nanowires and nanotubes. Materials that *are nanoscale in three dimensions* are particles, e.g., precipitates, colloids, and quantum dots (tiny particles of semiconductor materials). Nanocrystalline materials, made up of nanometer-sized grains, also fall into this category. The nanomaterial field includes subfields which study or develop materials having unique properties arising from their nanoscale dimensions [2]:

- Interface and colloid science has given rise to many materials which may be useful in nanotechnology such as carbon nanotubes and other fullerenes and various nanoparticles and nanorods. Nanomaterials with fast ion transport are also related to nanoionics and nanoelectronics.
- Progress has been made in using these materials for medical applications.
- Nanoscale materials are sometimes used in solar cells which combat the cost of traditional silicon solar cells.
- Development of applications incorporating semiconductor nanoparticles to be used in the next generation of products, such as display technology, lighting, solar cells, and biological imaging.

21.2.1 **Applications of Nanomaterials**

Most current applications represent evolutionary developments of existing technologies: e.g., the reduction in size of electronics devices.

21.2.1.1 *Sunscreens and Cosmetics*

Nanosized titanium dioxide and zinc oxide are currently used in some sunscreens, as they absorb and reflect ultraviolet (UV) rays and yet are transparent to visible light and so are more appealing to the consumer.

21.2.1.2 *Composites*

Nanoparticles and nanotubes are used in composites, materials that combine one or more separate components and which are designed to exhibit better overall properties than each of components.

21.2.1.3 *Clays*

Clays containing naturally occurring nanoparticles have long been important as construction materials and are undergoing continuous improvement. Clay–particle-based composites—containing plastics and nanosized flakes of clay—are also finding applications such as use in car bumpers.

21.2.1.4 *Coatings and Surfaces*

Coatings with thickness controlled at the nano- or atomic scale have been in routine production for some time. Recent applications include the self-cleaning window, which is coated in highly activated titanium dioxide, engineered to be highly hydrophobic (water repellent) and antibacterial, and coatings based on nanoparticulate oxides that catalytically destroy chemical agents.

21.2.1.5 Tougher and Harder Cutting Tools

Cutting tools made of nanocrystalline materials, such as tungsten carbide, tantalum carbide, and titanium carbide, are more wear and erosion resistant, and last longer than their conventional (large-grained) counterparts. They are finding applications in the drills used to bore holes in circuit boards.

21.3 BIOMEDICAL APPLICATIONS OF NANOTECHNOLOGY AND ITS LIMITATIONS

Three applications of nanotechnology in biomedicine [3] are focused on (i) targeted drug delivery, (ii) diagnostic techniques, and (iii) prostheses and implants. Interest is booming in biomedical applications for use outside the body, such as diagnostic sensors and "lab-on-a-chip" techniques, which are suitable for analyzing blood and other samples, and for inclusion in analytical instruments for R&D on new drugs. For inside the body, many companies are developing nanotechnology applications for anticancer drugs, implanted insulin pumps, and gene therapy. Other researchers are working on prostheses and implants that include nanostructured materials. Nanotechnology applications have not been marketed long enough for claims to be corroborated about risks to human health and environment. Still, small nanoparticles can enter the human body through pores and may accumulate in cells. The health effects of such nanoparticles are unknown. Historical experience with unintended consequences of technologies, such as drug resistance to antibiotics, or the persistence of chemicals, such as DDT in the environment, teaches us to take precautions.

21.4 NANOTECHNOLOGY APPLICATIONS IN DRUG DELIVERY SYSTEMS, NANODIAGNOSTICS, AND VARIOUS OTHER FIELDS

It is envisaged that nanotechnology should let us make every manufactured product faster, lighter, stronger, smarter, safer, and cleaner. The following are some areas in which nanotechnology can have significant impact in the future.

21.4.1 Drug Delivery System

21.4.1.1 Nanobots and its Uses

Nanobots are robots that carry out a very specific function and are ~50–100 nm wide. They can be used very effectively for drug delivery. Normally, drugs work through the entire body before they reach the disease-affected area. Using nanotechnology, the drug can be targeted to a precise location which would make the drug much more effective and reduce the chances of possible side effects. Figure 21.1 shows a device that uses nanobots to monitor the sugar level in the blood [4]. Special sensor nanobots can be inserted into the blood under the skin where microchips, coated with human molecules and designed to emit an electrical impulse signal, monitor the sugar level in the blood.

The drug carriers have walls that are just 5–10 atoms thick and the inner drug-filled cell is usually 50–100 nm wide. When they detect signs of the disease, thin wires in their walls emit an electrical pulse which causes the walls to dissolve and the drug to be released. A great advantage of using
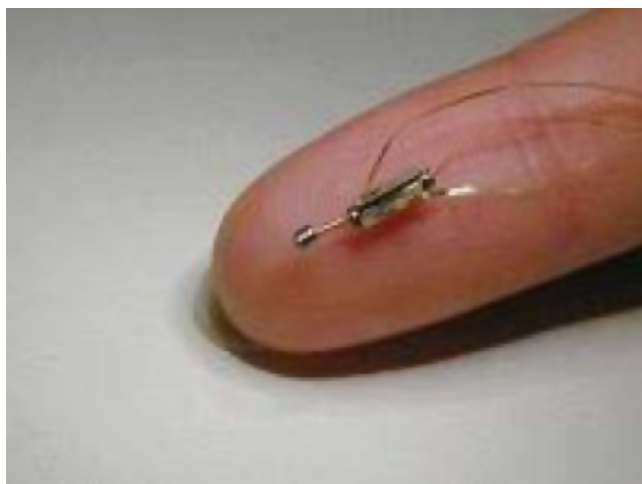
FIGURE 21.1

Device using nanobots for checking blood contents.

Amazing Nanobots [4].

nanobots for drug delivery is that the amount and time of drug release can be easily controlled by controlling the electrical pulse [5]. Furthermore, the walls dissolve easily and are therefore harmless to the body. Elan Pharmaceuticals has already started using this technology in their drugs Merck's Emend and Wyeth's Rapamune [6]. Nanomedicine could make use of these nanorobots (e.g., Computational Genes), introduced into the body, to repair or detect damages and infections. Using nanotechnology, the drug can be targeted to a precise location which would make the drug much more effective and reduce the chances of possible side effects. In the future, these nanorobots could actually be programmed to repair specific diseased cells, functioning in a similar way to antibodies in our natural healing processes.

21.4.1.2 Use of Nanorattles

Tang and her collaborators [7] demonstrated that silica nanorattles (rattle-type nanoparticles consisting of a spherical shell encapsulating a freely moving core particle in solvent) show advantages for *in vivo* enhancement of therapy efficacy and reducing the systematic toxicity of antitumor drugs. The enhanced tumor inhibition of the docetaxel-loaded silica nanorattles may be attributed to the sustained docetaxel release from the nanorattles *in vivo* as well as the accumulation of drug-loaded nanorattles in the intratumor due to enhanced permeability and retention effect once intravenously administered (Figure 21.2).

21.4.2 Nanodiagnostics and Disease Prevention

21.4.2.1 Biosensors

Nanodiagnostics utilizes biosensor technology, which is one of the most promising, compact systems consisting of a composite analysis of biological recognition element (DNA, protein, etc.). Detecting
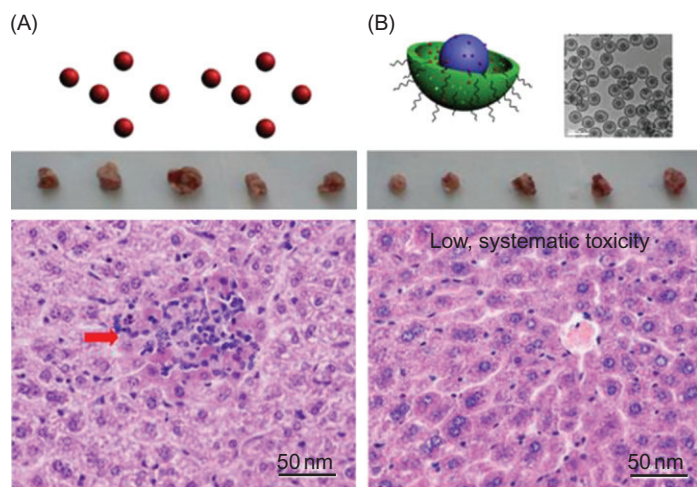
FIGURE 21.2

After encapsulation into the silica nanorattle, the antitumor drug docetaxel had increased therapy efficacy and decreased systematic toxicity for liver cancer therapy: (A) free drug and (B) drug-loaded silica nanorattle.

Tang Group, Chinese Academy of Sciences [7].

an analyte (glucose, antibiotics, etc.) using a transducer element or detector element to quantify the amount of analyte is the working principle of biosensors. The *transducer* or the *detector element* (works in a physicochemical way; optical, piezoelectric, electrochemical, etc.) transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transducers) that can be more easily measured and quantified.

21.4.2.2 Diagnosis Using Nanobots
Nanobiotechnology scientists have successfully produced microchips that are coated with biological molecules. The chip is designed to emit an electrical impulse signal when the molecules detect signs of a disease. Special sensor nanobots can be inserted into the blood under the skin where they can check blood contents and warn of any possible diseases. They can also be used to monitor the sugar level in the blood. Advantages of using such nanobots are that they are very cheap to produce and easily portable [5].

21.4.2.3 Quantum Dots
Quantum dots are nanomaterials that glow very brightly when illuminated by UV light. They can be coated with a material that makes the dots attach specifically to the molecule they want to track. Quantum dots bind themselves to proteins expressed in cancer cells, thus helping to visualize tumors [9] (Figure 21.3).
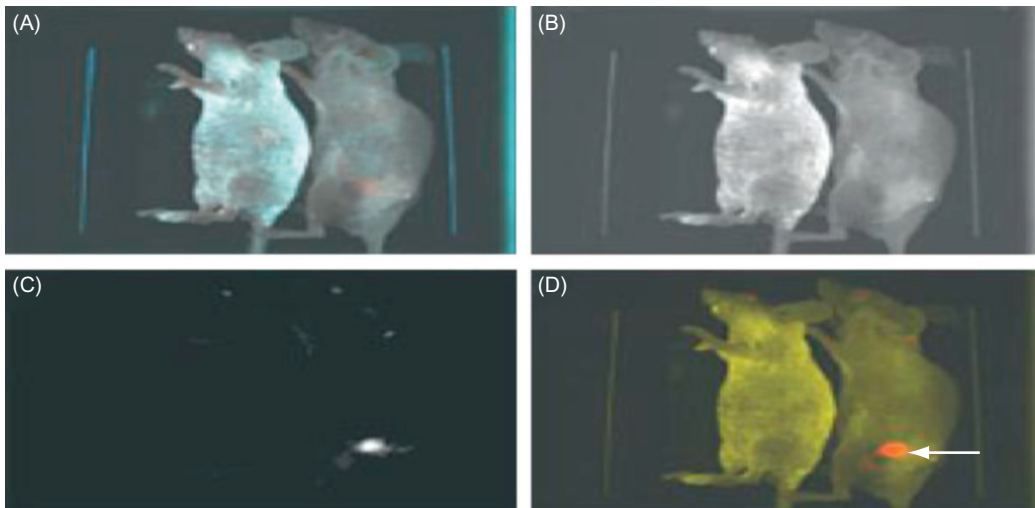
FIGURE 21.3

A light in dark places. Spectral imaging of quantum dots. White spot indicated with white arrow in 21.3D indicate a prostate tumor growing in a live mouse [4].

21.4.2.4 Regenerative Medicine

Nanotechnology will also play an important role in the field of regenerative medicine. This area is associated with the use of pluripotent stem cells that can develop into other various types, so that could, at least in theory, replace tissue destroyed by diseases such as diabetes, ischemic heart disease, Alzheimer's, Parkinson's, spinal cord injuries, muscular dystrophy, retinal degeneration, and many more [8].

21.4.3 Disease Prevention

21.4.3.1 Cardiovascular Interventions

It has been proposed that nanobots can also be used to prevent heart attack (cardiac failure) in the future. Cardiac failure is caused by fat deposits blocking the blood vessels. Nanobots can be directed to remove these fat deposits [5]. Figure 21.4 shows the artists imagination of nanobots removing the yellow fat deposits on the inner side of blood vessels.

While nano- and microparticle-based imaging of cardiovascular interventions is still in its developing phase, it has already presented the exciting potential to monitor primary interventional procedures for precise therapeutic delivery, enhance the effectiveness of delivered therapeutics, and monitor therapeutic efficiency after interventions performed to treat cardiovascular diseases [10].

21.4.3.2 Nanoparticles and the Blood–Brain Barrier: As Treatment Opportunity

Nanoparticles can be used as carrier systems to overcome the blood–brain barrier (BBB) and deliver specific medications to regions of the brain that would normally be inaccessible. Their surfaces can be coated with certain materials or manufactured such that they can bypass the BBB and transport
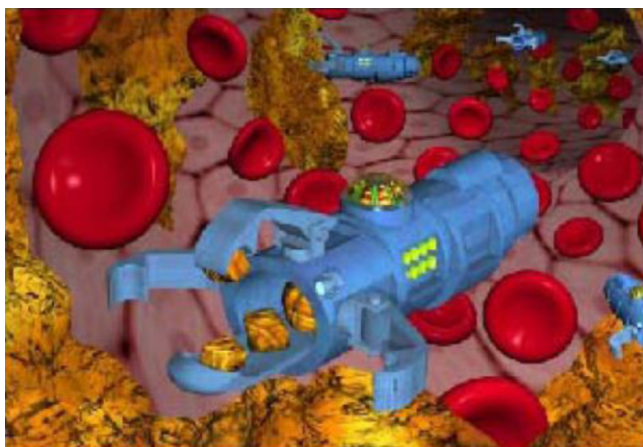
FIGURE 21.4

Nanobots preventing heart attacks (artist's view) [5].

the drug to the sites where they are needed. One currently used form of therapy, although it does not overcome the BBB, is the so-called hyperthermia therapy, which uses nanoiron particles to treat brain tumors such as glioblastoma [11] (Figure 21.5).

21.4.3.3 Tissue Reconstruction

Nanoparticles can be designed with a structure very similar to that of bone. An ultrasound is performed on existing bone structures and then bone-like nanoparticles are created using the results of the ultrasound [3]. The bone-like nanoparticles are inserted into the body in a paste form [6]. When they arrive at the fractured bone site, they assemble themselves to form an ordered structure which later becomes part of the bone [6].

21.4.3.4 Medical Tools

Nanodevices are nanoparticles that are created for the purpose of interacting with cells and tissues and carrying out very specific tasks [3]. The most famous nanodevices are the imaging tools. Oral pills can be taken that contain miniature cameras. These cameras can reach deep parts of the body and provide high-resolution pictures of cells as small as $1\,\mu m$ in width. (A red blood cell is $7\,\mu m$ wide [4].) This makes them very useful for diagnosis and also during surgeries. Figure 21.6 shows such cameras working with other nanoparticles to get rid of a disease.

21.4.4 Other Applications

21.4.4.1 Treatment of Injured Nerves

Another key application for nanoparticles is in the treatment of injured nerves. Samuel Stupp and John Kessler at Northwestern University in Chicago have studied on tiny rod-like nanofibers called *amphiphiles*. They are capped with amino acids and are known to spur the growth of neurons and
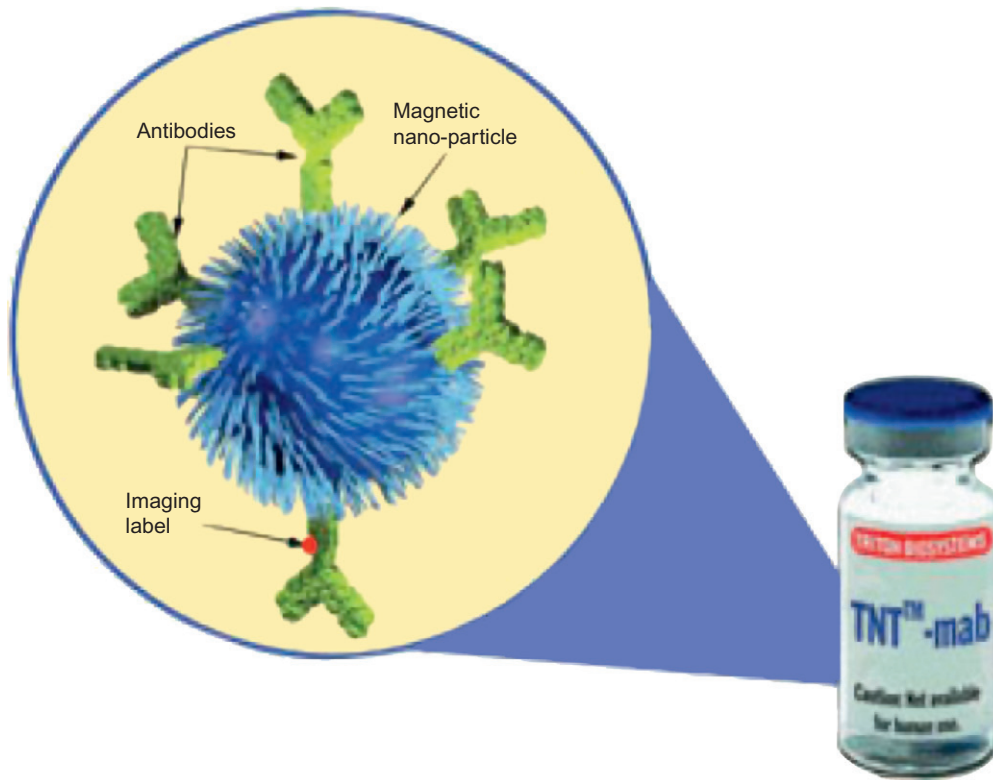
FIGURE 21.5

Cancer Cooker—Triton BioSystems is developing an anticancer therapy using antibody-coated iron nanoparticles [4].

prevent scar tissue formation. Experiments have shown that rat and mice with spinal injuries recovered when treated with these nanofibers [9].

21.4.4.2 Nanocapsules

A nanocapsule consists of shell and a space in which desired substances may be placed. Drug-filled nanocapsules can be covered with antibodies or cell surface receptors that bind to cancer or various cells and release their biological compound on contact with that specific tissue [12]. Polymeric nanocapsules can now be made in specific sizes and shapes. These can then be functionalized by inserting molecules with a particular property onto the shell of nanocapsules. These molecules cause the release of the contents of the nanocapsule in response to a particular biomolecule which would be the triggering mechanism in a targeted drug delivery system.

21.4.4.3 Nanotubes

Carbon nanotubes are tubes of graphite sheets with diameter in the nanoscale, including single wall carbon nanotubes and multiwall carbon nanotubes [12]. The ends of some nanotubes are open, the
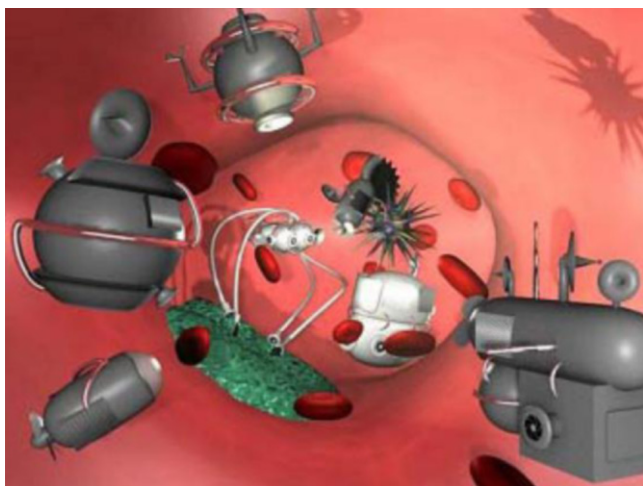
FIGURE 21.6

Miniature cameras inside blood vessels.

Blender Battles [4].

others are closed with full fullerene caps. Carbon nanotubes are named as the "king of nanomaterials." Nanotube drugs have been discovered that kill bacteria. These drugs are more effective than the traditional antibiotics. These nanotubes are about 3 nm in diameter and 6 nm in length. To make them into effective bactericides, they are attached with side chains of the amino acids that make up a tube.

21.4.4.4 Nanosomes

Nanosomes are capable of transporting active ingredients in a specific way and are used as nanoscale vehicles that penetrate into deep layers of skin to deliver vitamin E. Protein and hydrophobic drugs can be encapsulated within these nanosomes. Phospholipid nanosomes may find utility in the enhanced delivery of hydrophobic drugs such as recombinant proteins and nucleic acid as well as hydrophobic anticancer and anti-HIV drugs.

21.4.4.5 Nanowires

Nanowires are the artificial materials that consist of ultrafine wires or linear array of dots. Peptides rich in amino acid histidine have a high affinity for metal ions [13]. Histidines incorporated into nanowires could recruit metals to the surface of wire without attracting the reduced forms. Boron-doped silicon nanowires were used to create highly sensitive, real-time electrically based sensors for biological and chemical species [14].

21.4.4.6 Needle-Free, Painless Vaccinations with Nanopatches

Reporting their findings in a recent issue of *Small* ("Nanopatch-targeted skin vaccination against West Nile virus and chikungunya virus in mice"), Kendall and his collaborators [15] describe the nanopatch approach for directly targeting vaccines to thousands of viable skin antigen presenting cells.
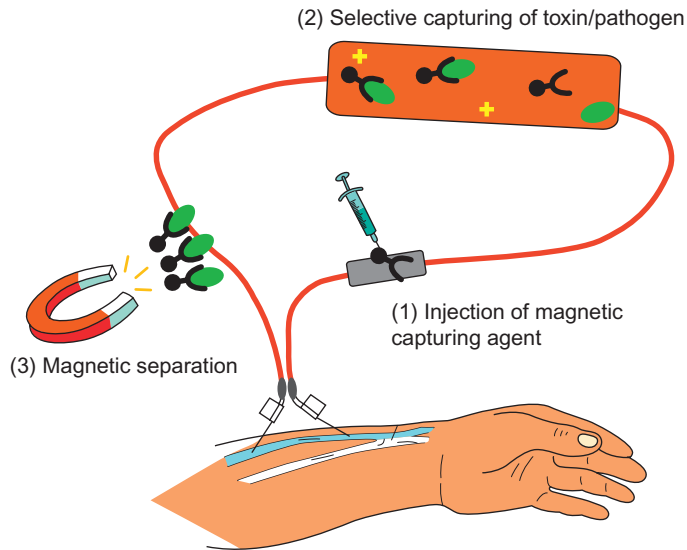
FIGURE 21.7

Schematic depiction of the process of pathogen removal from the blood using nanomagnets [16].

21.4.4.7 Nanomagnets Remove Pathogens from Blood

Demonstrating a novel use of nanomagnets, researchers in Switzerland have rapidly and selectively removed heavy metal ions, overdosed steroid drugs, and proteins from human blood. Rapidly accessible surface of the nanomagnets enables efficient adsorption in contrast to other blood purification techniques available on the market (Figure 21.7).

21.4.4.8 Nanocrystalline Silver

With most applications of nanotechnology in medicine still under development, nanocrystalline silver is already being used as an antimicrobial agent in treatment of wounds [17]. Silver works in a number of ways to disrupt critical functions in a microorganism. It has a high affinity for negatively charged side groups on biological molecules such as sulphydryl, carboxyl, phosphate, and other charged groups distributed throughout microbial cells. Silver attacks multiple sites within the cell to deactivate critical physiological functions such as cell wall synthesis, membrane transport, nucleic acid (RNA and DNA) synthesis and translation, protein folding and function, and electron transport. For certain bacteria, as little as one part per billion of silver may be effective in preventing cell growth [18]. Silver antimicrobial nanotechnology is effective against pathogens associated with biofilms including *E. coli*, *Streptococcus pneumoniae*, *S. pneumoniae*, *Streptococcus aureus*, and *Aspergillus niger* [19]. Local nanoscale characterization of cellular ultrastructure and functional properties is an important focus for probing cariogenic nature of oral microbes. The development of resistance to antimicrobial silver would be extremely rare because an organism would have to undergo simultaneous mutations in every critical function within a single generation to escape silver's influence. Silver

is more efficient than traditional antibiotics because it is extremely active in small quantities, as little as one part per billion of silver may be effective in preventing cell growth.

21.4.4.9 Nanospheres

Nanospheres are hollow nanoscale structures made of polymers. These nanospheres can be loaded with special molecules like anticancer drugs. Injectable nanospheres have important potential applications such as site-specific delivery and medical imaging.

21.5 CONTRIBUTION OF MICROBIOLOGY TO NANOTECHNOLOGY

Considering microbiology in general, nanoscience could be used both to understand and control processes at the molecular assembly level. Just as bacteria were the first models to understand genetics and biochemistry that were later applied to eukaryotic systems, the same is true for the processes of function, recognition, and assembly. Micro (Greek) meaning very small, usually smaller than 1 mm, denotes 10^{-6}, so a micrometer is one millionth of a meter. Nano (Greek) dwarf—goes from small to tiny; the word "nano" means 10^{-9}. So a nanometer is one billionth of a meter. If it is micro it is almost nano; however, going down from the micro- to the nanoscale is not a simple matter of size reduction. The transition between the "small" and the "tiny" involves a radical change in the scientific concepts applicable. The laws of physics that govern the macroscopic world of our everyday experience can be readily used to understand microscopic objects. However, they break down completely in the nanoworld because of the radically different length-scale hierarchies and the underlying quantum mechanical behavior of individual atoms, electrons, or photons.

The term "microbiology" generally describes the study of those organisms invisible to the human eye, in particular yeast, bacteria, and viruses. However, these three types of organisms are significantly different from each other. Yeast and bacteria are different cell types (eukaryotic and prokaryotic, respectively), whereas a virus is not strictly a living organism, being an obligate intracellular parasite. The tools to conduct research in microbiology can be separated into two main fields—microscopy, which is required for visualization, and molecular biology, which has been used to characterize (in some cases comprehensively) the genetic and proteomic makeup of these organisms. The initial steps in opening the field of microbiology came with the advent of the first microscopes. Bacteria were first visualized by Antony van Leeuwenhoek, using a simple, self-built microscope, around 1676. The microscopes built by Leeuwenhoek were not compound microscopes, relying instead on a single lens, more like a very powerful magnifying glass. One of his first descriptions of bacteria (referred to as animalcules) was from samples scraped from the teeth of van Leeuwenhoek himself.

Advancements made in light microscopy by the combined work of Ernst Abbe and Carl Zeiss in the 1880s further extended the research of the microbiological world. However, as Abbe himself had described, there is a limit to the resolution of light microscopy, dependent on the wavelength of the illuminating light and the numerical aperture of the lens. In reality, the resolution of light microscopy is limited to half the wavelength of light, or around 250 nm. As such, the study of the structure of viruses had to waft for the advent of the electron microscope in 1931 by Ernst Ruska and the subsequent crystallization of the tobacco mosaic virus (TMV) in 1935 by Wendell Stanley (Figure 21.8).
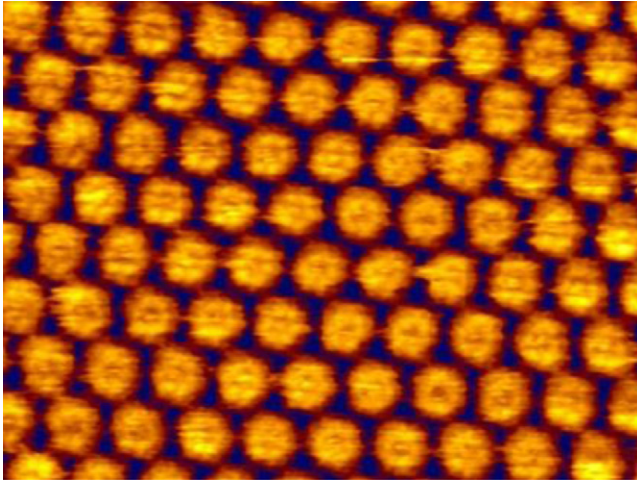
FIGURE 21.8

Hexagonally packed intermediate (HPI) layer from *Deinococcus radiodurans* [20].

21.6 AFM IMAGING OF MICROORGANISMS

More recently, the atomic force microscope has opened a new path for the investigation and manipulation of structures on a very small scale. One of the most often cited advantages of the atomic force microscopy (AFM) in the study of biological structures is the fact that, unlike electron microscopy, high-resolution images can be obtained under physiological conditions. However, there is more to the AFM than just its capacity for high-resolution imaging. The mechanical nature of AFM means that the cantilever, used for imaging, can also be used to measure interaction forces in the piconewton range.

As such, not only can the AFM image the surface of microorganisms at high resolution, under physiological conditions, it can also be used to investigate the binding forces between microorganisms and target surfaces.

21.6.1 Yeast

Saccharomyces cerevisiae, also known as budding yeast, is not only of use in industrial processes from bread making to beer brewing, but it is also a type organism used in the study of eukaryotic cells. The yeast *S. cerevisiae* is surrounded by a cell wall composed of proteins, polysaccharides, and small amounts of chitin. Electron microscopy has shown that this cell wall is a layered structure ranging up to 300 nm thick. When imaged with AFM, the surface of the cell appears very smooth and is easily deformed, necessitating careful scanning at minimal force (Figure 21.9). The only apparent surface feature is the bud scar at the opposite end of the mother cell to the newly forming daughter cell. The surface appears smooth as the sugars obscure the membrane.
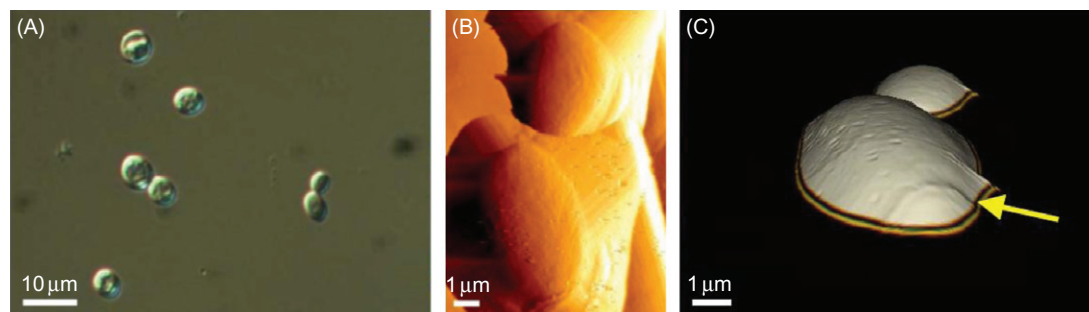
FIGURE 21.9

Imaging of *S. cerevisiae*. Yeast cells were located using DIG microscopy (A) and then imaged in contact mode in fluid with the AFM (B). In (C) a 3D image generated from the height channel is displayed, highlighting the bud scar on the mother cell (white arrow) [20].

21.6.2 Bacteria

As AFM is a surface imaging technique, it has been used to characterize surface structures at high resolution. The most commonly used laboratory bacteria is *E. coli*, a gram-negative bacteria. Gram-negative bacteria have a plasma membrane, surrounded by a periplasmic space in which there is a rigid but highly porous cell wall of peptidoglycan. This is then surrounded by an outer membrane, from which lipopolysaccharides of varying length extend.

Here, two strains of *E. coli*, DH5a and OP50, have been imaged. The images of DH5a show the classic, rod shape of many gram-negative bacteria. The cells were scanned in air (Figure 21.10) and in buffer (Figure 21.11). When imaged in air, the surface of the bacteria appears highly patterned. In addition, a halo around the bacterium is apparent. These structures likely correspond to pill, which are found at the surface of *E. coli*. In contrast when imaged in fluid (Figure 21.11), the surface of the bacterium appears much smoother. In this case this is due to the fact that the surface structures would be easily displaced by the movement of the tip during scanning, as they are not fixed in place.

The OP50 strain, while also *E. coli*, appears quite different. OP50 was originally isolated as a strain that could be used to feed *Caenorhabditis elegans*. It is a uracil-requiring strain that is more fragile and smaller than other *E. coli* strains. When imaged in air (Figure 21.12), these bacteria do not exhibit the same structured surface as seen for the DH5a. In addition, the cells are more fragile and must be carefully imaged to avoid removing them from the surface. In fluid, the surface is also less structured than that of DH5a, and regions of the cell surface are displaced in the scan direction, likely corresponding to the displacement of the sugars and other flexible structures at the surface of the cell.

21.6.3 AFM Study of the Structure–Function Relationship of the Biofilm-Forming Bacterium *Streptococcus mutans*

AFM has garnered much interest in recent years for its ability to probe the structure, function, and cellular nanomechanics inherent to specific biological cells. In particular, AFM has been used to probe the important structure–function relationships of the bacterium *Streptococcus mutans*.
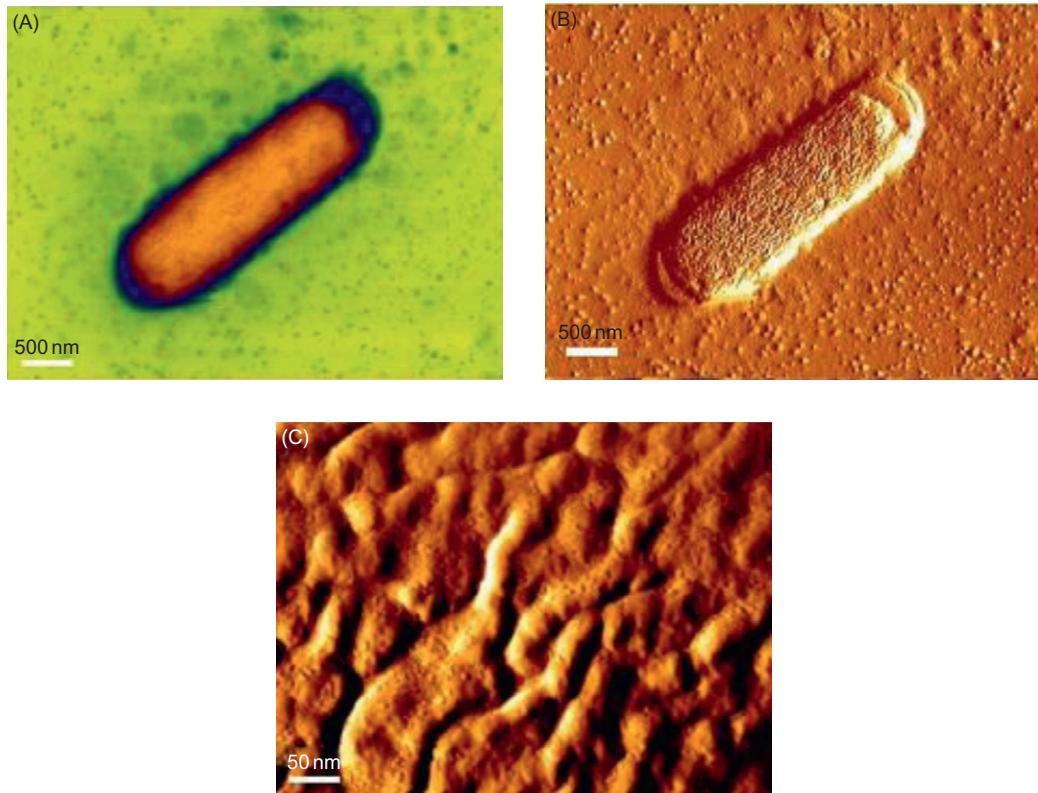
FIGURE 21.10

Intermittent contact mode images of DH5a cells. Overview height (A) and error signal (B) images, and a higher-magnification error signal image (C) of the surface of the bacterium [20].
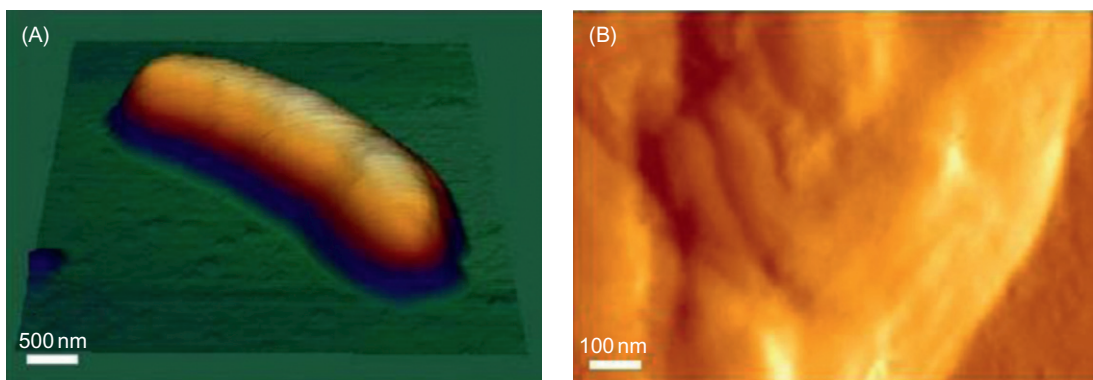
FIGURE 21.11

Intermittent contact mode images of DH5a in fluid. A 3D image generated from topographic data (A) and a higher-magnification error signal image (B) are displayed [20].
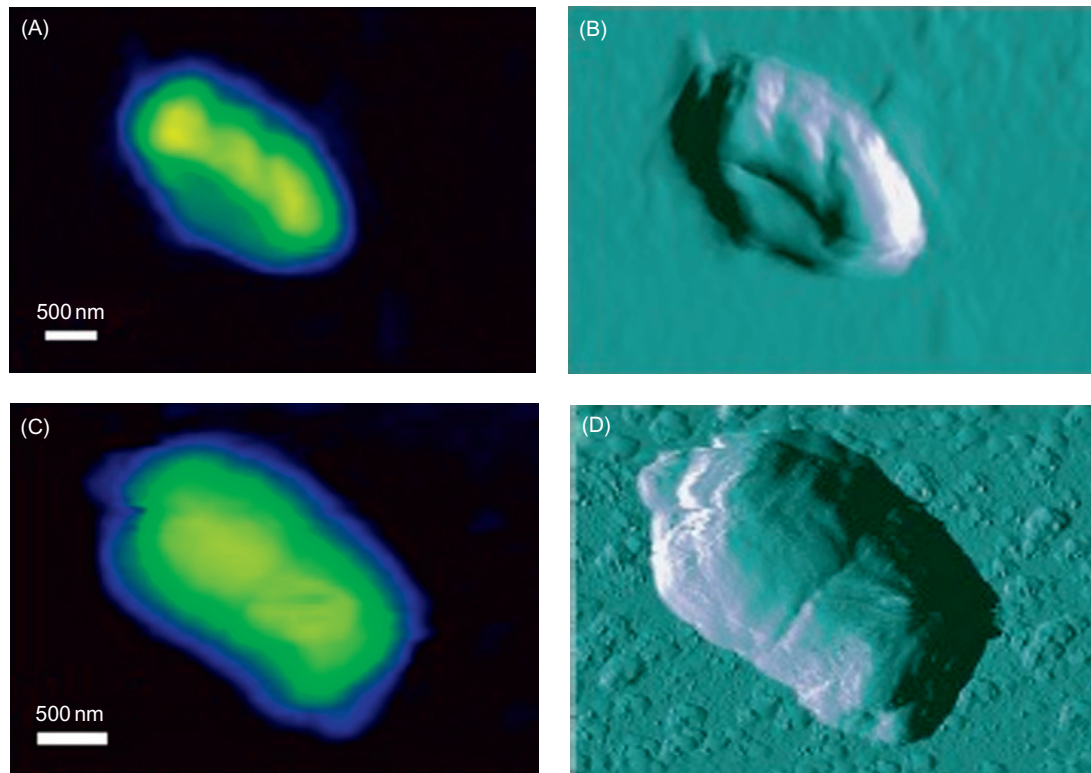
FIGURE 21.12

Images of OP50 bacteria imaged in air (A, topography; B, error signal) and fluid (C, topography; D, error signal) [20].

S. mutans is the primary etiological agent in human dental caries (tooth decay), and it is of medical importance due to the virulence properties of these cells in biofilm initiation and formation, leading to increased tolerance to antibiotics. AFM has been used to characterize the unique surface structures of distinct mutants of *S. mutans*. These mutations are located in specific genes that encode surface proteins, thus using AFM characteristic surface features have been resolved for mutant strains compared to the wild type. Ultimately, characterization of surface morphology has shown distinct differences in the local properties displayed by various *S. mutans* strains on the nanoscale, which is imperative for understanding the collective properties of these cells in biofilm formation [19].

21.6.4 Viruses

Viruses are obligate intracellular parasites composed of an outer protein coat surrounding genetic material that consists of either DNA or RNA. This genetic material does not contain all the information required for replication; instead the virus needs to subvert the cellular machinery of the host cell
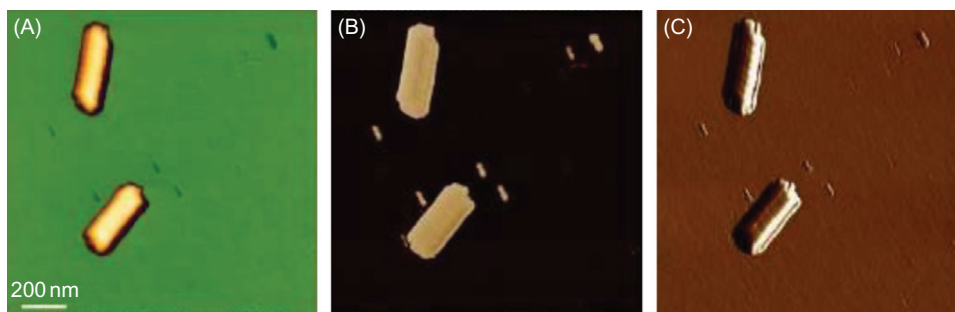
FIGURE 21.13

Intermittent contact mode images of TMV particles. Topography (A), phase (B), and error signal images (C) of the same sample region are presented [20].

to propagate. Viruses are extremely small, around 20–400 nm. This means that the characterization of viral structure requires high-resolution imaging techniques, such as AFM. Here TMV has been imaged, the first virus imaged using electron microscopy.

The TMV particles were adsorbed to mica and imaged using intermittent contact mode. These virus particles are known as helical capsids, where the coat protein stacks in a helical pattern around the genetic material. This helical stacking can be seen in the height, phase, and error signal channels (Figure 21.13). One significant advantage of using a BioAFM, such as the JPK Nanowizard®, to image biological samples is that the imaging can be conducted in fluid. As such, virus particles can be imaged on the surface of their target cells in fluid. Here, influenza virus has been imaged, attached to the surface of red blood cells, in fluid, using intermittent contact mode. The virus particles are clearly imaged at the surface of the cells (Figure 21.14).

21.7 NANOPLASMONIC SENSORS DETECTING LIVE VIRUSES

The recent emergence of H1N1 and H5N1 flu viruses and severe acute respiratory syndrome has highlighted the importance of rapid detection and accurate diagnosis in health-care and preventive medicine. The problem in these areas is that many virus detection platforms have limitations because they are not easily compatible with point-of-care use without the existence of significant infrastructure. Cell culturing is a time-consuming, highly specialized, and labor-intensive process. Therefore, highly sensitive/specific, compact, rapid, and easy to use virus diagnostics are needed to prevent further spread at the onset of a viral epidemic.

Unlike techniques based on external labeling, such resonance shifting operates as a reporter of the molecular binding phenomena in a label-free fashion and enables transduction of the capturing event directly to the far field optical signal. Specific detection of viruses in a label-free fashion requires an effective method to distinguish nonspecific binding of the viruses to the plasmonic sensor surface. Selectivity is achieved by surface immobilized highly specific antiviral immunoglobulins showing strong affinity to the viral membrane proteins. Correspondingly, with the use of antibodies, viruses
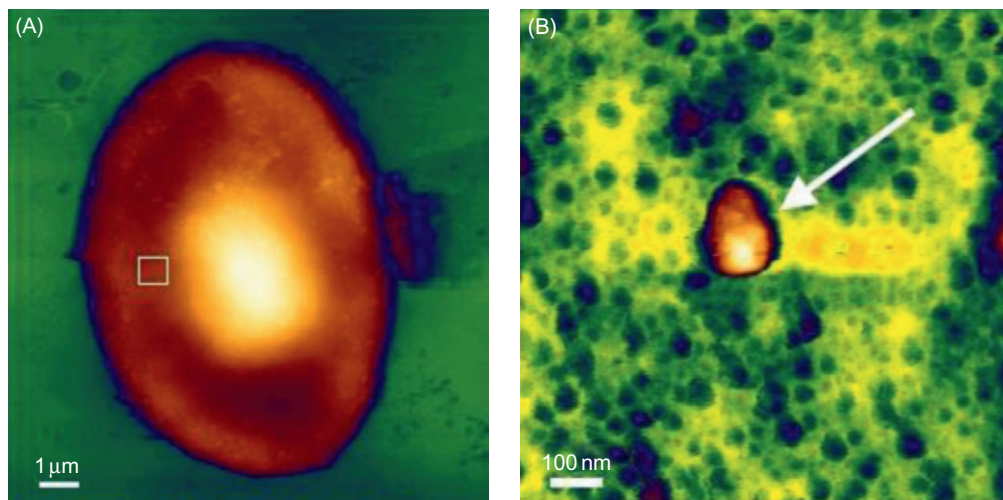
FIGURE 21.14

Influenza virus particles associated with the surface of an erythrocyte. The overview image (A) and higher-magnification image (B) of the red blood cell surface show influenza virus particles (white arrow) [20].

are specifically captured from a sample solution on the surface of our sensor [21]. This is illustrated in Figure 21.15.

21.8 NANODENTISTRY

There are significant advances in dentistry that illuminated the road for the shift from "macro" to "nano" in dental sciences. It is evident that increases in the versatility of scientific knowledge and the ability to control physical processes at a finer resolution naturally lead to more information and, henceforth, to more questions. The broader our knowledge, the more amazement arises in face of natural wonders [22,23]. The same could certainly be said for the field of dentistry. The historic progress in this area naturally goes hand in hand with many new questions and challenges that provide opportunities for improvement. The progress, admittedly, has been slower than might be considered desirable for those who would wish to put a cutting-edge technology to clinical use. For example, early descriptions of the extraction of teeth with the use of forceps by Hippocrates and Aristotle date back to 500–300 BC, a technique that has remained essentially unchanged till date. Likewise, restorations with amalgam and gold date back to years 700 and 1746, respectively, and are still a part of our clinical setting without much change.

21.8.1 The Impact of Nanotechnology

It is, on the other hand, valid to point out that nanotechnology slowly had made its way from the lab bench to any other technological or medical field. This is hampered not only by slow progress
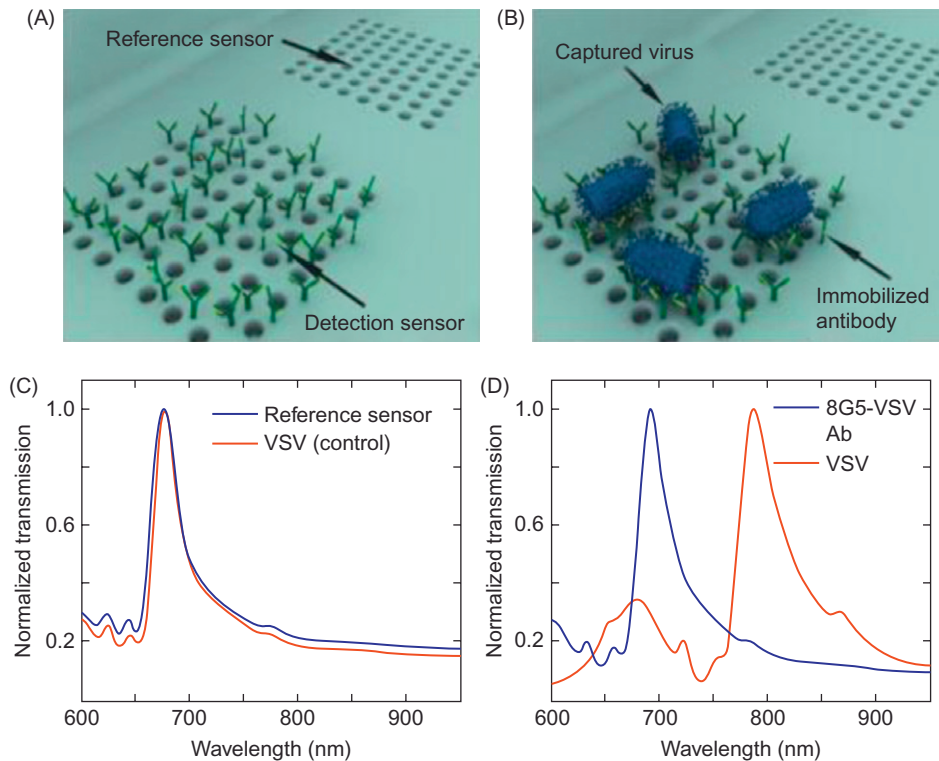
FIGURE 21.15

3D renderings (not drawn to scale) and the experimental measurements illustrate the detection scheme using optofluidic-plasmonic biosensors based on resonance transmissions due to extraordinary light transmission effect. Detection of the vesicular stomatitis virus (VSV) is experimentally observed with a strong red shifting of the plasmonic resonances.

Hatice Altug Research Group, Boston University [21].

in understanding the basics in controlling the nanoscale phenomena, but by strict regulations in the translational stages too [24–26]. This, however, offers a controlled environment for the timely identification of weaknesses and strengths, which are all critical when it comes to introducing a new material or technology into the clinical setting. As we see, nanotechnologies have favored our understanding of dental tissues at the nanoscale and enabled the design of materials with ultrafine architecture. There is a prospect that probing the structure of dental tissues at ever finer size scales and using the dynamic resolution capabilities of advanced nanotools will give us answers to some of the puzzles that occupy dental researchers.

21.8.1.1 Dynamic View of Dental Tissues

The significant advances in dental research that revealed the nanoperspective of dental tissues have been explored over the past decade. When viewed at the nanoscale resolution, these structures, otherwise considered as static and stagnant, have fascinating structural dynamism.

Enamel is composed of 92–94 vol% fibrous apatite crystals with ~20 nm in diameter and ultrahigh length-to-width aspect ratio [27]. Precise spatial arrangement of these fibers gives rise to a super-structural organization with symmetry displayed at various size scales, ranging from nano to micro [28]. Morphogenesis of enamel proceeds through interaction between nanospheres or nanofibers of amelogenin, the main protein of the developing enamel matrix, and the growing crystals of apatite [29]. With the advancement of real-time nanoscale visualization techniques [30,31], there is a prospect that a clearer picture of these highly dynamic phenomena that govern the formation of dental and other biological tissues can be visualized. Fostering understanding of the dynamical nature of enamel should thus give rise to finer reparative techniques. For instance, understanding what molecules are able to pass through the biological barrier that enamel offers against the oral environment, as first investigated by Dibdin [32], might offer insights into drug delivery techniques that can give guidance in reaching more advanced approaches for enamel tissue engineering and regeneration.

The dentin matrix is mainly composed of type I collagen fibrils with associated noncollagenous proteins, forming a three-dimensional organic scaffold that is reinforced by mineral. The mineral is a nanocrystalline hydroxyapatite that is partitioned according to its location with respect to the collagen fibrils into extrafibrillar mineral, which is located in the spaces separating the collagen fibrils [33–35] and intrafibrillar mineral, which is mainly in the gap regions of the fibrils extending between tropocollagen molecules [36–38]. It is noteworthy that the current concepts of restorative dentistry ignore most of the recent findings that elucidate the structure and function of dentin at a nanometer scale, which suggests that the modernity of the technologies currently used may be brought into question.

21.8.1.2 What Are We Really Bonding to?

Hybrid layers, responsible for dentin bonding, form when adhesive comonomers infiltrate demineralized (i.e., acid-etched) dentin collagen fibrils. However, many studies have shown that nanoleakages, which are only around 20–100 nm wide [39,40] compared to 10–20 μm wide microleakage gaps, occur within the hybrid layer even in the absence of gap formation. Although the spaces are too small to allow for bacterial penetration, they are large enough for enzymes and water to enter and cause degradation of resin/dentin bonds over time. The original interpretation of nanoleakage was that the silver dye used for the leakage studies occupied nanometer-sized spaces around naked collagen fibrils, where resin failed to infiltrate, or where residual water had not been displaced by the adhesive resin [41]. An improved understanding of the nanostructure of collagen fibrils in dentin would lead to reinterpretation of nanoleakage occurrence and may provide insights into the fundamental limitations of adhesives used in the restorative dental materials.

21.8.1.3 "Small Is Beautiful" of Dental Science: Small Structures, Great Strength

Nanotechnology has been applied in dentistry in the early 1970s with the beginning of the era of microfills. Microfilled composites comprises silicon dioxide filler particles with less than 100 nm in diameter in conjunction with prepolymerized organic fillers, aggregated by crushing them into larger filler particles. Nowadays, the most commonly used resin composites, i.e., microhybrids and nanofilled composites, comprise filler particles ranging from ~20 to 600 nm. Other dental nanotechnologies rely on the delivery of molecules that facilitate tooth structure remineralization by means of noninvasive dental techniques that forestall caries, the latter being an active area of nanoresearch in dentistry. In agreement with Saunders [42], the most tempting venue for speculation on the next

phase of nanorestoration of tooth structure is biomimetics, i.e, mimicking processes that occur in nature [43,44].

In the case of biomaterials, nanoparticulate materials appear to strongly influence the host response at both cellular and tissue levels, which makes nanotechnology particularly attractive for dental implants [45]. Processes such as sol–gel deposition [46], pulsed laser deposition [47], sputtering coating techniques [48,49], ion beam assisted deposition [50], and electrophoretic deposition [51]are a few examples of nanotechnology-based approaches that have been used to develop bioceramic thin-film coatings for implant surfaces. The main objective of these newer technologies is to reduce the thickness and the particle size of the coating layer and thereby increase its specific surface area and reactivity, thus improving the interaction with the surrounding living tissue.

21.8.1.4 Biofilm Formation and Treatment

Nanoscience has also recently promoted emerging concepts in oral microbial ecology, which may soon redefine our understanding of biofilm formation and treatment. Recent analyses with ribosomal RNA-based technologies have revealed the diversity of bacterial populations within dental biofilms and have highlighted their important contributions to oral health and disease [52]. In enamel, the aim appears to be unraveling the ways to mimic nature's own nanotechnological mechanism by which the cooperative interaction between the nanoscale self-assemblies of amelogenin and the uniaxially oriented apatite crystals proceeds [42,53]. Dentin, on the other hand, is linked to far more challenging scenarios. There appears to be a long and tortuous path to make a step from promising results to the actual transition of dental tissue engineering methodology from the lab to the clinical setting. The field of diagnostics of oral diseases is also a subject of rapid evolution. Proteomic analyses by mass spectrometry with their ability to identify proteins at ultralow concentration levels have a chance of drastically improving the diagnostic sensitivity and efficiency [54].

Saliva is now recognized as an excellent diagnostic medium for the detection of malignant tumors that are either within or are remote from the oral cavity [55]. Containing biomarkers for various diseases, the identification of which is currently under investigation, saliva holds great promises for early detection of disease and/or monitoring therapeutic outcomes through a noninvasive approach [56]. Other oral components, such as gingival crevicular fluid, epithelial cells, breath, and dental plaque, also have diagnostic potential [57]. The future of dentistry will thus undoubtedly witness routine and mechanistic restorations ceding place to a more holistic clinical practice where each particular case is analyzed in the context of the organism as a whole.

Finally, in parallel with the strong shift in the field of chemistry away from the traditional reference to strong, chemical bonding effects to the control of weak physicochemical interactions [58] (that has given rise to the prosperous practical framework of self-assembly and soft/wet chemistry [59]), a similar shift away from the mechanically interfering reparative methods toward soft remineralization techniques can present one of the most promising streams in the modern dental science. Despite the seemingly slow development of the dental field, we should keep in mind that scientific fields develop in waves. Computer science has rapidly expanded in the previous two decades or so, whereas the theoretical physics set the quantum mechanical fundaments for its slow subsequent development in only a few decades at the turn of the twentieth century. Let us hope that one such big breaking wave is on the horizon for the world of dental science. In our opinion, to surf on that promising wave, learning the art and know-how offered by modern nanotechnologies will be a must.

21.8.2 **Nanotechnology in Periodontics**

Some of the futuristic applications of nanotechnology in dentistry have been outlined as follows. Freitas has described how medical nanorobots might utilize specific motility mechanisms to crawl or swim through human body tissues with navigational precision, acquire energy, sense and manipulate their surroundings, achieve safe cytopenetration [60]. Functions may be controlled by an onboard nanocomputer executing programmed instructions in response to local sensor stimuli. Alternatively, the dentist may issue strategic instructions by transmitting his orders directly to *in vivo* nanorobots via acoustic signals (e.g., ultrasound).

21.8.2.1 *Local Anesthesia and Hypersensitivity Cure*

Colloidal suspension containing millions of active analgesic micron-size dental nanorobots will be instilled on the patient's gingiva. After contacting the surface of the crown or mucosa, the ambulating nanorobots will reach the dentin by migrating into the gingival sulcus and passing painlessly through the lamina propria or the $1–3\,\mu m$ thick layer of loose tissue at the cemento-dentinal junction. Upon reaching the dentin, nanorobots enter $1–4\,\mu m$ diameter dentinal tubule holes and proceed toward the pulp, guided by a combination of chemical gradients, temperature differentials, and positional navigation, all under onboard nanocomputer control. Assuming a ~10mm total path length from tooth surface to pulp, a very modest nanorobot travel speed of $100\,\mu m/s$ completes the journey into the pulp chamber in ~100s. The presence of natural cells that are constantly in motion around and inside the teeth suggests that such journeys should be feasible.

Once installed in the pulp and having established control over nerve impulse traffic, the analgesic dental nanorobots may be commanded by the dentist to shut down all sensitivity in any particular tooth that may require treatment. When the dentist presses the icon for the desired tooth on the handheld controller display, the selected tooth immediately numbs. After the oral procedures are completed, the dentist orders the nanorobots (via the same acoustic data links) to restore all sensation, to relinquish control of nerve traffic, and to egress from the tooth by similar pathways used for ingress, followed by aspiration. Reconstructive dental nanorobots would selectively and precisely occlude selected tubules in minutes, using native biological materials, offering patients a quick and permanent cure.

21.8.2.2 *Natural Tooth Maintenance and Repair*

The appearance and durability of tooth may be improved by replacing upper enamel layer with covalently bonded artificial materials such as sapphire or diamond, which have 20–100 times the hardness and strength of natural enamel. It can be made more fracture resistant possibly including embedded carbon nanotubes.

Major tooth repair may evolve through several stages of technological development:

- first using genetic engineering,
- tissue engineering and
- later growing whole new teeth *in vitro* and installing them.

Ultimately, the nanorobotic manufacture and installation of a biologically autologous whole replacement tooth including both mineral and cellular components (e.g., complete dentition replacement therapy) should become feasible to undertake within the time and economic constraints of an ordinary office visit, using an affordable desktop manufacturing facility in the dentist's office.

21.8.2.3 *Nanorobotic Dentifrice (Dentifrobots)*

The futuristic proposals of Dentifrobots include subocclusal-dwelling nanorobotic dentifrice delivered by mouthwash or toothpaste could patrol all supragingival and subgingival surfaces at least once a day, metabolizing trapped organic matter into harmless and odorless vapors and performing continuous calculus debridement (invisibly small ($1–10\mu m$) dentifrobots, 103–105 nanodevices/oral cavity and crawling at $1–10\mu m/s$).

It would be inexpensive purely mechanical devices which can safely deactivate themselves if swallowed and programmed with strict occlusal avoidance protocols.

Properly configured dentifrobots could identify and destroy pathogenic bacteria residing in the plaque and elsewhere, while allowing the ~500 species of harmless oral microflora to flourish in a healthy ecosystem.

Dentifrobots could also provide a continuous barrier to halitosis, since bacterial putrefaction is the central metabolic process involved in oral malodor.

With this kind of daily dental care available from an early age, conventional tooth decay and gum disease will disappear into the annals of medical history.

21.9 CONCLUSIONS

Nanotechnological advances should be viewed in the context of other expected developments relevant to oral health in the coming decades. Nanodentistry faces many significant challenges in bringing its promises to fruition. It is hoped that dental nanorobots in due time will make fast, painless, and precision dentistry a reality. At the same time, continual refinement of traditional methods, development of advanced restorative materials, and new medications and pharmacologic approaches will continue to improve dental care. Nanotechnology is still in its early stages. A few applications discussed in this chapter have already been developed and are already helping patients all over the world. As further research continues in this field, more treatment methodologies will be discovered. Many diseases that do not have a cure today may be cured by nanotechnology in the future. Some of the concerns were also discussed, but with proper care these problems can be avoided. Scientists who are against the use of nanotechnology also agree that advancement in nanotechnology should continue because this field promises great benefits, but testing should be carried out to ensure the safety of the patients. In the near future, nanotechnology will one day become part of our everyday life and will help prevent a lot of dental pathological conditions.

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