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Bio-Nanotechnology
A Revolution in Food, Biomedical and Health Sciences

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Dedicated to my well respected and beloved guruji, Dr. Basudeb Achari, PhD.

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Dedicated to my beloved daughter Dipanjali Bagchi, and my mother Bakul Bardhan, for always giving me inspiration.

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Dedicated to my beloved daughter Atsue, and son, Takanobu.

Hiroyoshi Moriyama

To the memory of my beloved parents.

Fereidoon Shahidi
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Foreword

Predictions indicate that well over a million scientists and technologists will work in nanoscience and nanotechnology-related areas over the next decade. Indeed, nanoscale advances in science and technology promise applications in almost every area, with revolutionary socio-economic impacts. We can, for instance, expect major developments in research areas as diverse as nanocomposite materials for solar power generation to nanoscale devices with precise function for future medical strategies.

The drug industry has hardly started and is still locked in a Neanderthal mindset, focused mainly on relatively simple molecules to combat diseases. The new field of nanoscience and nanotechnology indicates that we should in future be able to develop medical weapons that are of commensurate sophistication with that of ‘the enemy’. We should be able to develop complex and clever molecular machines which will be able to combat on more even terms viruses and bacteria, which employ subtle strategies for infection. Penicillin is a miracle drug which led to the antibiotics revolution, but in comparison with these enemies it is really a very, very simple system.

The all-carbon hollow cage molecules, the fullerenes, and their elongated cousins, the carbon nanotubes (CNTs) are stable allotropes, which in addition to graphene, graphite and diamond show fascinating promise as basic materials for novel nanoscale applications. The morphology of materials is a fascinating field, and structure-related properties are of key interest in nanoscale engineering, promising nanoscale devices exhibiting advanced performance in sustainable, environmentally friendly applications. As we improve our chemical synthetic capabilities and are able to construct molecular devices with complex function, we can expect these unusual carbon-based systems also to be applied in medical situations. Drug delivery is but one area where fullerene cages promise to be non-toxic carriers of radioactive elements in chemotherapy application.

Particularly exciting is the promise of paradigm-shifting advances in medical strategies. This volume contains one of the first collections of articles addressing this fascinating and challenging area. If all these exciting advances are to be realized, then the next cohort of young biologists and medical practitioners must have a sound education in nanoscale science and technology and this education needs to be integrated into the undergraduate and graduate curricula in student biological and medical courses. This text is a welcome and highly effective response to this challenge, that must be met if we are to develop the effective biomedical technologies we shall certainly need to survive into the next century.

Harold Kroto
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Bio-nanotechnology is the key functional technology of the 21st century, which is emerging around the world. The possibility of exploiting the structures and processes of biomolecules for novel applications in materials, biosensors, bioelectronics and medical applications has created the rapidly growing field of nanobiotechnology. At the nano level, atoms demonstrate extreme diversity and uniqueness. The term ‘bio-nanotechnology’ is a fusion of bioscience and nanotechnology based on the principles and chemical pathways of living organisms, and refers to the functional applications of biomolecules in nanotechnology. It encompasses the study, creation and illumination of the connections between structural molecular biology, nutrition, food science and nanotechnology, since the development of techniques of nanotechnology might be guided by studying the structure and function of the natural nano-sized molecules found in living cells.

The bio-nanotechnology of ‘biomimetic membranes’ describes the current state of research and development in biomimetic membranes for their versatile applications in bio-nanotechnology. The application areas in bio-nanotechnology range from novel nanosensors, to novel methods for sorting and delivering bioactive molecules, to novel drug delivery systems. The success of these applications relies on a good understanding of the interaction and incorporation of macromolecules in membranes and the fundamental properties of the membrane itself.

The biological and physical sciences share a common interest in small structures (ranging from 1 nm to 1 mm). The development of nanoscience around new materials and tools (largely from the physical sciences) and new phenomena (largely from the biological sciences) is already happening. The physical sciences offer tools for the synthesis and fabrication of devices for measuring the characteristics of cells and subcellular components, and of materials useful in cell and molecular biology; biology offers a window into the most sophisticated collection of functional nanostructures that exist.

The present situation regarding the biomaterials that are currently used differs greatly from the situation a decade ago. Although implantable medical devices are still immensely important, medical technologies now encompass a range of drug and nanodelivery systems, tissue engineering and cell therapies, organ printing and cell patterning; and also nanotechnology-based imaging and diagnostic systems and microelectronic devices. These technologies still encompass metals, ceramics and synthetic polymers, but also biopolymers, self-assembled systems, nanoparticles, carbon nanotubes and quantum dots. These changes imply
that our original concepts of biomaterials and our expectations of their performance may have to change. It may be concluded that many substances which were not regarded as biomaterials may now be considered as traditional structural biomaterials. Hence, substances have been engineered and developed to perform functions within health-care, where they are directly controlled by interactions with cells and tissue components. These include engineered tissues, cells, organs and even viruses.

This book is intended for health professionals, nutritionists, food scientists, biologists, physicians and a diverse scientific community. Sir Harold Kroto, eminent Nobel Laureate, Professor at Florida State University, and the inventor of fullerene, wrote the Foreword for this book. Professor Kroto’s support and encouragement gave us the highest level of enthusiasm to complete this book.

The book is divided into nine main sections with forty-four chapters as follows:

- Introduction
- Nanotechnology in Nutrition and Medicine
- Nanotechnology, Human Health and Applications
- Nanotechnology and Other Versatile Diverse Applications
- Nanomaterial Manufacturing
- Applications of Microscopy and Magnetic Resonance in Nanotechnology
- Applications in Enhancing Bioavailability and Controlling Pathogens
- Safety, Toxicology and Regulatory Aspects
- Future Directions in Bio-Nanotechnology

Each chapter gives a detailed description of currently available methods, and contains numerous references to the primary literature, making this the perfect ‘field guide’ for chemists, biologists, biochemists and materials and food scientists who want to explore the fascinating world of bio-nanotechnology.

The book starts with a Foreword, highlighting the importance of bio-nanotechnology in the field of biomedical sciences and applications in human health. There are three chapters in the Introduction section. The first chapter provides a review on the biomedical applications of nanomaterials, while the second chapter highlights the challenges of nanotechnology-derived foods with a special emphasis on addressing the concerns of the public. The third chapter deals with nanotechnology and public health.

The second section emphasizes the applications of nanotechnology in nutrition and medicine. There are four chapters in this section: the first covers functional nanomaterials for biomedical research with an integral focus on bio-functionalization and biomedical applications, and the second provides an overview of nanoparticle-assisted polymerase chain reaction technology. The third chapter demonstrates the medical applications of micellar nanoparticles, and the fourth illustrates the uses of nanotechnology for regenerative medicine.

The third section is entitled Nanotechnology, Human Health and Applications, and comprises seventeen chapters. The first chapter gives an overview of novel technologies for the production of functional foods, the second illustrates nanomedicine, which is described as ‘the revolution of the big future with tiny medicine’, and the third describes the application of γ-cyclodextrin in nanomedicinal foods and cosmetics. The fourth chapter illustrates the application of polymer-based nanocomposites for food packaging, the fifth discusses ultrasound-mediated delivery systems combined with nano/microbubbles of bubble liposomes, the sixth describes nanoprobe and quantum dots, which are described as ‘a novel device to watch biology’, and the seventh highlights enhanced optical biosensors based on nanoplasmonics. The eighth chapter discusses nanobiosensors for mimicking gustatory and olfactory senses, and the ninth chapter describes nanoparticles that induce biointeractive reactions into living organisms, and the tenth chapter discusses novel technology to analyse immunological reactions in nanoscale food. The eleventh chapter gives an overview on green nanotechnology, the twelfth provides a detailed technique for the characterization of bio-polymers and chitosan-based nanocomposites with antimicrobial activity, and the thirteenth discusses the application of nanotechnology in the agriculture and food sectors. The fourteenth chapter highlights the applications of polymeric nanoparticles with steroids, the fifteenth gives an overview on nanocomposites for food packaging, and the sixteenth illustrates the application of nanotechnology in cosmetics. Finally, the seventeenth chapter provides a vivid overview of the potential
medical applications of fullerenes – and we are very proud to have the approval of Sir Harold Kroto, the prime discoverer of fullerene.

The fourth section highlights other versatile and diverse applications of nanotechnology. Two dedicated chapters discuss the biomedical applications of carbon-based nanomaterials and carbon nanotubes. The third chapter discusses the application of the nanoparticle cyclodextrin as an emulsifier, and the fourth chapter highlights the application of poly(γ-glutamic acid)-based nanoparticles as an antigen delivery carrier in cancer immunotherapy. The fifth chapter demonstrates the potential applications of nanobubbles.

The fifth section elaborates on the different nanomaterial manufacturing applications. There are three chapters in this section. The first describes the formulation and characterization of nanodispersions composed of dietary materials for the delivery of bioactive substances. The second illustrates the production of nanoscale food using high-pressure emulsification technology, and the third demonstrates the production of monodisperse fine dispersions by micro/nanochannel emulsification.

The sixth section provides a discussion on the applications of microscopy and nuclear magnetic resonance in nanotechnology. The first chapter discusses the use of atomic force microscopy (AFM) in food nanotechnology, and the second discusses the applications of nuclear magnetic resonance in biomolecular interaction systems.

The seventh section deals with applications in enhancing bioavailability and controlling pathogens. The first chapter demonstrates the bioavailability and delivery of nutraceuticals and functional foods using nanotechnology; the second demonstrates the encapsulation of bioactive compounds into micron/submicron-sized dispersions using microchannel emulsification or high-pressure homogenization, and the third describes nanometric-size delivery systems of bioactive compounds for the nutraceutical and food industries.

The fourth chapter deals with nanoemulsion technology for the delivery of nutraceuticals and functional foods, and the fifth chapter is an application note on nanotechnology and nonpolar active compounds in functional foods.

The eighth section examines the safety, toxicology and regulatory aspects of bio-nanotechnology. The first chapter describes the standardization of nanotechnologies in the USA; the second ties up US FDA with nanotechnology and discusses various salient features on regulatory uncertainty. The third chapter provides a vivid description of the toxicology and environmental risks of nanomaterials. The fourth chapter covers nanoparticle-lung interactions and their potential consequences to human health.

In the final section, the Editors have provided an account of the future directions and expected advancements of bio-nanotechnology in the near future and named the chapter ‘Bio-nanotechnology: a journey back to the future’.

Overall, we have covered a broad spectrum of areas in the field of bio-nanotechnology and human health. First of all, our special thanks go to Nobel Laureate Sir Harold Kroto. Our sincere gratitude and appreciation go to all the eminent scientists, researchers, doctors and authors who worked very hard to contribute to this book. Finally, all four editors sincerely extend their heartfelt gratitude and thanks to Catriona Cooper of Wiley-Blackwell for her unstinting help and cooperation.

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Part 1

Introduction
1 Biomedical Applications of Nanomaterials: An Overview

Sunil K. Singh, Paresh P. Kulkarni, Debabrata Dash
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1.1 Introduction

Nanotechnology (the Greek word *nano* means “dwarf”) is the creation and utilization of materials, devices, and systems through the control of matter at the nanometer-length scale, i.e., at the level of atoms, molecules, and supramolecular structures. It is the popular term for the construction and utilization of functional structures with at least one characteristic dimension measured at nanometer scale—a nanometer (nm) is one-billionth of a meter ($10^{-9}$ m). This is roughly four times the diameter of an individual atom. The width of DNA is approximately 2.5 nm and protein molecules measure 1–20 nm. It is essential to understand nanomaterials and their properties in order to develop innovations in biological systems and medicine. However, it is only in the last 5 years that a new branch of science, known as “nanomedicine,” has emerged as a distinct field, and it has since grown exponentially. The late Nobel physicist Richard P. Feynman had the visionary idea that tiny nanorobots could be designed, manufactured, and introduced into the human body to perform cellular repairs at the molecular level. In his prescient 1959 talk, “There’s plenty of room at the bottom,” he proposed using machine tools to make smaller machine tools, which could be used in turn to make still smaller machine tools, and so on all the way down to the atomic level [1]. Feynman was clearly aware of the potential medical applications of the new technology he was proposing. As perceived by Feynman, it is extremely likely that nanomedicine, a multidisciplinary field that embraces biology, chemistry, physics, engineering, and materials science, will play a major role in the betterment of the human condition.

Nanomedicine offers examples of how nanotechnological tools are being utilized in biomedical research. The overall goal of nanomedicine is the same as it always has been in medicine: to diagnose as accurately and early as possible, to treat as effectively as possible without side effects, and to evaluate the efficacy of treatment noninvasively. The promise that nanotechnology brings is multifaceted, offering not only improvements to current techniques but also providing entirely new tools and capabilities. By manipulating drugs and other...
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materials at the nanometer scale, their fundamental properties and bioactivity can be altered. These tools can permit control over characteristics of drugs or agents such as solubility, blood pool retention times, controlled release over short or long durations, environmentally triggered controlled release, or highly specific site-targeted delivery. Furthermore, by using nanometer-sized particles, the increased functional surface area per unit volume can be exploited in various ways. This chapter presents some of the more recent successes in applying various nanomaterials and tools in the biomedical field. It also gives researchers a comprehensive overview of the present status and suggests future directions for employing nanomaterials to attain as yet unmet biomedical goals.

The unique optical, magnetic and electronic properties of nanomaterials provide promising platforms for a wide variety of biomedical applications [2–8] including biosensing, imaging, and drug delivery (see Fig. 1.1). As all the properties of nanomaterials are size- and shape-dependent, the study of methods for their preparation is one of the primary research areas. Traditionally, synthetic approaches to nanomaterials have been divided into two categories: “top-down” and “bottom-up.” A typical “top-down” procedure – also called a physical method – involves the mechanical grinding of bulk material and the subsequent stabilization of the resulting nanosized particles by the addition of colloidal protecting agents [9–10]. A “bottom-up” procedure attempts to build nanomaterials and devices one molecule/atom at a time, much in the same way that living organisms synthesize macromolecules. In this chapter we provide an overview of synthetic approaches to carbon-based nanomaterials, wet chemical methods for the fabrication of metallic nanoparticles (NPs) that
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for anticancer therapy [43–45], antioxidant and antiapoptotic agents for the treatment of amyotrophic lateral sclerosis [46] and Parkinson’s disease [47], and in many other applications.

- **Carbon nanotubes (CNTs):** CNTs are one of the most widely used nanomaterials because of their remarkable physical, chemical, and biological properties. There are two classes of CNTs: single-walled (SWCNTs) and multi-walled (MWCNTs). Theoretically, nanotubes are viewed as rolled-up structures of single or multiple sheets of graphene to give SWCNTs and MWCNTs, respectively. These one-dimensional carbon allotropes have a large surface area, high mechanical strength with ultra-light weight, rich electronic properties, and excellent chemical and thermal stability. Because of their unique physiochemical properties, researchers have been exploring their potential in biological and biomedical applications [48–54]. CNTs can easily be surface functionalized to bind proteins and nucleic acids, and hence are emerging as novel components in nanoformulations for the delivery of therapeutic molecules [5, 55].

- **Nanodiamond particles (NDP):** Diamond NPs have been investigated as single-particle bio-markers for fluorescence imaging [56–58]. The surface of NDP can be functionalized to bind proteins and nucleic acids, allowing NDP to be used as a carrier for pharmaceutical agents or oligonucleotides [59–62].

- **Graphene:** The distinct structural properties of graphene, in particular its high aspect ratio, propensity to functional modification, unique electronic and optical properties, as well as its potential biocompatibility, makes it an extremely attractive candidate for biomedical applications such as biosensor development, imaging, drug delivery, bacterial inhibition, and photothermal therapy [16, 63–72].

- **Quantum dots (QDs):** QDs are semiconductor nanocrystals with spatially confined excitation states. Crucial for their biological applications is the need to coat them with other materials to allow their aqueous dispersion and to prevent leakage of the toxic heavy metals. QDs have been widely used in imaging and cell labeling either in vitro or in vivo [73–75]. QDs have also been successfully used to quantify fluorescence rely on the chemical reduction of metal salts, and biocompatible synthesis procedures for quantum dots and other biomedically important nanomaterials. A large variety of stabilizers such as donor ligands and surfactants, as well as surface modifications or functionalization, are used to control the growth of the primarily formed nanoclusters and to prevent them from agglomerating [11–16]. Depending upon the nature of the materials and their unique properties, nanomaterials can be categorized as follows.

- **Metallic NPs:** Among the different nanomaterials employed in biomedical research, metallic NPs have proved to be the most convenient and suitable. Based on their unique optical, physical, and electrical properties, metallic NPs have found significant applications in a wide spectrum of biomedical utilities such as imaging, sensing, drug delivery, and gene targeting [17–22]. Reports from our laboratory and others suggest that some of these NPs also have significant therapeutic potential [23–38]. Their applications are constantly increasing in view of the relatively lesser toxicity reported with these NPs. In the second section of this chapter we discuss biomedically important and extensively studied metallic NPs such as silver and gold nanoparticles.

- **Carbon-based nanomaterials:** Carbon nanomaterials (CNs) include fullerenes, nanotubes, nanodiamonds (ND), and graphene.

- **Fullerenes:** Fullerenes are novel carbon allotropes with a polygonal structure made up exclusively of 60 carbon atoms. Soluble derivatives of fullerenes such as C60, with a soccer ball–shaped arrangement of 60 carbon atoms per molecule, show great promise as pharmaceutical agents. Nanostructures are characterized by the presence of numerous points of attachment whose surfaces can be functionalized for tissue binding. These derivatives, many of which are already in clinical trials, have good biocompatibility and exhibit low toxicity even at relatively high dosages. Fullerene compounds can be employed as antiviral agents, most notably against human immunodeficiency virus [39], antibacterial agents (e.g., *Escherichia coli* [40], *Streptococcus* [41], *Mycobacterium tuberculosis* [42]), photodynamic agents for anticancer therapy [43–45], antioxidant and antiapoptotic agents for the treatment of amyotrophic lateral sclerosis [46] and Parkinson’s disease [47], and in many other applications.
bio-nanotechnology changing the relative influence of interfacial properties and bulk properties through the characteristic dimensions of the components. The principal parameters of NPs are their shape, size, and the morphological substructure.

As most properties of metallic NPs are size- and shape-dependent, methods for their preparation are one of the primary areas of interest for nanoscientists. Nanostructured metal colloids could be obtained by “top-down” and “bottom-up” approaches as discussed in Introduction section. Synthesis of biocompatible or biogenic NPs is one of the major challenges to be overcome so as to realize their biomedical application. In this context, some major advances have been made by employing methods based on chemical reactions in solution (often termed “wet chemistry”). A wet chemical procedure involves growing nanoparticles in a liquid medium containing various reactants, in particular reducing agents such as sodium borohydride [29], potassium bitartrate [90], methoxypolyethylene glycol [91], or hydrazine [92]. However, in most of these procedures the strong chemical reductants have now been replaced by more biocompatible reagents such as glucose. Stabilizing agents such as donor ligands, polymers, and surfactants are often employed to prevent NPs from agglomerating and to make them easily miscible under cellular conditions. A surfactant is a molecule that is dynamically adsorbed to the surface of the NPs under the reaction conditions. It must be mobile enough to provide access for the addition of monomer units, while remaining stable enough to prevent the aggregation of NPs. Some examples of suitable surfactants or stabilizing agents include alkyl thiols, phosphines, phosphine oxides, phosphates, amides or amines, carboxylic acids, sodium dodecyl benzyl sulfate, or polyvinyl pyrrolidone [12, 13, 92]. As most of the surfactants used have low compatibility with cells and tissues, bovine serum albumin is a popular choice for use as a stabilizing agent. Scientists have recently endeavoured to make use of microorganisms as possible eco-friendly nanofactories for the synthesis of metallic NPs [93] such as cadmium sulfide [94], gold [95], and silver [96]. In recent years, metallic NPs and their alloys have been studied extensively in various fields such as sensor technology [18], optical devices [3], catalysis [97], biological labeling [98], drug delivery systems [21], and

1.2 Metallic NPs

Among the different nanomaterials used in biomedical research, metallic NPs have proven to be the most convenient and suitable. The properties of NPs depend on their structure and composition, and can typically be engineered or modified by

Other biomedically important nanomaterials: Apart from the nanomaterials discussed above, various other nanoparticles have widespread acceptance in the biomedical field, including magnetic nanoparticles, which provide many exciting opportunities in biomedical applications. The ease of optimization of size according to requirement, manipulability by external magnetic force, contrast enhancement in magnetic resonance imaging (MRI), and other such desirable properties of magnetic NPs have recently been exploited in various applications in the field of biology and medicine, including protein purification, drug delivery, imaging, tagging, sensing, and separation [81, 82].

Polymeric nanoparticles are colloidal structures composed of synthetic or semisynthetic polymers. Polymers such as polysaccharide chitosan–polyacrylic acid, polyacrylic acid cogenic acid, poly-caprolactone, and chitosan nanoparticles have been used as drug carriers [83–85]. The drug is dissolved, entrapped, encapsulated, or attached to a nanoparticle matrix.

Liposomes are nanoparticles comprising a lipid bilayer membrane surrounding an aqueous interior. The amphiphilic molecules used for the preparation of these molecules have similarities between the biologic membranes and so have been used for improving the efficacy and safety of new drugs [86–88].

Solid lipid nanoparticles have been proposed as a new type of colloidal drug carrier system suitable for intravenous administration [89]. The system consists of spherical solid lipid particles in the nanometer range, which are dispersed in water or in surfactant solution.

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treatment of some cancers [99]. Metallic NPs are suitable as markers for the optical detection of biomolecules due to their excellent SPR (surface plasmon resonance) properties. Reports from our laboratory and others suggest that some of these NPs can also have significant therapeutic potential as antimicrobial [24, 29] and antiplatelet agents [23] as well as for the stabilization of proteins, drug delivery, and photothermal tumor ablation. These are extremely promising prospects in the field of health and medicine. Here we discuss the biomedical applications of two extensively exploited metallic NPs: silver and gold.

1.2.1 Silver NPs and their biomedical applications

Nanosilver particles are generally smaller than 100 nm and contain 20–15,000 silver atoms (see Fig. 1.2). Silver NPs (AgNPs) have been receiving considerable attention as a result of their unique physical, chemical, and biological properties, and have found important applications in optics, electronics, and medicine. In addition, nanosilver has innate antimicrobial [24, 29] and antiparasitic activity [100]. Silver NPs support surface plasmons, attributed to the collective oscillation of electrons on the particle surface upon excitation with electromagnetic radiation, which contributes to their unique optical properties. At specific wavelengths of light, the surface plasmons are driven into resonance and the AgNPs have a distinct color that is a function of their size, shape, and environment. The plasmon resonance of silver NPs is responsible for the yellow color of the solution. Any visible change in the color of NPs in solution typically indicates a change in the extent of aggregation of these NPs. The peak absorption wavelength of AgNPs is in the range of 405–430 nm and is evidence for the formation of AgNPs.

As described in previous reports, we have successfully synthesized biocompatible AgNPs with enhanced stability and significant antibacterial activity by using glucose as the reducing agent [24] and bovine serum albumin as the stabilizing agent [101]. As mentioned above, another approach to the synthesis of biocompatible silver NPs that is a biogenic process involves the utilization of microorganisms. Several attempts have been made in this direction. When the bacterium Pseudomonas stutzeri AG259, isolated from a silver mine, is placed in a concentrated aqueous solution of silver nitrate, it brings about reduction of Ag$^+$ ions and the formation of AgNPs of well-defined size and distinct topography within its periplasmic space [102]. Ahmad et al. (2003) and Nanda and Saravanan (2009) have also successfully synthesized silver NPs using the fungus Fusarium oxysporum [96] and the bacterium Staphylococcus aureus [103], respectively. Based on their unique physiochemical properties, AgNPs have found significant applications in a wide spectrum of biomedical utilities such as imaging and therapeutics, especially as antimicrobial agents.

1.2.1.1 Antimicrobial properties

Silver nanoparticles have been shown to exhibit significant antimicrobial activity as well as enhancing the action of conventional antibiotics [104]. They have cidal activity against several bacterial species, and are in fact more efficient and broad-spectrum than the majority of conventional antibiotics. Recent studies have also shown that nanosilver has higher microbicidal activity than ionic silver. The study demonstrated the ability of colloidal silver to inhibit the growth and multiplication of
bacterial strains including those that are multi-drug resistant. The antimicrobial activity of colloidal silver particles is influenced by the dimensions as well as the shape of the particles; the bactericidal activity increasing with a decrease in size of the particles. Recently one group has successfully synthesized AgNPs having broad-spectrum activity with efficacy against both Gram-positive and Gram-negative bacteria [104]. Thus, NPs of silver have extensively been investigated for their antibacterial properties. Considerable efforts have been made to explore this activity through electron microscopy, which has revealed a size-dependent interaction of AgNPs with bacteria [105]. The study concluded that nanosilver particles mainly in the size range of 1–10 nm attach to and disrupt the cell membrane. They were also found distributed within the cell, affecting other important cell organelles. NPs of silver have been used as a medium for antibiotic delivery, and to synthesize composites for use as disinfecting filter and coating materials [32]. However, the bactericidal properties of these NPs depend on their stability in the growth medium, since this provides a greater retention time for bacterium–nanoparticle interaction. It has proved challenging to prepare NPs of silver stable enough to significantly restrict bacterial growth. In our earlier report [24], we demonstrated the synthesis of highly stable NPs of silver endowed with significant antibacterial properties (see Fig. 1.3).

Efforts have been made to understand the underlying molecular mechanism of such antimicrobial actions. In this report we have for the first time shown that silver NPs can change the protein profile of bacteria by interacting with protein molecules that are involved in bacterial cell signaling. Bactericidal properties of the NPs are related not only to the direct effects of silver NPs accumulating intracellularly or at the cell membrane, but also to the ionic or dissolved silver derived from NPs, which also possesses significant antibacterial properties.

In addition, NPs of silver also exhibit antiviral and antifungal properties. It has been reported by Elechiguerra et al. (2005) that AgNPs in the size range 1–10 nM bind with HIV-I in a size-dependent fashion [26]. These authors have shown that silver NPs inhibit HIV-1 infection in CD4+ MT-2 cells and cMAGI HIV-1 reporter cells. Kim et al. evaluated the efficacy of AgNPs as an antifungal agent against yeast [29].

1.2.1.2 Bio-imaging

Due to good surface characteristics, ease of preparation, and easily accessible excitation wavelengths in the visible range, silver is the most preferred metallic substrate for surface enhanced Raman spectroscopy (SERS). SERS is a powerful and sensitive analytical tool for the detection and identification of a wide range of molecules and is even suitable for single molecule detection. Due to its high sensitivity and the fact that water has very weak Raman scattering, SERS has been recognized as one of the most effective tools for biomolecule detection. Aqueous solutions of AgNPs have been extensively used for identifying proteins, studying the interactions of various drugs with proteins for drug discovery, understanding the effects of pH and other factors on the conformation of proteins, for DNA detection at concentrations down to \(10^{-13}\) M, for developing microarray type gene probes, and for biodetection and biolabeling. Novel fabrication techniques to develop unique nanostructured silver-based SERS substrates to fully exploit the tremendous potential of SERS in biomedical research are currently ongoing. Metal-enhanced fluorescence (MEF) is the increase in fluorescence emission intensity of fluorescent molecules when placed near metallic NPs. The

![Fig. 1.3](image-url)  
**Fig. 1.3** Dose-dependent inhibition of growth rate (µ) of different bacterial strains by silver nanoparticles.
quantum yield, excitation rate, and photostability of weakly fluorescing species can be significantly increased by silver nanoparticles. Therefore, MEF could considerably improve the performance of current fluorescence-based techniques by using silver NP-coated substrates and could make a significant impact in areas such as drug discovery, high throughput screening, immunoassays, clinical diagnostics, and protein–protein detection. Aptamer-based silver nanoparticles are used in intracellular protein imaging and single NP spectral analysis, where the AgNPs act as an illuminophore and the aptamer as a biomolecule-specific recognition unit [106].

1.2.1.3 Therapeutics

Nanosilver is also used as a biocide to prevent infection in burns, traumatic wounds, and diabetic ulcers [37]. Other uses include improved surface coating for indwelling catheters and other medical devices implanted on/within the body [38]. Tian et al. investigated the effect of AgNPs on wound healing and scar tissue formation using thermal injury, diabetic wound, and chronic wound models in mice [107]. This study also demonstrated the efficacy of AgNPs in controlling local and systemic inflammatory responses following burn injury by cytokine modulation. Recently, we have for the first time shown that nanosilver has innate antiplatelet properties and that it effectively prevents integrin-mediated platelet responses, both in vivo and in vitro, in a concentration-dependent manner (see Fig. 1.4) [23]. Our findings further suggest that these NPs do not possess any lytic activity against platelets and hold the potential to be promoted as antiplatelet/antithrombotic agents after a careful evaluation of toxic effects. Thus, nanosilver is expected to have increasing applications in medicine and related disciplines.

1.2.2 Gold nanoparticles and their biomedical applications

In past decades, gold NPs (AuNPs) have aroused considerable interest and have been explored as a model platform for biomedical research because of their unique physical and chemical properties [4, 17,108, 109]. Gold particles are inert, which makes them relatively more biocompatible. The synthesis of AuNPs with diameters ranging from a few to several hundred nanometers is well established in aqueous solution as well as in organic solvents. Like AgNPs, AuNPs are synthesized through a chemical reduction method. In a typical synthesis, gold salts such as HAuCl₄ are reduced by the addition of a reducing agent that leads to the nucleation of Au ions to NPs. Turkevich et al. (1951) for the first time synthesized the colloidal gold Au⁰ from AuIII by using citric acid as the reducing agent [110], a method that is still used nowadays after subsequently replacing the citrate ligand of these AuNPs with the appropriate ligands of biological interest [19]. Recent modifications of the Turkevich method have allowed better size distribution and size control within the 9–120 nm range [111]. In addition, stabilizing agents are also required, which are either adsorbed or chemically bound to the surface of the AuNPs. These agents (also known as surfactants) are typically charged, so that like-charged NPs repel each other, rendering them colloidal stable. Although AuNPs can be stabilized by a large variety of stabilizers (ligands, surfactants, polymers, dendrimers, biomolecules, etc.), the most robust AuNPs, discovered by Giersig and Mulvaney (1993), were stabilized by thiols using the strong Au–S bond between the soft acid Au and the soft thiolate base [112]. Along these lines, by far the most popular synthetic method using such sulfur coordination for AuNP stabilization is the Shiffrin–Brust biphasic synthesis using HAuCl₄, a thiol, tetraoctylammonium bromide, and NaBH₄ in water–toluene, yielding thiolate-AuNPs [113]. Since the solubility of these AuNPs is controlled by the solubilizing properties of the terminal group of the thiolate ligands, AuNPs can be transferred from an aqueous phase to an organic phase or vice versa by appropriate ligand exchange.

Water-soluble AuNPs typically contain terminal carboxylate groups at their periphery. The carboxyl group is used to attach to the amino groups of biomolecules using 1-ethyl-3(3-dimethylaminopropyl)-carbo diimide-HCl (EDC) [114]. With related strategies almost all kinds of biological molecules can be attached to the particle surface. Although such protocols are relatively well established, bioconjugation of Au nanoparticles is still not trivial and characterization of the synthesized conjugates is necessary, in particular
to rule out aggregation effects or non-specific binding during the conjugation reaction. In particular, the number of attached molecules per gold nanoparticle is only a rough estimate in many conjugation protocols, as no standard method for determining the surface coverage of particles modified with molecules has yet been established [115]. Interestingly, it is possible to synthesize not only spherical AuNPs but also other geometries such as rod-shaped particles or hollow shells by using the appropriate techniques. Au nanoparticles have been primarily used for labeling applications. In this regard, the particles are directed and enriched at the region of interest and provide contrast for the observation and visualization of this region.

Gold particles strongly absorb and scatter visible light. Upon absorption, the light energy excites the free electrons in Au particles to a collective oscillation, the so-called surface Plasmon resonance (SPR). Generally, the optical properties of small metal NPs are dominated by SPR [19, 116, 117]. For gold, it happens that the resonance frequency is governed by its bulk dielectric constant and lies in the visible region of the electromagnetic spectrum.

Fig. 1.4 Transmission electron micrographs demonstrating inhibition of platelet activation by silver nanoparticles.