Antimicrobial Coatings for Biodegradable Polymers

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The Medical Nanorevolution

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Cambridge
Scholars
Publishing

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This book first published 2023

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-3648-3
ISBN (13): 978-1-5275-3648-7

To Jure and Ernest

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PREFACE

In an era where the relentless march of science and technology shapes the contours of our world, the realm of medicine and healthcare stands as a testament to the remarkable progress humanity has achieved. Yet, amid these strides towards healthier lives, a persistent challenge continues to cast a shadow on our achievements – the escalating threat of antimicrobial resistance. As we grapple with the evolution of pathogens that defy conventional treatments, the need for innovative and effective solutions has never been more pressing.

This book delves into a groundbreaking approach to combating microbial threats: the world of antimicrobial nanoparticles. These minuscule entities, engineered at the nanoscale, harness the power of physics and chemistry to interact with microbes in ways previously unimagined. By exploring the intersection of nanotechnology and microbiology, we embark on a journey that promises to redefine how we wage war against infections.

The pages that follow offer a comprehensive exploration of antimicrobial nanoparticles – from their origins and fundamental principles to their cutting-edge applications in medicine, industry, and beyond. We will uncover the science behind their antimicrobial prowess, unravel the mechanisms that grant them their potency, and dissect the intricate dance between nanoparticles and microbes on scales invisible to the naked eye.

The scope of this book extends beyond the laboratory bench, delving into the ethical considerations and societal implications that accompany the emergence of these novel technologies. As we venture into this frontier, questions of safety, regulation, and responsible innovation loom large. How do we balance the extraordinary potential of antimicrobial nanoparticles with the need to ensure their judicious and sustainable use? What challenges must be surmounted to harness their benefits without unintended consequences?

The journey through these pages will introduce you to the pioneers and visionaries who have dedicated their lives to unraveling the secrets of antimicrobial nanoparticles. It will take you through the collaborative efforts of interdisciplinary teams that bridge the gap between nanoscience, medicine, and engineering. It will shed light on the breakthroughs that have already saved lives and transformed industries, while also illuminating the challenges that lie ahead on this uncharted path.

Whether you are a seasoned researcher seeking to expand your horizons, a student eager to explore the cutting edge of science, a healthcare professional grappling with the realities of antimicrobial resistance, or simply a curious mind intrigued by the promises of nanotechnology, this book invites you to join us on a voyage of discovery.

In an age where microbes are ceaselessly evolving, adapting, and challenging our ingenuity, the potential of antimicrobial nanoparticles offers a glimmer of hope. Together, let us embark on this expedition to unlock the potential of these tiny sentinels and forge a new chapter in our ongoing battle for a healthier, safer world.

Welcome to the world of antimicrobial nanoparticles.

Iva Rezić

ACKNOWLEGEMENTS

This book is dedicated to my precious teacher(s) Gyurme Chokyi Sengye Rabjam Rinpoche and Dilgo Khyentse Yangsi Rinpoche, who are an eternal inspiration in becoming a better person, and to my first teachers, mother Renata Penzar and father Josip Penzar, as well as to all mentors and professors I was privileged to work over many years until now.

Many results could have not been discovered without the efforts of multidisciplinary teams and collaboration with dear coworkers from different institutions and universities, so my gratitude goes to all of them.

Last but not least, this book is for you, dear reader, I hope you will find it useful, providing advice, support, and inspiration that will help you in your everyday work.

CHAPTER ONE

NANOREVOLUTION IN MEDICINE[1]

Introduction

Nanoparticles, possessing dimensions spanning 1 to 100 nm, manifest either naturally or through artificial means. They pervade various technological breakthroughs, spanning from intricate electronics to cutting-edge medical treatments. The remarkable strides in nanotechnology have fundamentally transformed the role of nanoparticles, both within the scientific realm and, more recently, across society at large. The realm of medicine has duly acknowledged the significance of polymer-coated nanoparticles, harnessing their exceptional physical, chemical, antibacterial, antimicrobial, and protective attributes. Notably, their biomedical application takes precedence, given that their nanoscale structures align with numerous biological molecules. This alignment bestows them with a myriad of crucial functionalities, encompassing targeted drug conveyance, imaging, photothermal therapy, and sensory applications. Furthermore, adept manipulation at the "nano-scale" level permits the tailoring of their attributes, thus achieving the precise properties requisite for distinct biomedical domains, encompassing electronics, optics, surface plasmon resonance, and physicochemical characteristics.

> Impact of nanotechnology extends deeply into both society and the scientific community. At present, nanomaterials stand at the forefront of advancements in enhancing the functionalities of diverse polymer surfaces, ranging from textiles to items created through 3D printing.

The progress we witness today in the realm of biomedical nanoparticle applications is a direct outcome of the advancements in engineered nanoparticle synthesis and utilization. Within this context, a diverse array of polymeric and metallic nanoparticles is under intense scrutiny for potential biomedical uses. This exploration serves as a pivotal area of

[1] Rezić, I. Nanoparticles for Biomedical Application and Their Synthesis, Polymers 2022, 14, 4961. https://doi.org/10.3390/polym14224961

extensive research, centring on the characterization and refinement of inherent attributes encompassing electronic, optical, and physicochemical traits, alongside the manipulation of surface plasmon resonance. The alteration of specific nanoparticle characteristics, such as size, shape, or aspect ratio, leads to transformative changes in all the aforementioned properties. This process underscores the dynamic nature of nanoparticle modification and its crucial role in shaping their suitability for various biomedical applications. [1]. Nanoparticles exhibit a remarkable capacity for facile synthesis and modification, enabling the induction of unique electronic, optical, magnetic, medical, catalytic, and mechanical attributes. This potent transformation process is responsible for generating a significant surface-to-volume ratio and triggering the quantum size effect, both of which are profoundly influenced by nanoparticle dimensions, configuration, and morphology.

The convergence of nanoparticles and polymers gives rise to materials poised to revolutionize the biomedical landscape. The list of nanoparticles applied on materials for biomedical application is presented in Table 1.1.

Table 1.1. Biomedically applied nanoparticles [1, 2,3].

Nanoparticle	Size	Nanoparticle	Size
Silver, Ag	1.5–350 nm	Magnesium, MgO	20–100 nm
Aluminium, Al	18 or 80 nm	Manganese, Mn_2O_3	30–60 nm
Gold, Au	50–150 nm	Molybdenum, Mo	70 nm
Boron, B_2O_3	40–80 nm	Nickel, Ni	20–50 nm
Barium, $BaSO_4$	1–5 um	Praseodymium, Pr_6O_{11}	15–30 nm
Carbon, C	3–400 nm	Silicon, Si	30–70 nm
Cerium, CeO_2	15–105 nm	Silicon, SiO_2	15–80 nm
Cobalt, Co	28 nm	Samarium, Sm_2O_3	15–55 nm
Chromium, Cr	50 nm	Tin, SnO_2	45–60 nm
Copper, Cu	25–500 nm	Strontium, $SrTiO_3$	100 nm
Dysprosium, Dy_2O_3	30 or 55 nm	Titanium, Ti	30–50 nm
Erbium, Er_2O_3	20–53 nm	Titanium, TiO_2	200 nm
Europium, Eu_2O_3	30–58 nm	Wolfram, W	50 nm
Iron, Fe	25–250 nm	Yttrium, Y_2O_3	20–40 nm
Gadolinium, Gd_2O_3	15–80 nm	Zinc, Zn	80–130 nm
Indium, In_2O_3	30–50 nm	Zinc, Zn	200 nm
Lanthanum, La_2O_3	15–30 nm	Zirconium, ZrC	30–60 nm

Through the strategic incorporation of precise nanoparticles into polymers, a realm of novel materials envisioned as biomedical devices takes shape. These materials find their application in a diverse range of contexts,

including woven and nonwoven medical textiles, polymers, and various other practical implementations.

Among the spectrum of diverse engineered nanoparticles, silver nanoparticles are estimated to hold the highest degree of commercialization. [2]. A great diversity of synthetic bottom-up and top-down methods is used for developing and producing nanoparticles of various chemical compositions, sizes, and shapes [3]. Nonetheless, a majority of these techniques rely on wet chemical processes rooted in solution-phase colloidal chemical reactions, frequently entailing rigorous conditions. In addition to these conventional methodologies, nanoparticles can also be synthesized through environmentally friendly approaches that minimize water consumption and operate at lower temperatures. Notably, enzyme-mediated reactions exemplify such green methods that offer a promising alternative for nanoparticle production.

> Production of nanoparticles by green methods efficiently enables industrial application and production in large quantities. Among others, enzymes such are urease or cellulase are very efficient in synthesis of nanoparticles, but have a drawback of being very expensive.

The production of nanoparticles by enzymes offers many advantages: reactions are performed at ambient temperature, moderate pH values are sufficient for an effective enzymatic reactions, the control of reactions is easy, produced nanoparticles can be easily combined with other organic or heat sensitive materials (e.g., proteins), the crystalline phase of the produced nanoparticles can be different from the one(s) obtained by conventional methods and lead to new products, and the morphology of nanoparticles can be controlled by enzymatic reaction engineering.

> Interactions of nanoparticles with living cells are numerous, and currently are under investigation of many multidisciplinary and interdisciplinary researchers. Their toxic effects and influence on the mechanisms of bacteria cells leads to very efficient antimicrobial effects. Such effects are recorded not only on model bacteria systems (gram positive and gram negative) but also against fungi. In addition, the effects were achieved also against microbial strains that are resistant to antibiotics.

A fundamental attribute defining nanoparticles is their scale. Comparisons highlight this significance, as novel engineered nanomaterials match the dimensions of established biological nanostructures. For instance, the DNA double-helix boasts a 2 nm diameter, while the bacteria extend to a length of 200 nm. Nanoparticles encompass precisely this size range. This unique property enables the engineering of colloidal nanoparticles to

function as "living nanodrugs," effectively emulating bacteria. Expanding on this concept, the prospect arises for the creation of "robotic nanodrugs." These envisioned entities could be meticulously designed to orchestrate molecular processes within the bodies of humans or animals. Such a paradigm holds the potential to revolutionize medical interventions by precisely manipulating molecular events at the nanoscale. [4].

Interactions of nanoparticles with living organisms

The interaction between nanoparticles and living organisms can be scrutinized across various tiers, spanning from molecular and cellular levels to higher, more systemic tissue levels. This interaction is intricately influenced by diverse routes of administration, encompassing inhalation, intranasal, intravenous, dermal, muscular, and other modalities. This multidimensional interplay engages the exploration of multiple interdisciplinary domains. Researchers from both toxicological and therapeutic perspectives are actively investigating the dynamics of these interactions. Their focus centers on the development of a new breed of nano-immuno-therapeutic drugs, facilitated through comprehensive in vitro and in vivo preclinical trials. However, it's essential to underscore that while the primary interaction of nanoparticles with immune cells hinges on the nanoparticles' physical and chemical characteristics, the mechanisms underlying these interactions remain elusive and cannot be conclusively predicted. [4,5]. Synthetic polymers crafted by human ingenuity undergo meticulous control during their production process, resulting in end products endowed with precisely delineated attributes. These encompass well-defined features such as specific molar mass, distinct architecture, hydrophobic or hydrophilic properties, crystallinity, and strategically positioned functional groups within the molecular structure. Within this context, particular attention is dedicated to gold nanoparticles (Au Nanoparticles), whether unadorned or coated with a polymer layer. This heightened interest stems from the remarkable advantages that position Au nanoparticles as prominent contenders within the diverse realm of nanoparticles tailored for bio-applications.

The pivotal qualities that underscore the prominence of Au nanoparticles are their inherent biocompatibility and the innate simplicity with which their surfaces can be tailored through a wide spectrum of molecules. These attributes bestow upon them a special stature, elevating them to the status of "stars" within the realm of nanoparticles suitable for diverse bio-related applications. (Figure 1.1).

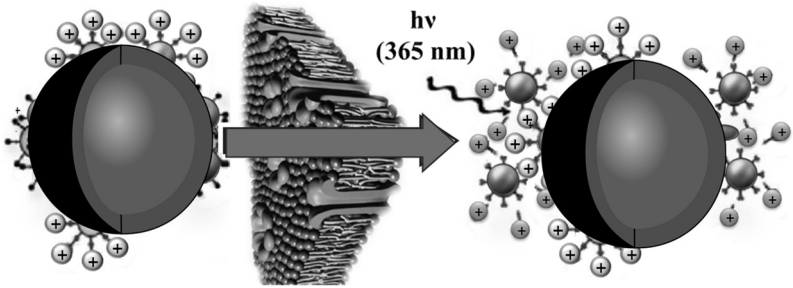

Figure 1.1. Schematic illustration of the photo-induced intracellular controlled release of gold nanospheres. Upon UV irradiation, the photo-labile linker on the gold nanoparticles is cleaved, changing the surface charge of gold nanoparticles from positive to negative and allows the release of the drugs for cancer therapy [5].

The pivotal qualities that underscore the prominence of Au nanoparticles are their inherent biocompatibility and the innate simplicity with which their surfaces can be tailored through a wide spectrum of molecules. These attributes bestow upon them a special stature, elevating them to the status of "stars" within the realm of nanoparticles suitable for diverse bio-related applications. (Figure 1.1). Prominent synthetic methods employed for the production of Au nanoparticles encompass the following techniques: Seed-Mediated Method (Zsigmondy Nuclear Method):

This approach involves a two-step process for generating nano-rods and nano-prisms. It leverages seed particles as a foundation for controlled growth. Thermal Decomposition (Polyol Synthesis): This method utilizes polyvinylpyrrolidone (PVP) as a surface-capping agent. Thermal decomposition within a polyol medium induces nanoparticle formation. Template Mediated Synthesis: Non-spherical Au nanoparticles are crafted in situ, shaped to match a template. Templates can include porous materials' channels, organic surfactants or block polymers, and even biological entities like DNA or viruses. Galvanic Replacement Reaction: Introduced by Brenner and Riddell, this process involves the spontaneous reduction of metal ions into metallic particles and films, operating independently of an external electric field. Distinct from these techniques, enzymatically catalyzed reactions stand out for their environmentally friendly and cost-effective attributes in nanoparticle production.

Within this vast array of controlled synthesis methods, several Au nanoparticle forms hold particular significance due to their yield and reproducibility: Nanorods, Prisms, Shells, Cages: These varied morphologies are meticulously crafted, yielding high-quality nanoparticles with controlled shapes. Hollow Nanostructures (e.g., Stars): These intricate

structures boast empty interiors, augmenting their potential applications. Of notable importance are Gold Shells, spherical nanoparticles characterized by a dielectric core (e.g., silica, polystyrene, or sodium sulfide) encased in a thin gold layer. Such techniques and nanoparticle varieties collectively contribute to a diverse toolkit for tailored Au nanoparticle production.

Applications of Au nanoparticles can be categorized into different technological areas (energy, environment, information technologies, and bio-applications) and are based on their outstanding properties [6], such as, for example, optical property to absorb and scatter light with extraordinary efficiency [7] (Genet and Ebbesen, 2007). Recent applications cover the application of Au nanoparticles in photovoltaic devices and environmental conversion of CO into CO_2, as well as in the design of antennas, lenses, and resonators [5]. Important medical applications of Au nanoparticles today are present in drug delivery [8]. Those are proposed for the use of laser irradiation, pH change, ionic concentration change, and similar reactions that trigger a drug release (Figure 1.1).

The exceptional biocompatibility of these nanoparticles renders them amenable to a compelling medical application: their utilization alongside a near-infrared laser source for the controlled thermal ablation of cancerous tissue. This technique holds the promise of minimizing harm to adjacent healthy tissue. Consequently, gold nano shells have made substantial progress and are currently undergoing advanced clinical trials under the scrutiny of the Food and Drug Administration (FDA).

Beyond their role in drug delivery, Au nanoparticles find versatile application within the medical realm. These encompass: Medicine Diagnosis and Therapy: Au nanoparticles play a pivotal role in diagnostics and therapeutic interventions. Their unique properties contribute to precise disease detection and targeted treatments.

Imaging of Tumor Cells: Leveraging the distinctive characteristics of Au nanoparticles, they are employed to facilitate imaging techniques that enhance the visualization of tumor cells.

Additional Diverse Applications: The potential of Au nanoparticles extends to various other medical applications, unlocking innovative approaches in disease management and healthcare.

The multifaceted medical applications of Au nanoparticles underscore their pivotal role in the advancement of medical science and treatment paradigms, as is listed in Table 1.2.

Table 1.2. Imaging and biomedical capabilities of Au nanoparticles [5-10].

Imaging Techniques and Therapy	
Biomedical application	**Nanoparticle morhpology**
Optical scattering	Nano-Au: shells, rods, cages
PET, SPECT	Radioisotope Au^{198}
CT	Au Nanoparticles
Photothermal	Nano-Au: shells, cages
Photoacoustic	NIR-absorbing Au nanoparticles
Chemotherapy	Au nanoparticles loaded with anticancer drugs
Gene therapy	Au nanoparticles loaded with RNA, DNA

There are many methods for characterization of nanoparticles [9]. However, only some of them can reveal the particle size, morphology, and size distribution [2,3]. Among the mostly used methods are scanning electron microscopy (SEM), transmission electron microscopy (TEM), photon correlation spectroscopy (PSC), surface area analysis (BET), and X-ray diffraction (XRF) peak broadening analysis [10]. Characterization methodology is crucial for targeting of special nanoparticles for desired application (Table 1.2). However, specific targeting using desired ligands can be hard to achieve, and the comparison between targeted and non-targeted nanoparticles obtained similar results [4]. Moreover, the interaction of nanoparticles with biological fluids at the contact point site of drug administration (oral, intranasal, or intravenous) makes the problem even more complex. Some strategies are implemented to manipulate this effect, such as PEG nanoparticles that have the ability to avoid interactions with other molecules. Secondly, there are nanoparticles coated with cell membranes that are able to reduce their blood clearance and, thirdly, there are nanoparticles that are able to drain lymph nodes. The array of properties exhibited by nanoparticles profoundly influences their efficacy in reaching their intended targets. Notably, insights garnered from our comprehension of immune system functionality spark innovative concepts for immunotherapeutic applications through alternative administration routes. Examples include intraperitoneal injection or subcutaneous nanoparticle delivery for vaccination purposes. Intriguingly, certain nanoparticle compositions inherently manifest immunostimulatory or immunosuppressive effects, even without the addition of drugs or immune modulators.

Numerous instances substantiate these phenomena, such as hyaluronic acid, PLGA, chitosan nanoparticles, and porous silicon micro particles. These materials have demonstrated the capacity to function as self-adjuvants or elicit M1 or Th1 immune responses.

Nanoparticles offer a plethora of advantages, including their bioactive attributes, seamless integration with other polymeric materials, straightforward composite and bio-composite formation mechanisms, and facile steps for modifying and functionalizing various carriers. However, challenges arise, encompassing strong hydration shells, precipitation-induced turbidity, surface aggregation, dispersive composition, size-related intricacies, and other properties that impede their operational and biomedical applications. Moreover, the diminutive dimensions of nanoparticles give rise to certain drawbacks. Their capacity to readily traverse cell walls, be it in skin or lung cells, raises concerns. Furthermore, their ability to breach the blood-brain barrier adds another layer of potential impact on human health.

In summary, while nanoparticles present remarkable potential for diverse applications, their inherent properties, both advantageous and disadvantageous, necessitate thorough consideration in their deployment for technological and biomedical pursuits.

Polymeric nanoparticles

Certain specialized polymeric nanoparticles are constructed with a central core housing an inner content that could encompass dyes, other inorganic nanoparticles, or drug molecules. Surrounding this core, an outer shell typically consists of hydrophilic polymers. This outer shell can be tailored to incorporate additional functional components, enhancing the nanoparticle's capabilities.

The World Health Organization (WHO) has identified several prevalent chronic diseases afflicting the global human population, including Alzheimer's disease, cardiovascular diseases, cancer, chronic obstructive pulmonary disease, and type 2 diabetes. Chronic inflammation is recognized as a pivotal underlying factor in most chronic diseases. Prolonged immune system activation leads to the production of pro-inflammatory cytokines, causing detrimental effects on organisms. Traditional treatments, involving non-steroidal and anti-inflammatory drugs, lack specificity and often result in various side effects. Furthermore, administering anti-inflammatory drugs presents challenges in achieving optimal bioavailability. Conversely, purposefully designed nanocarriers offer avenues to surmount these hurdles. They allow for targeted drug delivery, addressing specific sites and improving drug solubility. This concept of nano-carrier design is influenced by the inflamed tissue's microenvironment, characterized by heightened permeability, acidic pH, and a significant presence of reactive oxygen

species (ROS). These factors contribute to the optimization of treatment outcomes using nanocarriers.

> The World health organization has recognized the problem of antimicrobial resistance as one of the most important problems affecting global human health. Polymeric nanoparticles have a very important role in fighting the most prominent diseases of today human kind.

Nanoparticles in Cancer Treatment

Cancer claims the lives of over 10 million individuals annually, establishing it as the second most common cause of death worldwide. A notable challenge faced by conventional medical treatments is the intricate task of distinguishing between cancerous and healthy cells. However, unique characteristics intrinsic to cancer cells offer novel avenues for targeted approaches. Notably:

Acidic Extracellular Environment: The extracellular milieu of cancer cells tends to be acidic.

Elevated Cell Temperature: Cancer cells often exhibit a higher temperature compared to their surroundings.

Cancer-Associated Enzymes and Surface Molecules: Distinct enzymes and surface molecules are prevalent on cancer cells. These, in conjunction with hypoxic conditions and reactive oxygen species (ROS), define cancerous characteristics.

To address this distinct landscape, polymeric nanoparticles emerge as a versatile solution, amenable to easy modification and manipulation in terms of size and surface properties. Augmenting the effect of passive targeting, these particles are tailored within the size range of 10 to 200 nm, enhancing localization and drug delivery through active targeting mechanisms involving cancer-recognizing molecules.

Diverse polymeric nanoparticle delivery systems for anticancer drugs are under investigation, each targeting specific cancer types. Moreover, curcumin, a natural compound with potential anticancer properties, can be combined with efficient cancer-targeting delivery systems employing biocompatible polymers like PLGA, lecithin, chitosan, silk fibroin, and Eudragit®. Nano-formulated curcumin demonstrates substantial advancements in bioavailability compared to free curcumin, attributing to enhanced adhesion to cancer cells, particularly in the colon.

Incorporating these strategies into the realm of cancer treatment holds promise for improving therapeutic outcomes and enhancing the efficacy of anticancer interventions.

Nanoparticles in Treatment of Infectious Diseases

The escalating resistance of microorganisms to antibiotics poses a grave concern, as underscored by the World Health Organization, which has categorized this issue as a major threat to public health. Within hospital settings, various items with which patients come into contact, such as bedding, towels, sheets, bandages, catheters, and medical equipment, can serve as potential sources of danger if they are not properly sterilized. Consequently, there is an urgent need for technological advancements aimed at producing materials with protective and preventive properties against antibiotic-resistant microorganisms.

Staphylococcus aureus, a bacterium, is capable of causing a range of infections, particularly affecting the skin, soft tissues, bones, and blood vessels. Postoperative surgical procedures frequently result in bloodstream infections, with certain strains even leading to specific symptoms like toxic shock syndrome. The emergence of antibiotic-resistant strains of microorganism's dates back to the 1960s, primarily surfacing within hospital environments. However, over the past decade, the reach of Methicillin-Resistant Staphylococcus Aureus (MRSA) has significantly expanded across multiple countries globally. This proliferation underscores the urgency for preventive measures and novel material development.

Hospitalized individuals with open wounds, those using inhaled devices, and those with compromised immune systems face heightened susceptibility to infections within a broader population. Although MRSA infections post-surgery are relatively uncommon, they may manifest in wounds, the chest, or even the bloodstream, endangering lives and prolonging hospital stays. MRSA infections occur in a range of 1% to 33% of surgical cases, signifying the gravity of the situation and the imperative for effective preventive strategies and materials development.

Addressing the challenge of antibiotic resistance and enhancing infection control within healthcare settings remains a crucial endeavor in safeguarding public health and patient well-being.

> *Staphylococcus aureus* that is resistant to the antibiotics is called the "golden bacteria". It is known to cause severe health problems, such are infections on skin, soft tissues, bones, and blood vessels, which is the most common consequence of postoperative surgery. Some strains of *S. aureus* can cause various specific symptoms including toxic shock syndrome and lead to thousands of deaths all over the world.

Figure 1.2. (**a**) Graphical illustration of the spread of infections by *S. Aureus* (MRSA), WHO 2014; (**b**) SEM microphotographs of silver nanoparticle;

The initial emergence of penicillin-resistant strains of Staphylococcus aureus (S. aureus) occurred in the mid-1940s. Consequently, S. aureus has been categorized into strains that are either resistant *(MRSA - Methicillin-Resistant Staphylococcus Aureus*) or sensitive (*MSSA - Methicillin-Sensitive Staphylococcus Aureus*) to methicillin. Notably, MRSA infections in hospital settings are associated with a 50% higher likelihood of death compared to MSSA infections. The heightened incidence of MRSA infections within hospitals has prompted increased reliance on alternative antibiotics like vancomycin. However, this elevated usage has contributed to the emergence of vancomycin-resistant S. aureus (VRSA) microorganisms. The evolution of antibiotic-resistant strains continues to pose significant challenges in the realm of healthcare and necessitates vigilant efforts to combat and control their spread. The first such case was described in Japan in 1996 and afterwards in England, France, and the United States [11 -15]. Furthermore, *S. aureus* causes many types of infections: skin (McCaig et al., 2006, [16]); food poisoning (Wieneke et al., 1993 [17]); soft tissues, bones (Sheehy et al., 2010 [18]); blood vessels (Khatib et al., 2009 [19]); endocarditis (Fowler et al., 2005 [20]); or pneumonie [21]. Untreated bacteriemia of *S. aureus* has a mortality rate of 80% [19,22]; most patients do not survive the first year [23] and, in Western European countries, very often patients in hospitals do not receive adequate therapy at the time [22- 26].

A major problem in hospitals is non-sterile materials, because MRSA is easily transmitted through contaminated hands, clothing, or nesterious medical supplies after contact with a patient infected with MRSA either directly (after contact with a patient, blood, tissue fluid, secretions, and excretions) or indirectly (through contaminated instruments, objects, equipment, surfaces, and similar materials). The spread of MRSA can be prevented by the usage of disposable gloves, capes, and masks, but this is not always feasible. Therefore, new materials need to be developed that are active against microorganisms resistant to antibiotics. In the development of new antimicrobial materials, as well as in the functionalization and modification of existing ones, the application of nanoparticles of metal and metal oxides plays a very important role. The nanoparticles have excellent new medical, mechanical, optical, magnetic, catalytic, and electronic properties owing to a specific surface that is highly dependent on their size, structure, and shape. The global demand for nanoparticles of metal and metal oxide is projected to grow to 1700 tons in 2020 [5].

Nanoparticles have strong antibacterial activity on a wide range of Gram-positive and Gram-negative bacteria, including strains resistant to antibiotics [24]. They have great application because of their chemical stability, catalytic activity, and high conductivity. Rezić et al. have shown that silver nanoparticles have higher antibacterial activity owing to their high surface-to-volume ratio, which ensures better contact with microorganisms [4]. Furthermore, Al-Dhabi et al. have demonstrated visible antimicrobial activity against pathogenic wound infections such as *Bacillus subtilis, Enterococcus faecalis, Staphylococcus epidermidis,* multidrug-resistant *Staphylococcus aureus,* and *Escherichia coli* [25]. Silver nanoparticles with their own antimicrobial activity in combination with antibiotics (such as penicillin G, amoxicillin, erythromycin, clindamycin, or vancomycin) enhance the action of antibiotics in the treatment of resistant *Staphylococcus aureus* and *Escherichia coli* infections [26]. The interaction between nanoparticles and microorganisms is complex. Two interrelated mechanisms are described in the literature: (1) membrane potential disturbance and (2) production of reactive oxygen species (ROS), where nano-particles act as nano-catalysts [27,28]. Microbial membrane damage occurs when nanoparticles electrostatically bind to the surface of the bacterium, resulting in changes in the membrane wall and membrane potential and loss of integrity. Reactive oxygen species (ROS) are the cytotoxicity of nanoparticles in vivo and in vitro conditions where oxidative stress on the surface damages the integrity of microbial membranes by lipid peroxidation, further damaging the protein and enzyme function and damaging DNA and RNA. In some cases, ROS is induced by either visible

or UV light and photocatalytic nanoparticle toxicity, such as, for example, TiO_2 nanoparticles, which, under UV light, cause lipid peroxidation, respiratory dysfunction, and death of methicillin-resistant *Staphylococcus aureus* cells [29]. Other mechanisms of toxicity of bacterial cell nanoparticles include direct inhibition of specific essential enzymes, induction of nitrogen reactive species [30], and induction of programmed cell death or apoptosis [31]. Table 1.3 shows the activity of silver nanoparticles of various dimensions on microorganisms.

Table 1.3. Antimicrobial activity of silver nanoparticles on microorganisms [3,9,32].

Silver Nanoparticles size, nm	Activity	Microorganisms
5–30	Antibacterial	*Acinetobacter baumannii*
~20–100	Growth inhibition	*Acinetobacter*
~20–100	Growth inhibition	*Aeromonas*
104.9	Antifungal	*Aspergillus foetidus*
5–30	Antifungal	*Aspergillus fumigatus*
4.75–8.31	Antifungal	*Aspergillus niger*
104.9	Antifungal	*Aspergillus oryzae*
7–20	Antifungal	*Aspergillus*
Not specified	Antifungal	*Aspergillus terreus*
120	Antibacterial	*Bacillus cereus*
24	Antibacterial	*Bacillus megaterium*
16	Bactericide	*Bacillus mycoides*
Not specified	Bactericide	*Bacillus pumulis*
~100	Antibacterial	*Bifidobacterium*
~20–~30	Growth inhibition	*Bordetella pertussis*
~20–45	Antifungal	*Candida albicans*
Not specified	Antifungal	*Candida glabrata ATCC90030*
Not specified	Antifungal	*Candida tropicalis*
~20–100	Growth inhibition	*Citrobacter*
50–70	Antifungal	*Cryptococcus neoformans*
8–12	Bactericide	*Eschericia coli MTCC 443*
8–12	Antibacterial	*Enterobacter aerogenes*
~89	Antibacterial	*Enterococcus faecalis*
104.9	Antifungal	*Fusarium oxysporum*
~6	Antibacterial	*Klebsiella pneumoniae*
16	Bactericide	*Lactobacillus acidophilus*
17–29	Antibacterial	*Listeria monocytogenes*
198–595	Growth inhibition	*MRSA*
~20–~30	Growth inhibition	*Micrococcus luteus*

20–30	Inhibition of biofilm formation	*multidrug resistant Pseudomonas aeruginosa*
~6	Antibacterial	*Multidrug Resistant Escherichia coli*
~6	Antibacterial	*Multidrug Resistant Klebsiella pneumoniae*

Table 1.3 continues. Antimicrobial activity of silver nanoparticles [3,9,32].

Silver Nanoparticles size, nm	Activity	Microorganisms
~10–~100	Bactericide	*Staphylococcus aureus*
~10	Antibacterial	*Paenibacillus koreensis*
>42	Antifungal	*Penicillium*
~100	Antibacterial	*Peptostreptococcus*
9–10	Antifungal	*Pichia pastoris*
20–70	Antibacterial	*Propionibacterium acnes*
Not specified	Cytotoxic	*Proteus mirabilis*
50–70	Antibacterial	*Proteus vulgaricus*
1–12	Antibacterial	*Pseudomonas aeruginosa*
~2–40	Antifungal	*Rhizoctonia bataticola*
~15	Antifungal	*Saccharomyces*
63–90	Antibacterial	*Salmonella paratyphi*
Not specified	Antifungal	*Scedosporium JAS1*
Not specified	Antibacterial	*Serratia marcescens*
17–29	Antibacterial	*Shigella flexneri MTCC 1475*
1–12	Antibacterial	*Staphylococcus aureus*
5–25	Antibacterial	*Staphylococcus aureus 25923*
~89	Antibacterial	*Staphylococcus aureus BAA-1721*
20–70	Antibacterial	*Staphylococcus epidermidis*
15	Antibacterial	*Streptococcus*
~15	Antifungal	*Trichopyton rubrum*
5–25	Antibacterial	*Vibrio cholerae*
~54	Antibacterial	*Xanthomonas campestris*
Not specified	Antibacterial	*Yersinia enterocolitica*

Polymers containing metal nanoparticles and metal oxides have antimicrobial effects [32], but their effects on many pathogenic microorganisms have not yet been sufficiently explored.

As can be seen from the table 1.3. in the effects against different strains of the same bacteria, not only the chemical composition of the nanoparticles plays a significant role (since all nanoparticles presented in Table 1.3. are silver nanoparticles), but the efficiency strongly depends on the size of the particular nanomaterial.

Antibiotics usually target bacterial cell membrane synthesis, translation, and DNA replication [33]. The resistance to antibiotics can occur on each of these antimicrobial effects.

The primary mechanism through which nanoparticles exert their effects involves direct interaction with the bacterial membrane. This process does not necessarily entail membrane penetration or breaching the cell wall. This unique mode of action provides microorganisms with less opportunity to develop resistance against nanoparticles. Currently, silver nanoparticles find application in various coatings, such as those used on surgical instruments, catheters, masks, orthopaedic implants, and dental materials. They are incorporated into these materials to enhance their properties. Additionally, silver nanoparticles contribute to diagnostics by increasing the sensitivity of bio-detection techniques. These nanoparticles are employed in highly sensitive clinical tests, ranging from diagnosing myocardial infarction to fluorescence-based detection of RNA. The versatile applications of silver nanoparticles underscore their potential to advance diverse fields, from healthcare to diagnostics and beyond. [34] (Table 1.4).

Table 1.4. Application of silver nanoparticles in medicine items [35].

Treatment	Effect of Silver Nanoparticles
Anaesthesiology	Coating on breathing masks, endotracheal tubes for mechanical ventilation assistance
Cardiology	Coating on the tracking catheter
Stomatology	Adhesives in dental materials, silver-filled SiO_2 nanocomposite resins
Diagnostics	Ultra-sensitive and ultra-fast platform for clinical tests for myocardial infarction diagnosis, fluorescence detection of RNA
Drug release	Remote laser induced opening microcapsules
Eye care	Contrasts on contact lenses
Visualisation	Nomenclatures for marking cells
Neurosurgery	Coating of the catheter for the drainage of cerebrospinal fluid
Orthopaedics	Bone cement additives, joint replacement implants, orthopaedic socks
Drugs	Treatments for dermatitis, ulcerative colitis and acne, HIV-1 inhibition

Surgery	Coats in medical textiles—surgical suits and masks
Urology	Plastered on surgical nets for pelvic reconstruction

In addition to the pharmaceutical industry, nanoparticles of silver are used in the cosmetic industry thanks to their strong antibacterial and anti-inflammatory properties, in cosmetic products such as deodorants, anti-aging creams, and so on. Nanotechnology can solve the problem of bio-film formation by using an antibacterial active surface with a combination of ZnO and MgO nanoparticles that is activated in the dark and is effective against *MRSA* species [36].

Considering the new solutions, it is not surprising that resistance to nanoparticles by microorganisms has already occurred; Panaček et al. demonstrated that gram-negative bacteria *of Escherichia coli 013, Pseudomonas aeruginosa CCM 3955* and *E. coli CCM 3954* can develop resistance to silver nanoparticles after repeated exposure and resistance results from the production of adhesive flagellin protein, which activates the aggregation of nanoparticles [37]. Research into antimicrobial nanoparticle coatings has found intriguing applications in the realm of space exploration. Notably, the International Space Station (ISS) has conducted experiments with coatings composed of silver and ruthenium. These coatings exhibited remarkable efficacy against both Gram-negative and Gram-positive bacterial strains, including highly resistant pathogenic bacteria such as *MRSA, Enterococcus faecalis, Staphylococcus epidermidis, E. coli, Pseudomonas aeruginosa, Acinetobacter baumannii*, and *Legionella.* The ISS, characterized by its confined and isolated environment, presents unique challenges in terms of bacterial growth. In this environment, bacteria can evolve specialized antibiotic defence mechanisms, such as developing thicker cell walls or upregulating virulent genes. Furthermore, microgravity and cosmic radiation can contribute to enhancing the virulence of microorganisms, potentially transforming them into more potent pathogens. The distinct conditions within the ISS, coupled with reduced immune defences among astronauts due to microgravity and cosmic radiation, create an environment that is particularly conducive to infections. This susceptibility is further exacerbated by the psychological stress associated with space travel. As a result, astronauts become more vulnerable to infections, necessitating innovative approaches such as antimicrobial nanoparticle coatings to mitigate the risks posed by various microorganisms in the unique space environment. [38].

Nanoparticles in Implants and Prosthetics

Nanoparticles find extensive application in the realm of implantable devices, where specific attributes are essential. Implantable devices necessitate qualities such as excellent biocompatibility, tissue compatibility, resistance to corrosion, and notably, antibacterial properties. A diverse array of classical medical devices, implants, and prosthetics encompass this category, including catheters, dental implants, pacemakers, prostheses, and more. These materials engage in prolonged, direct contact with biological tissues, making their biocompatibility a pivotal consideration that governs their usability.

Problems frequently encountered with implantable devices are linked to their biocompatibility, giving rise to issues such as toxicity, allergic reactions, inflammation, and, at times, bacterial infections. The integration of nanoparticles in the design and fabrication of these devices offers a multifaceted approach to address these challenges. By bestowing antibacterial properties, nanoparticles enhance the safety and effectiveness of implantable devices, effectively mitigating the risks associated with microbial infections. The utilization of nanoparticles in the creation of implantable devices marks a significant advancement in biomedical engineering, poised to revolutionize the field by improving the longevity, functionality, and overall compatibility of these critical medical tools [39].

Nanoparticles as Theragnostic Devices

Therapeutic systems exhibit a dual role, serving both as a therapeutic and diagnostic agent. These systems are meticulously engineered to achieve target-specific drug delivery while concurrently monitoring the drug release process. Furthermore, they possess the capability to track the progression of disease and the response to treatment. In contrast to traditional diagnostic imaging techniques employing inorganic contrast agents for methods like magnetic resonance imaging, computed tomography, and angiography, organic fluorophores emerge as a promising alternative due to their favorable safety profile.

These systems find diverse applications, extending to formulations for skin, hair, nails, and various other contexts. For specific applications, nanoparticles of varying sizes (ranging from 50 to 500 nm) are employed, capitalizing on their ability to penetrate the skin and reach deeper layers within the organism. This attribute has facilitated the development of "transdermal drugs," wherein therapeutic agents are administered through the skin, enabling efficient and controlled delivery of treatments.

By merging therapeutic and diagnostic functionalities, these innovative systems pave the way for more precise and personalized medical interventions, advancing the frontiers of both diagnosis and treatment in the field of healthcare [39]. Polymers used as carriers are chitosan, albumin, PLGA, or PLA. In addition, inorganic nanoparticles are useful in antibacterial coating; thus, for example, TiO_2 coated with silver-doped hydroxyapatite or silver-coated collagen proved to possess antibacterial activity useful for catheters and dental implants effective against *S. aureus* and *P. aeruginosa* [40].

Nanoparticles Synthesis by Enzymes

The first attempt of synthesis of metallic particles by enzymes was performed by McConnel and Frajola (1961), when the synthesis of carbonate apatite was carried out in the presence of carbon anhydrase. Today, the advents and developments in nanotechnology resulted in a wide range of microorganisms and their enzymes, which can be utilized in the production of nanoparticles. Some examples of synthesis of biomedically applicable nanoparticles are presented in Table 1.5

Table 1.5. Nanoparticles synthesized by microorganisms [41–44].

Microorganisms	Nanoparticle	Temperature, °C	Size (nm)	Shape
Rhodococcus species	Au	37	5–15	sphere
Shewanella oneidensis	Au	30	12 ± 5	sphere
Plectonemaboryanum	Au	25–100	<10–25	cube
Plectonema boryanum	Au	25	10–600	octahedral
Escherichia coli	Au	37	20–30	triangle
Yarrowia lipolytica	Au	30	15	triangle
Rhodopseudomonas capsulate	Au	30	10–20	sphere
Brevibacterium casei	Au, Ag	37	10–50	sphere
Trichoderma viride	Ag	27	5–40	sphere
Phaenerochaete chrysosporium	Ag	37	50–200	pyramidal
Bacillus cereus	Ag	37	4–5	sphere
Lactobacillus species	Ba, TiO_3	25	20–80	tetragonal
Fusarium oxysporum	CdSe	10	9–15	sphere
Escherichia coli	Cd,Te	37	2.0–3.2	sphere
Fusariumoxysporum	$CdCO_3$, $PbCO_3$	27	120–200	sphere