NANOTECHNOLOGY IN DRUG DISCOVERY

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Nanotechnology in Drug Discovery

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Nanotechnology in Drug Discovery

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PREFACE

Drug discovery is a critical step in the treatment and cure of diseases, involving identifying drug targets, lead identification, modification, synthesis, characterisation, validation, optimization, screening, and tests for therapeutic efficacy. Drug research and development have been greatly impacted by nanotechnology, resulting in the development of novel medicines for diseases that were previously incurable. At present, the pharmaceutical industry is attempting to minimize the time needed for medication development in response to the growing demand for fast drug development. Nanotechnology has allowed for the evolution of critical processes in traditional drug discovery, with an emphasis on enhancing lead identification, modifications, synthesis, stability, and target selectivity. There has been a surge in nanomedicine research over the last few decades, which is now being translated into commercialization endeavours throughout the world, leading to the marketing of various nano-drugs.

This book is intended for students and researchers who are just starting out in the modern drug development sector, where nanotechnology has taken up a significant space. The book will progressively expose readers to the topic of nanotechnology-based drug research by first examining the fundamentals of nanoparticles. Then this book will cover the utilization of nanotechnology throughout the drug development process from lab to market, focusing on lead identification and synthesis, drug delivery, nano-drug toxicity, in-vivo fate of NPs, and finally regulations on NPs-based drugs in various countries. The work then finally focuses on the future perspective of nanotechnology in drug discovery. Eventually, the readers will have an overall idea of how nanotechnology has improved the conventional drug development process. The abstract and conclusion given at the beginning and end of each chapter will provide the readers with concise information that is elaborated throughout the chapters.

We anticipate that this book will serve as a reference book, offering an in-depth account of how nanotechnology has revolutionized the drug development process while highlighting the intriguing recent findings in the field.

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CHAPTER 1

Fundamentals of Nanotechnology

Abstract: Nanomaterials, a category of materials with a dimension in the nanometric range (1 nm-100 nm), were first recognized in 1959. They have unique physical, chemical, and mechanical properties, with nanoparticle size affecting properties like melting temperature, ionization potential, colour, electron affinity, electrical conductivity, and magnetism which is different from their bulk material. Nanotechnology improves biomarker development and aids in developing more sensitive treatments in medicine using nanodevices which enhances drug discovery by improving the understanding of biological processes, disease mechanisms, and signalling pathways.

This chapter provides an overview of nanomaterials and examines their distinct properties. The key top-down and bottom-up methods for synthesizing nanomaterials are also explained along with specific examples. The chapter will also include a summary of several nanoparticle characterization methods and the attributes associated with each method. In addition, comprehensive information about advanced devices that have been inspired by nanotechnology to increase the efficiency of the drug development process through a better understanding of the biological mechanisms underlying diseases, signalling pathways, and the precise effects of medications have also been discussed. The chapter will conclude by outlining the advantages and challenges of using nanotechnology in drug development and treatment.

Keywords: Challenges, Characterisation, Drug discovery, Nanomedicine, Nanomaterials, Nanodevices, Opportunities, Synthesis.

1. INTRODUCTION TO NANOMATERIALS

Although nanomaterials are not a new phenomenon in nature, interest in engineering at a very tiny scale arose following Richard P Feynman's legendary talk titled “There's plenty of space at the bottom” on 29th December 1959, at the annual meeting of the American Physical Society, when he spoke of manipulating and controlling things on a microscopic scale. Feynman is often considered as the first visionary of nanotechnology due to his clairvoyance. Unfortunately, it took the scientific community more than three decades to turn his vision into reality due to a lack of suitable tools and processes.

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The prefix "nano" is derived from the Greek word for "dwarf." Nanomaterials represent a category of special materials that have at least one dimension in the nanometric range (1 nm-100 nm). The size comparison of nanomaterials is given in Fig. (**1**). Nanomaterials are classified into four types as zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) nanostructures. All three dimensions are present at the nanoscale in zero-dimensional nanostructures. These nanoparticles resemble point particles and display quantum confinement. 1D nanoparticles have at least one dimension bigger than nanoscales (*i.e.* 100 nm), with the remaining dimensions occurring within the nano range. Nanofibers, nanotubes, and nanorods are the most frequent types of one-dimensional nanoparticles. The most popular examples of 2D nanomaterials are nanofilms, nanolayers, and nanocoatings, which are plate-like structures having two dimensions larger than the nanoscale. Although the constituents of 3D nanomaterials are smaller than 100 nm, none of their dimensions are less than the nanoscale. Nanomaterials with three dimensions are formed when the nanoscale particles are combined. These substances are typically nonporous and have a wide range of uses. The most common types of three-dimensional nanomaterials include nanocomposites, bundles of nanofibers, and multi-nanolayer structures [1, 2].

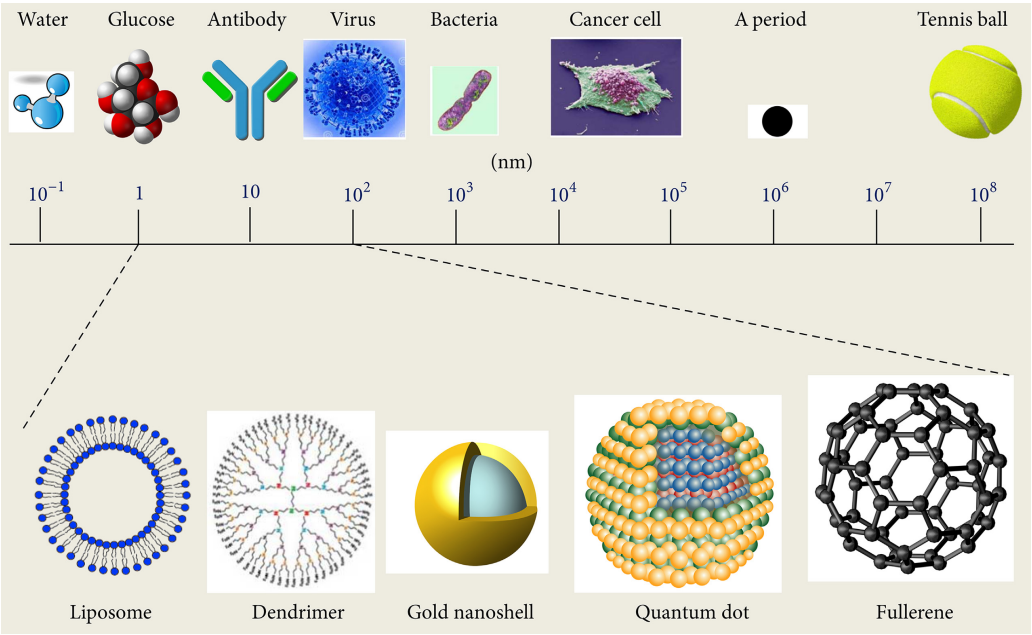

Fig. (1). Length scale showing size of nanomaterials. Reproduced with permission from [6] © 2015, Neha Pradhan *et al.* published by Hindawi Publishing Corporation, distributed under the terms of the Creative Commons Attribution 3.0 International. https://creativecommons.org/licenses/by/3.0/.

Beyond mere miniaturisation, nanotechnology has other applications. Materials at the nanoscale have distinctly different physical, chemical, and mechanical properties from bulk materials. When grain size is reduced to nanoscale dimensions, considerable changes are made to the properties, and the resulting qualities typically outperform those of conventional materials. The fact that nanoparticles are used in so many different applications is not surprising. The potential applications of nanomaterials are being discovered in ever-greater quantities.

Nature is undoubtedly the most important source of inspiration for nanoscientists and nanotechnologists. Many nanoparticles and nano-based systems have been refined by nature over millions of years through the process of evolution. Practically all fields of science and technology, including nanoscience and nanotechnology, can benefit from simple observation of the natural occurrences occurring around us. The cell membranes, as well as various other functioning organelles and enzymes, which are responsible for all metabolic activity in the body, are all nanometric in size. It is unsurprising that nanoparticles are employed in so many different applications [3 - 5].

Quantum phenomena, increased surface area, and self-assembly are mostly credited for the special features of nanomaterials. At the nanoscale, quantum effects may appear to govern how matter behaves, influencing how materials behave electrically, optically, and magnetically. This is because, at the nanoscale, matter no longer complies with Newtonian physics but rather with quantum mechanics, which is explicated by quantum confinement, size effect, and density of states. The bulk properties of a material are set by the average of all the quantum forces acting on all the atoms simultaneously. Yet, as structures get progressively smaller, eventually there comes a moment where averaging is no longer effective. Moreover, compared to bulk materials generated from the same mass, nanoparticles have a considerably greater surface area. When the fraction of surface atoms becomes greater, reactivity of the material is enhanced due to an increased number of active sites. In certain circumstances, inert materials in their bulk form turn out to be reactive when manufactured at the nanoscale level. All nanomaterials, regardless of their shape, including nanoparticles, nanowires, nanotubes, and nanocoatings, are affected by the increasing surface area. Finally, self-assembly is a process that relies on the arrangement of individual components to create structured or ordered patterns.

It reflects the information contained in each individual molecule, including shape, charge, polarizability, and other characteristics that affect their attracting or repelling interactions, particularly at the nanoscale. Whilst it can also exploit kinetically labile covalent connections, molecular self-assembly often benefits

CHAPTER 2

Nanotechnology in Drug Development

Abstract: Nanotechnology plays a key role in the development of new drugs, from start to end through target identification, lead identification, lead optimization, and synthesis of active pharmaceutical ingredients (API) as well. Nanodevices and nanoparticles have been extensively utilized in discovering new drug targets in illness sites or blood and for swift screening of interactions of molecular compounds with therapeutic targets for lead identification/optimization. In addition, API development employing nanoparticle catalysts to expedite the drug development process and investigating pure nanomaterials as drugs are two further areas on which the pharmaceutical industry is concentrating. This chapter will go into great detail on how nanotechnology is used in the drug development process, starting with the identification of drug targets, moving on to the identification and optimization of leads, and concluding with the synthesis of API.

Keywords: Active pharmaceutical ingredients, Drug target, Lead optimization, Nanotechnology, Nanoparticles, Nanosensors, Nanocatalysts, Nano-drugs.

1. INTRODUCTION

Due to the availability of several competing alternative medical markets, pharmaceutical companies are always challenged to create improved drug discovery technologies. For the efficient treatment of a wide range of disorders, the pharmaceutical industry needs to find and develop novel medications. Nanotechnology has become an area of enormous relevance to pharmaceutical corporations and their drug development programs.

Nanotechnology plays a key role in the development of new drugs, from start to end through target identification, lead identification, lead optimization, and synthesis of active pharmaceutical ingredients (API) as well. Nanotechnology is focused on developing better medication formulations and diagnostic techniques for more efficient and effective therapy in the realm of drug research and development. The utilization of nanodevices and nanoparticles has been widespread in the search for new drug targets because of their unique advantages. These advantages include enhanced biomarker detection sensitivity for early identification of diseases and diagnosis, the production of safer drug formulations, and the development of intriguing medical devices.

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Nanotechnology has been utilized for the swift screening of interactions of molecular compounds with therapeutic targets for lead identification/optimization as well. For instance, nanosensors to detect protein activity in drug-treated cells have been created. By effectively suppressing background noise from irrelevant sources and drastically enhancing signals for specific biomolecular binding, these nanosensors have higher sensitivity and consume less sample. Investigating real-time dynamic processes in living cells, such as protein interactions inside cells, intracellular signal transmission systems, and cell proliferation, is made possible by fluorescent nanoparticles' special optical feature. This expedites the process of optimizing lead compounds chemically by the use of in vivo biological consequences. Such nanosystems are appropriate for probing biological disorders at the cellular pore and receptor levels. Furthermore, there has been a noticeable decrease in the degree of harmful and negative consequences because of the small size of diagnostic materials. New dimensions of diagnostic tools that have been explored with the aid of these cutting-edge nanotechniques will be discussed in this chapter. In addition, API development employing nanoparticle catalysts to expedite the drug development process and investigating pure nanomaterials as drugs are two further areas on which the pharmaceutical industry is concentrating. To meet the increased demand of drug production, it is extremely promising to restructure and optimize the development process of APIs as early as feasible. In comparison to small-molecule medicines, which frequently have poor pharmacokinetic profiles and extensive secondary effects, nanotherapeutics have been proven to improve therapeutic effectiveness and decrease off-target toxicity by modifying drug biodistribution.

2. NANOTECHNOLOGY IN DRUG TARGET IDENTIFICATION

The first crucial steps in the process of discovering new drugs are the identification and validation of targets. Therefore, this first stage of the drug development process is of utmost importance to researchers. The goal of target identification is to find new targets, which are typically hormones, proteins, enzymes, genes, and DNA/RNA, whose modulation might slow or reverse disease development. In the past, scientists tended to focus on a small number of preferred genes that could be studied using low-throughput methods and were often discovered in the literature by academia. Due to the relatively modest number of protein classes that have proven susceptible to pharmaceutical development, most of the effective drug discovery initiatives have focused on these selected classes. With the use of modern technology, researchers can look for novel targets by attempting to link variations in gene (genomics) and protein (proteomics) expressions with human ailments [1]. To find new targets in illness sites or blood, nanotechnology is a potential technique in the biomedical industry.

Single-molecule fluorescence (SMF) nanosensors have been widely used to identify a variety of drug targets, including proteins, nucleic acids (including DNAs and microRNAs), enzymes, viruses, and living cells [2]. A tricyclic ligase chain reaction (LCR)-mediated QD-based Förster resonance energy transfer (FRET) nanosensor, for example, was created by Wang *et al.* to detect DNA methylation, an epigenetic mutation linked to a number of hereditary illnesses in humans [3]. Changes in microRNA expression are strongly associated with a number of human disorders, including cancer [4]. Zhang *et al.* developed an enzyme-free toehold-mediated strand displacement cascade-based QD-SMF nanosensor to detect microRNA [5]. Without any protein enzyme requirement, for target signal amplification, in this nanosensor, microRNA itself operates as the signal catalyzer. A SMF nanosensor based on AuNP and the same above mechanism was created by Liu *et al.* to detect microRNA [6]. Additionally, as one disease is typically associated with the deregulation of numerous microRNAs, simultaneous detection of multiple microRNAs is crucial for clinical diagnosis [7]. In this regard, Zhang *et al.* created a siRNA-directed self-assembled QDs nanosensor for the simultaneous single-molecule detection of many microRNAs [8]. TdT is a DNA-modifying enzyme that has the potential to serve as a biomarker for the leukaemia illness disease [9], since it can catalyze the random integration of nucleotides into the 30 -OH termini of DNA strands without the need of any DNA templates [10]. To measure TdT activity, Wang *et al.* created a QD-based SMF nanosensor [11].

SMF nanosensors have also been used in the detection of deregulated proteins and protein-modifying enzyme biomarkers associated with various diseases as drug targets. A single-molecule immunosorbent nanosensor based on upconversion nanoparticles (UCNPs) was created by Farka *et al.* to detect prostate-specific antigen (PSA), a crucial oncological biomarker for the detection of prostate cancer [12]. In addition, Wang *et al.* developed an SMF nanosensor based on a magnetic nanobead-assisted dual bar-code method to concurrently detect the tumour necrosis factor-α (TNF-α) and cytokines interferon-γ (IFN-γ) [13]. As the deregulation of OGT activity is intimately associated with several human disorders including malignancies and Alzheimer's disease, Zhang *et al.* developed a single QD-based fluorescent nanosensor to detect OGT activity [14]. For label-free quantitative detection of Acetylcholinesterase activity, which is directly related to Alzheimer's disease, a fluorescent conjugated polymer nanoparticle and manganese dioxide (MnO_2)-based SMF nanosensor have been created [15]. It has been discovered that inhibiting acetylcholinesterase activity is a successful method for treating the symptoms of Alzheimer's disease [16].

For the clinical diagnosis and treatment of illnesses caused by viruses, reliable viral detection is crucial [17]. Using fluorescent magnetic multifunctional

CHAPTER 3

Development of Nanomaterials as Drug Candidates

Abstract: Nanomaterials, with their unique therapeutic traits such as antioxidant, anti-inflammatory, antibacterial, antiviral, and anticancer properties, can be used as drug candidates to treat a wide range of diseases. Nano complexes like dendrimers, carbon nanotubes, fullerenes, graphene-based nanomaterials, carbon quantum dots, nanohydrogels, peptide nanostructures, MXenes, Silicene, and Antimonene have been distinguished by researchers, among the many nanomaterials because of their lower toxicity, ease of tuning to the desired end use, complex interactions with biological macromolecules, and solubility properties. This chapter will present the most recent research details on nanomaterials that have been developed as therapeutic candidates to treat a number of illnesses.

Keywords: Dendrimers, Fullerenes, Graphene based nanomaterials, Peptide nanostructures, Nano-drug candidates, Nanohydrogels.

1. INTRODUCTION

Contemporary processes of discovering and developing drugs encompass a progression of consecutive steps, commencing with fundamental research and advancing towards targeted activities, ultimately resulting in the creation of novel medications for addressing human ailments. A potential drug must fulfil precise requirements, such as selectively binding to the intended receptor site, triggering the desired functional response, ensuring proper bioavailability and distribution in the body, and exhibiting the desired effects in animal models of human diseases without causing toxicity. Additionally, a molecule must meet fundamental criteria for future production and storage to be considered a viable drug candidate.

Nanomaterials fulfilling most of the above criteria can be employed as medication candidates to treat a variety of diseases and ailments due to their special intrinsic therapeutic capabilities, such as antioxidant, anti-inflammatory, antibacterial, antiviral, and anticancer properties. The physicochemical properties of these nanodrug candidates, such as size, hydrophobicity, and surface charge, have proven to increase the cellular uptake than larger ones.

Nano complexes like dendrimers, carbon nanotubes, fullerenes, graphene-based nanomaterials, carbon quantum dots, nanohydrogels, peptide nanostructures,

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MXenes, silicene, and antimonene have been distinguished by researchers, among the many nanomaterials because of their lower toxicity, ease of tuning to the desired end use, complex interactions with biological macromolecules, and solubility properties. This chapter will focus on the most recent research details on nanomaterials that have been developed as therapeutic candidates to treat a number of illnesses.

2. DENDRIMERS

Multidrug-resistant bacterial infections have the potential to become the leading cause of mortality by 2050 [1]. A potential alternative infection control method is the development of antibacterial dendritic polymers. Development of antibacterial dendritic polymers is the potential alternative infection control method. The class of synthetic polymers known as dendrimers is notable for its typical symmetry, high branching, and monodispersed nature. There is an enormous potential of their usage in medicinal applications because of their distinctive characteristics, which include condensed branching structures with exact control of the size, form, and various functional groups on their outer layer. It has been demonstrated that the quantity of ligands on their outer shell has a considerable impact on inhibiting the multivalent adhesion activities of viruses, bacteria, cells, proteins, and combinations. Their branching architecture appears to be crucial to their use. Dendrimers can be used as medications because of their extensive and multifaceted interactions with biological macromolecules as well as their higher binding affinities. Multivalent substitutions in dendrimers are a powerful alternative for single molecular drugs [2].

Dendrimers offer a platform for antibacterial agents and antiviral agents. The permeabilization of bacterial membranes over the time and rupturing their lipid bilayer *via* the electrostatic attraction between the positively charged dendrimer and the negative surface of the bacterium could be the reason for the underlying antibacterial activity. This means that, like other antimicrobial materials, the positive charge multivalency of high-generation cationic dendrimers is a critical factor in determining their antibacterial efficacy [3]. The most extensively studied antibacterial dendrimer is poly(amidoamine) (PAMAM) (Fig. **1**). For instance, a recent study evaluated the connection between dendrimer formation and dendrimer charge type when used against *S. aureus* [4]. Here, antibacterial experiments were performed on three different types of polyanionic dendrimers, including those with the terminal groups; succinamic acid, sodium carboxylate, and hydroxyl, as well as polycationic dendrimers with primary amine ended PMAM, to assess their inhibitory zone and antibacterial activity. Anionic dendrimers were weaker than cationic dendrimers in terms of potency. Primary amine dendrimers with the formula G(5)-128NH_2 and G(4)-64NH_2 had the

greatest inhibition, respectively. The effects of succinamic acid carboxylate, and hydroxyl dendrimers were weaker.

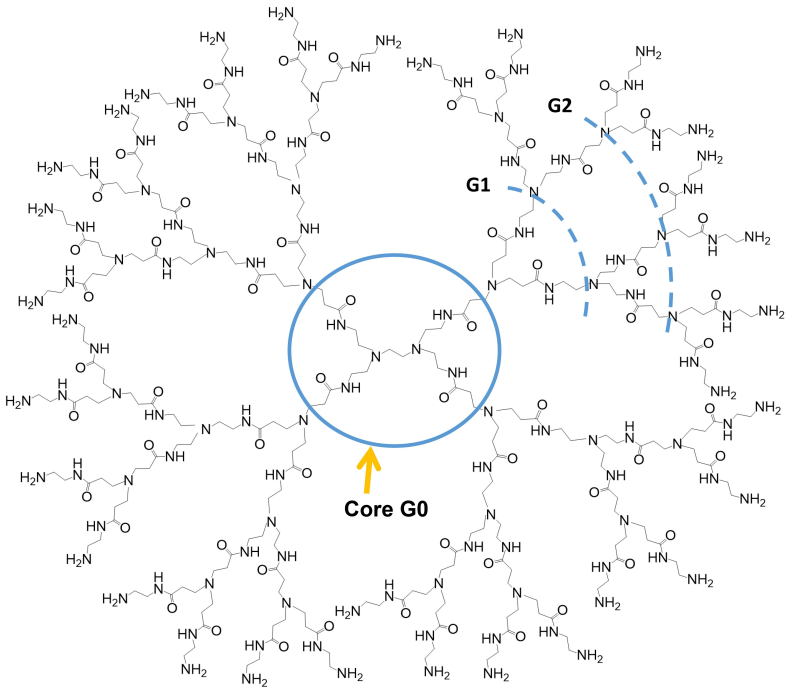

Fig. (1). Generation 2 (G2) PAMAM dendrimer.

In a recent study, the role of several peripheral groups in dendrimer penetration into *P. aeruginosa* biofilms was examined [1]. At the beginning, the penetration of the dendrimers with NH_3^+ groups at their periphery to the acidic environment of *P. aeruginosa* biofilms was faster than those with OH^- or COO^- groups. Additionally, the negatively charged biofilm components and the peripherally charged dendrimers with NH_3^+ developed an electrostatic attraction that allowed the dendrimer to accumulate close to the top of the biofilm. Dendrimers containing peripheral OH- and COO- groups, however, accumulate more strongly and uniformly throughout the biofilm depth, compared to NH_3^+ based dendrimers. The surface composition of dendrimers regulates the depth of penetration and accumulation in biofilms. This finding will have significant implications for the ongoing development of new antibacterial or antimicrobial-carrying polymers [1].

Since cationic dendrimers are harmful to mammalian cells, nitric oxide (NO)-releasing dendrimers were developed in order to lower the concentration of cationic dendrimers while maintaining enough antibacterial activity. This preserved the antibacterial effect while significantly reducing toxicity on

CHAPTER 4

Nanotechnology for Drug Design and Drug Delivery

Abstract: The development of ideal, secure, efficient, non-invasive drug delivery systems is now a top priority in this field of drug delivery. Nanoparticles are being employed more frequently for effective medication delivery, exerting the desired therapeutic effect at the expected site of action with the least amount of activity or volume loss. Size, surface chemistry, biological destiny, toxicity, *in vivo* dispersion, and targeting capabilities all play a role in these systems. The stability and interactions of nanoparticles with cells are regulated by their surface chemistry, and they can access a greater variety of targets. The development of nano-drug delivery systems has opened up new avenues for the treatment and prevention of disease, as well as for enhancing pharmacological properties, enhancing targeting, overcoming drug resistance, and lowering immunogenicity and toxicity. This chapter will first discuss the desirable characteristics of an effective drug delivery system and will cover recent developments in nano drug delivery systems used in clinical research, including dendrimers, solid lipid nanoparticles, nanogels, nanoemulsions, polymeric micelles, and polymer nanofibers.

Keywords: Dendrimers, Nano-drug delivery, Nanogels, Nanoemulsions, Polymeric micelles, Polymer nanofibers, Solid-lipid nanoparticles, Solid lipid nanoemulsions.

1. INTRODUCTION

Nanotechnology is being used in drug development at every level, from formulation to effective dosage to administration using the best possible delivery methods. Poor solubility, large molecular size, and inadequate bioavailability for clinical candidates are the primary issues that drug delivery experts face.

Nano-drug delivery systems (NDDS) are crucial in addressing the challenges of conventional drug delivery. The focus is increasingly shifting towards nanoparticles for precise and targeted drug delivery. The effectiveness of nanoparticle targeting relies on factors such as size, surface charge, hydrophobicity, and surface modification. NDDS enhances drug solubility by downsizing drugs to the nanoscale, ensuring sustained and controlled release for improved patient compliance. Furthermore, nano-drug delivery formulations offer

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a viable alternative for unstable formulations with shorter shelf lives, reduce the required dosage, thereby increasing safety by mitigating side effects. These systems enhance therapeutic outcomes by prolonging drug action, enhancing efficacy and specificity, combating drug resistance, and decreasing immunogenicity and toxicity.

This chapter will first discuss the desirable characteristics of an effective drug delivery system and will cover recent developments in NDDS used in clinical research, including liposomes, dendrimers, solid-lipid nanoparticles, nanogels, nanoemulsions, polymeric micelles, inorganic NPs, and polymer nanofibers.

2. ATTRIBUTES OF AN EFFECTIVE DELIVERY SYSTEM

Nanoparticles designed as drug delivery systems work by trapping pharmaceuticals or biomolecules inside their internal structures, adhering them to the surfaces of the particles. Currently, drugs, proteins, genes, vaccines, polypeptides, nucleic acids, and other substances are delivered using nanoparticles (NPs). Different uses of the NP-based drug delivery system have seen tremendous growth in recent years in industries including pharmaceutical, medical, biological, and others. Considering the impact of NPs in drug delivery systems, this section will brief on how NP delivery systems circumvent the limitations of conventional delivery methods, the characteristics of NPs that affect on being an efficient delivery system, different targeting methods, and then the existing nano drug delivery systems will be discussed.

While it might be challenging to deliver free drugs in traditional dosage forms to the target place at the recommended doses, during the right time period or after it, drug targeting to specific organs and tissues has emerged as one of the key research efforts. Thus, one of the breakthrough research fields is the hunt for novel drug delivery systems and therapeutic mechanisms [1]. Poor solubility, large molecular size, and inadequate bioavailability for clinical candidates are the major constraints experienced by drug delivery experts. Drug administration for children and the elderly, drug delivery for proteins and peptides, and other issues are concerns in this discipline. The development of ideal, secure, efficient, non-invasive drug delivery systems is now a top priority in this field of drug delivery.

To treat chronic human illnesses, regulated drug release and tailored drug delivery have greatly benefited from nanotechnology. Given that they are composed of substances that have been created at the atomic or molecular level, nanoparticles are frequently very small nanospheres [2]. They might therefore move through the body with more suppleness than larger materials. Nanoparticles have different structural, chemical, mechanical, magnetic, electrical, and biological properties. *In vivo* instability, poor body absorption, issues with target-specific distribution,

low bioavailability and solubility, issues with therapeutic effectiveness, and perhaps even negative pharmacological effects are major obstacles when employing large-scale materials for drug administration [1, 3]. Additionally, investigations claim that nanostructures enhance the delivery of medications that are only weakly water-soluble to their target locations and prevent pharmaceuticals from being contaminated in the gastrointestinal tract. Because they are often absorbed by absorptive endocytosis, nanopharmaceuticals have a higher oral bioavailability.

Due to their long-term persistence in the blood circulation system, nanostructures enable the release of integrated medicines at the specified dose. As a consequence, they have fewer adverse effects and less variability in plasma [4]. Due to their nanoscale size, these structures may easily enter the tissue system, allow more efficient drug delivery and administration to cells and ensure that the drug acts where it is targeted. Compared to larger particles between 1μm and 10 μm in size, cells absorb nanostructures at substantially faster rates [5, 6].

There are a number of things to take into account while developing a nanoparticulate drug delivery system. One of the most crucial characteristics of NPs is their particle size, which affects their biological destiny, toxicity, *in vivo* dispersion, and targeting potential. Additionally, drug loading, drug stability, and drug release are all impacted by NPs. Being smaller yet more mobile than microparticles, nanoparticles can reach a wider array of cellular and intracellular targets [7]. The release of drugs depends on particle size. Accelerated drug release is caused by the large surface area-to-volume ratio of small particles. Instead, bigger particles' massive cores enable the extra drug to be confined per particle, resulting in a delayed drug release [8]. The shape of NPs, in addition to particle size, influences biological processes such as endocytosis [9], transportation through the vasculature [10], consequent intracellular transport [11], circulation half-life [12], targeting efficiency [13], and phagocytosis [14], related to the therapeutic administration of a drug [15 - 17]. When compared to particles shaped like cubes or squat cylinders, it was discovered that rod-shaped nanoparticles, which resemble the form of bacteria that are adept at infecting cells, were faster in accessing the cells [18].

The surface chemistry of nanoparticles plays a significant role in the nanoparticulate drug delivery system in addition to size and shape. Particle stability and interactions with cells [19, 20] as well as opsonization, biodistribution, blood circulation, and phagocytosis [21] are affected by the surface charge and hydrophobicity of the particles. Highly positively or negatively charged NPs showed very high liver absorption in *in vivo* biodistribution studies, which is most likely because of the macrophages (Kupffer cells) in the liver,

CHAPTER 5

Fate of Nanoparticles

Abstract: Gaining insight into the process that ingested nanoparticles/nanodrugs is crucial to maximize therapeutic advantages and avoid side effects. In the process of drug development, it is critical to consider how nanodrugs are ingested, how they interact with body fluids, how particles are absorbed by cells, and how they are eliminated to achieve effective treatments. In addition, consideration of the toxicity of the ingested nanoparticles is of utmost significance.

Hence the fate of ingested nanoparticles within the body will be covered in this chapter, including ingestion, endocytosis, exocytosis, and lastly the toxicity of the ingested NPs *in vivo* and *in vitro*. Initially, the chapter will brief about how the ingested nanoparticles undergo interactions with proteins in body fluids to form a protein corona and then will discuss comprehensively the different endocytic routes. Then the nanoparticle's excretion from cells which is essential for preserving homeostasis and receptor function will be discussed. Finally, the toxicity such as DNA damage, protein damage, cell membrane damage, oxidative stress, inflammation, impaired protein synthesis, deregulated cellular functions, and neurotoxicity of some commonly used nanoparticles will be outlined.

Keywords: Cellular damages, Endocytosis, Exocytosis, *In-vivo* toxicology, *In-vitro* toxicity, Nanoparticles, Neurotoxicity, Oxidative stress.

1. INTRODUCTION

Understanding the destiny of NPs in the human body is a crucial step in evaluating their safety and effectiveness in various consumer or medicinal applications. To better understand real-time behaviour, enhance targeted region absorption, and improve diagnostic techniques and treatment outcomes, it is imperative to study NP absorption, distribution, metabolism, and excretion in vivo systematically. For the purpose of industrializing nanoparticle formulations and creating novel nanodrug formulations, it is crucial to comprehend the absorption processes and toxicity of nanoparticle formulations. Hence the fate of ingested nanoparticles within the body will be covered in this chapter, including ingestion, endocytosis, exocytosis, and lastly the toxicity of the ingested NPs *in vivo* and *in vitro*.

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2. CELL UPTAKE AND METABOLISM

The nanodrugs can be administered to the body *via* four major routes; orally, transdermally, intravenously, and by inhalation [1]. Serum proteins attach non-specifically onto the nanoparticle surface when they come into contact with blood, forming a protein corona. The protein corona affects the physicochemical characteristics of nanoparticles by giving them an unintentional biological identity; subsequently, affecting how it interacts with biological systems such as organs, tissues, cells, and subcellular organelles. As a result, rather than the intentionally created synthetic nanoparticle features, this biological identity predominantly regulates nanoparticle *in vivo* circulation and biodistribution. Engineering of nanomedicines faces substantial difficulties since a nanoparticle's physicochemical characteristics may alter considerably after being exposed to the biological environment [2]. In order to optimize the therapeutic advantages of nanomedicines while avoiding adverse effects, it is crucial to investigate what happens to nanoparticles after they are introduced to the body.

Nanoparticles' absorption in cells requires regulated systems and biomolecular interactions to penetrate the cell plasma membrane, which acts as a barrier. The membrane's negative charge with few cationic areas and selective permeability to ions, biomolecules, and nanoparticles make it crucial to understand their fundamental absorption processes, as they impact their function, intracellular fate, and biological response [3, 4].

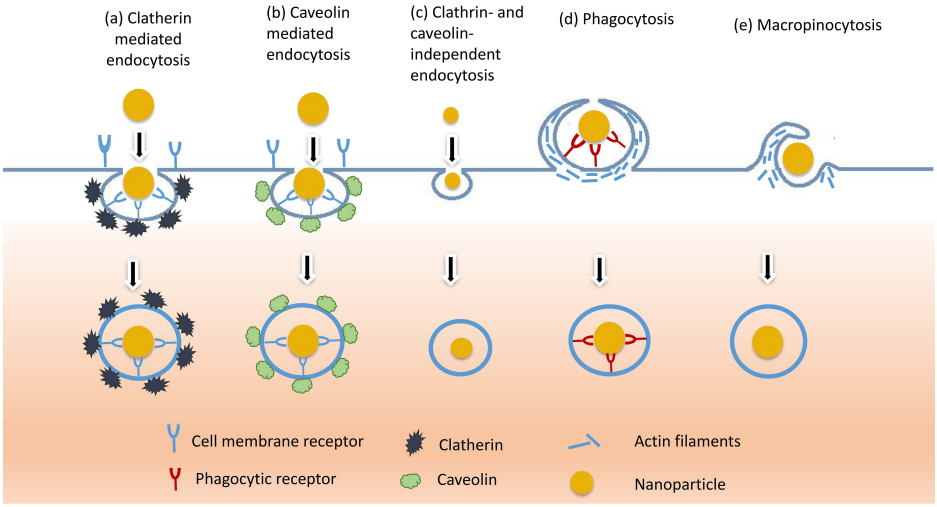

Fig. (1). Schematic diagram of endocytosis mechanisms (a) (a) clathrin-mediated; (b) caveolin-mediated; (c) clathrin- and caveolin-independent; (d) phagocytosis; and (e) macropinocytosis pathways.

The internalization mechanism significantly affects the rate of nanoparticle uptake, whereas the physical characteristics of the nanoparticles, such as size, shape, surface chemistry, including surface electrical charge, hydrophobicity, hydrophilicity, and ligand binding, influence the cell uptake process [5]. Furthermore, each cell has its own mode of internalization. Identifying the techniques of internalization can therefore assist scientists in determining which cells are more likely to ingest nanoparticles designed to target certain routes. When studying nanoparticles with different cell lines or shifting nanoparticles from *in vitro* to *in vivo* studies, a lack of understanding of internalization routes may result in poorly built nanoparticles with inadequate therapeutic efficacy. Additionally, diverse cell types may absorb a single nanoparticle in different ways [6, 7]. Direct fusion with the plasma membrane and endocytosis are the two primary routes into the cell.

2.1. Endocytosis

Nanoparticles may enter cells by a variety of different techniques and routes, which are referred to together as endocytosis. These processes are classified into five mechanistically distinct groups: (a) clathrin-dependent endocytosis, (b) caveolin-dependent endocytosis, (c) clathrin- and caveolin-independent endocytosis, (d) phagocytosis, and (e) macro-pinocytosis (Fig **1**). Several lipids and transport proteins, including lipid rafts clathrin, dynamin, caveolin, and pattern recognition receptors, regulate and govern various absorption pathways at the biomolecular level [8, 9].

Nanoparticle cell absorption mechanisms are controlled by biomolecules, affecting intracellular transport and influencing biological responses and therapeutic outcomes.

2.1.1. Clathrin-mediated Endocytosis

Clathrin-dependent endocytosis is characterized by the clustering and binding of nanoparticle surface ligands to cell membrane receptors such as transferrin receptors, β2 adrenergic receptors, low density lipoprotein receptors, and epidermal growth factor, resulting in complicated multistep processes [10]. It entails the formation of a clathrin-coated pit from the nucleation of cytosolic endocytosis-related proteins, bending and invagination of the plasma membrane, scission from the plasma membrane, which is then usually transported to endosomes with the aid of intracellular actin filaments, and uncoating and recovery of endocytic proteins from intracellular vesicles [11]. Nanoparticles with diameters between 100 and 500 nm are entrapped in intracellular vesicles through the clathrin-dependent endocytosis mechanism [12]. In intracellular vesicles, which are detached from the membrane with the aid of conformational changes

CHAPTER 6

Regulation, Development, and Commercialization of Nano-Based Drugs

Abstract: Nanopharmaceuticals necessitate rigorous, costly testing to address safety concerns, including cytotoxic effects. The lack of toxicity testing protocols and understanding of the interactions of nanomaterials make it difficult to make accurate assessments of health risks. To meet the purpose of regulating and monitoring nano products in pharmaceuticals, various nations have devised their suitable regulatory processes. Approximately two decades are required for drug development, which includes drug discovery, clinical testing, and production approval. However, only when a novel pharmaceutical product can be mass manufactured in industrially substantial quantities is its development considered to be accomplished. At present, nanodrugs have already been introduced successfully to the market, demonstrating their future potential. This chapter will provide comprehensive details about the drug development process covering regulations, development, and commercialization of nano-based drugs

Keywords: Crystalline NPs, Drug development, Drug commercialization, European medicines agency, FDA, Inorganic NPs, lipid-based NPs, Nano-drug regulation, Polymeric NPs.

1. INTRODUCTION

The advent of nanomedicine has been heralded ever since the U.S. Food and Drug Administration (FDA) granted its initial approval for a nanotherapeutic product back in 1995—Doxil, an anticancer medication utilizing PEGylated nano-liposomes. Contrary to popular belief, however, nanotechnology has not yet fundamentally altered how diseases are diagnosed and treated, despite a handful of clinical breakthroughs that have benefited cancer and cardiology in particular. Nanotechnology is expected to make a big contribution to medicines, *in vivo* imaging, and *in vitro* diagnostics, although the technology has not yet shown its full potential. Many contend that this is caused, at least in part, by the ambiguities surrounding the potential health dangers that nanomaterials could bring [1].

Nanopharmaceuticals undergo more thorough and expensive testing due to safety concerns, particularly those pertaining to the cytotoxic effects caused by nanomaterials [2]. Establishing reliable health risk assessments is difficult due to

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the absence of approved toxicity testing methodologies and the incomplete knowledge of how nanomaterials interact with biological systems [3]. Because of this ambiguity, nanomedicine may not have performed well in clinical trials, which is one of the main reasons it has taken so long for it to be an authorized therapeutic therapy [4, 5].

Different nations have devised appropriate regulatory procedures to achieve the goal of regulating and monitoring nano products. Countries like the United States (US), the United Kingdom (UK), European Union (EU), China, and Japan play a significant role in establishing and regulating standards and guidelines for the safe and successful commercialization of goods based on nanotechnology [6].

The Food and Drug Administration (FDA) in the US oversees the regulation of nanotechnology-related products, including nanomedicines, employing the legal and regulatory framework already in place as well as standards that are particular to each product. To address the challenge of regulating nanotechnology globally, the FDA established the Nanotechnology Task Force and Nanotechnology Interest Group, which was formed of officials from numerous regulatory centers. Although the FDA has not developed specific rules for nanomedicines, the Task Force anticipates that since they are subject to pre-market review and approval as part of the New Drug Application process, the current laws are sufficient for their safe development. This conclusion is predicated on the claim that current regulatory standards would be able to identify toxicity in nanoproducts [7]. In order to address the safety and efficacy issues of licensing complex medications including nanomaterials, the FDA at long last produced a non-binding draft guidance for industry in December 2017. Nevertheless, this creates a lot of ambiguities regarding potential approval paths [8]. In the US, until recent revisions by Frank R. Lautenberg Chemicals Safety for the 21st Century Act, a broad range of nanomaterials were under the Toxic Substances Control Act (TSCA) [9, 10]. They are subject to special regulations, such as premanufacture notices for nanoparticles and a rule requiring information collecting on both new and existing nanomaterials. Manufacturers are required to submit the precise chemical identification, production volumes, manufacturing processes, usage and exposure details, as well as any accessible health and safety data, in accordance with TSCA section 8(A) [11]. This new law applies to many pharmaceutical-grade excipients, and they must abide by its requirements. The Environmental Protection Agency (EPA) of the United States released a draft advice in January 2017 that lists commonly asked questions and the agency's responses to those queries from suppliers of nanoscaled materials [12].

The Medicines and Healthcare products Regulatory Authority (MHRA) handles the regulation of medications in the UK. There has been no specific guidance

provided with regard to nano medicine approval, and they appear to be handled on a case-by-case basis, as with the FDA. For assistance and direction throughout the process, researchers developing nano medicines are urged to communicate with the MHRA Innovation Office. Similar to the US, other institutions, including the UK- and EU-based European Nanomedicine characterisation Laboratory (EU-NCL), supply and continuously improve expertise on preclinical characterisation tests of nano medicine [13].

In accordance with European Union regulation (EC) No. 1907/2006 governing registration, evaluation, authorization, and restriction of chemicals (REACH), and regulation (EC) No. 1272/2008 on classification, labelling and packaging of substances and mixtures all chemicals, including excipients used in the manufacture of pharmaceutical products, are regulated by the European Chemicals Agency (ECHA) [14, 15]. These regulations are applicable to all compounds produced or imported at a rate of more than one ton annually and demand for the issuance of a dossier outlining the toxicological, physicochemical, and eco-toxicological features [11]. The second article of REACH specifically excludes from its scope pharmacological drugs, medicinal products, and invasive medical devices regulated by the European Medicines Agency (EMA) [15]. They are extensively assessed on a case-by-case basis throughout the medication approval procedure. In 2012 [16] and 2017 [17], the ECHA produced a number of nanomaterial-specific guideline sheets describing the regulations for chemical registration and testing, including nanomaterials, as defined by the European Commission (EC) [18]. The European Commission established new standards for the registration of nanomaterials on December 3, 2018. According to the new regulations, both the organization and ECHA must conduct a risk assessment of nanoparticles. From January 1st, 2020 onwards, these ECHa modifications become effective [19]. Numerous reflection papers on the standards for human-use nanomedicines, diagnostic iron oxide nanoparticles, generic liposomal products and surface coatings of nanoscaled parenteral dosage forms have been published by the EMA [11, 20, 21].

In South America, a committee of specialists in the field of nanoparticles was established by the Brazilian Health Surveillance Agency (ANVISA), with an emphasis on medications, medical devices, hygiene products, food and diagnostic tools and supplies. The objective was to create a questionnaire for manufacturers eager to register goods with nanomaterials. Without taking any more action, the effort was abandoned in 2016. All pharmaceuticals that include engineered nanoparticles are now assessed on an individual basis. The National Administration for Food, Drugs and Medical Technology (ANMAT) of Argentina's Nanotechnology Working Group published an article in January 2018 that provided an overview of the tests used to characterize and compare

CHAPTER 7

Future of Nanotechnology-Based Drug Discovery

Abstract: By enhancing drug administration and diagnostics, nanotechnology is transforming the healthcare industry. Novel approaches to drug design are being driven by combining cutting-edge technologies such as nanorobots and artificial intelligence. Healthcare can benefit from the potential of nanotechnology through the development of multifunctional nanotherapeutics, which could close gaps in the current therapeutic field.

Powered by integrated circuits, sensors, and data storage, nanorobots can increase efficiency and lessen systemic effects while follow-up care for cancer patients is made simpler by nanosensors. Additionally, nanotherapeutics have gained their way in developing novel therapeutics to overcome cancer drug resistance by targeting the mechanisms that induce the drug resistance. Another upcoming field in nanomedicine is the utilization of 3D printing techniques in order to create solid dosage forms based on nanomedicine. By enabling flexible design and on-demand manufacture of customized dosages, enhancing bioavailability, and other attributes, 3D printing technology has revolutionized the pharmaceutical industry. The futuristic applications of nanotechnology hybridized with novel techniques will be discussed in this chapter.

Keywords: Artificial intelligence, Drug resistance, Nanorobots, Nanosensors, Nanodrugs, 3-D drug printing, Patient follow-up care, Personalized medicine, Solid dosages.

1. INTRODUCTION

Nanotechnology is changing healthcare practices, and it is expected to have a huge impact in the future and enhance healthcare facilities. It has contributed to both diagnostics and the viability of therapeutic medication administration. The most cutting-edge technologies are being hybridized to take nanotechnology-based drug designing to new heights. For instance, by using artificial intelligence to assist the construction of nanostructures and the use of nanorobots, breakthroughs in nanotechnology have taken a fresh direction. In addition, the creation of multifunctional nano therapeutics has significant potential to close the gaps in the current therapeutic field. This section will discuss how pharmaceutical companies using nanotechnology are moving towards more sophisticated drug design strategies.

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2. FUTURE OF NANOTECHNOLOGY IN DISEASE DIAGNOSIS AND THERAPY

Pharmaceutical companies are among the first to benefit from artificial intelligence (AI), which has lately begun to ramp up its application in a variety of societal domains. AI and nanotechnology are two disciplines that are assisting in achieving precision medicine's objective of designing the optimum therapy for each patient. Recent crossover between these two fields has improved patient data collecting and improved nanomaterial design for precision cancer treatment.

The capacity to customize a nanomedicine for every patient is a commonly posed query when addressing precision medicine treatments. Although current technologies allow for the flexible attachment of any desired antibody to nanoparticles as well as versatility in the choice of cargo there are still a number of additional barriers that must be overcome before this strategy can be successfully used [1]. Along with the challenging clinical approval procedure for personalized nanomedicine, concerns must be raised about the drawbacks of present manufacturing methods and the expensive research and development expenses of nanomedicine. By creating a combinatorial therapy strategy for each patient that concurrently targets many pathways, more precise usage of already available medications may be achieved through the use of precision diagnostic platforms and customized drug-tailoring procedures. By doing so, it will be possible to overcome medication resistance and boost treatment effectiveness. The formation, development, and use of these nanomedications will heavily rely on artificial intelligence (AI) and other computational models in the future [2].

By adjusting the drug release rate to the unique pharmacokinetic and pharmacodynamic characteristics of the patient, nanomaterials can enable controlled drug dosage. External stimuli are utilized for this goal in a way similar to the targeted technique. In order to tailor the treatment, dosing management is not always enough, because patients with diverse pharmacogenomic profiles react differently to various medication dosages. In these circumstances, AI can be used to determine the relationship between drug dosage and the effectiveness of the treatment. For instance artificial neural networks that are capable of developing specific radiotherapy regimens for cancer patients in accordance with the treatment's objective, radiation's physical requirements and the patients' physiological and anatomical data have been generated [3].

Numerous issues with formulation development may be resolved by combining AI with nanotechnology [4]. By analyzing the energy released during the interaction between the drug molecules and keeping focus on any circumstances that can cause the formulation to aggregate, a methotrexate nano suspension was

created computationally [5]. The analysis of drug-dendrimer interactions and the assessment of drug encapsulation within the dendrimer can be aided by coarse-grained simulation in conjunction with chemical computation. Additionally, tools like LAMMPS and GROMACS 4 may be used to analyze how surface chemistry affects the ingestion of nanoparticles into cells [5]. The creation of silicasomes—a mix of irinotecan-loaded multifunctional mesoporous silica nanoparticles and the tumour-penetrating peptide iRGD— has been aided by AI. Since iRGD enhances silicasome transcytosis, improving treatment outcomes and overall survival, it consequently increased the absorption of silicasomes [6].

Although tailored omics data analysis has advanced, it is still challenging to predict a patient's reaction to treatment solely based on omics. This challenge is particularly evident in the realm of cancer, where it is necessary to take into consideration the heterogeneity of the tumour and metastases as well as the emergence of resistance over time when projecting the tumour's response [7]. The course of treatment can be optimized by evaluating a potential medicine within the patient's body to determine its response. Such *in situ* diagnostics are made possible by nanotechnology. The efficacy of a medicine may be predicted in a patient's tumour by using barcoded liposomes, which each containing a drug and a unique DNA barcode [8, 9]. For instances, murine breast cancer models were injected with unique DNA barcoded liposomes containing four potential medications (gemcitabine, cisplatin, doxorubicin, and caffeine) at dosages less than 0.1% [10]. After 24 hours, a biopsy of the tumour was performed, and the distribution of barcodes between live and dead tumour cells was examined. Higher concentrations of barcodes corresponding to medications with high efficacy were discovered in dead tumour cells compared to low amounts in living cells. Taking a direct count of the barcodes and the percentage of the liposomes delivered to the live and dead cells was the method of analysis in this approach. By taking into account intricate patterns of barcode distribution, integrating AI algorithms into the data analysis process might broaden its application and enable the detection of combinatorial treatment effects [2].

The outcomes of the *in situ* nano-based screening can also be enhanced by using computational approaches for in silico drug screening as the first step [11]. For instance a researcher used a random forest classifier learning-based algorithm to categorize mutational cancer drivers according to their mode of action [12, 13]. The foundation of this method is a mix of decision trees that assess certain attributes once the algorithm has been trained on prior data. An automated fitting of therapeutic agents to each patient's mutational landscape was done after creating a dataset of medications that are currently on the market and are capable of targeting these cancer drivers based on drug-target interactions. This computational technique gives a distinct viewpoint on potential treatment

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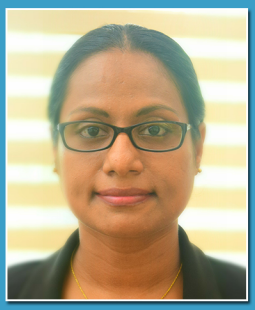
With his independent research contributions, he published research papers in peer reviewed journals, book chapters and also received President's Award for Scientific Research in 2020.



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