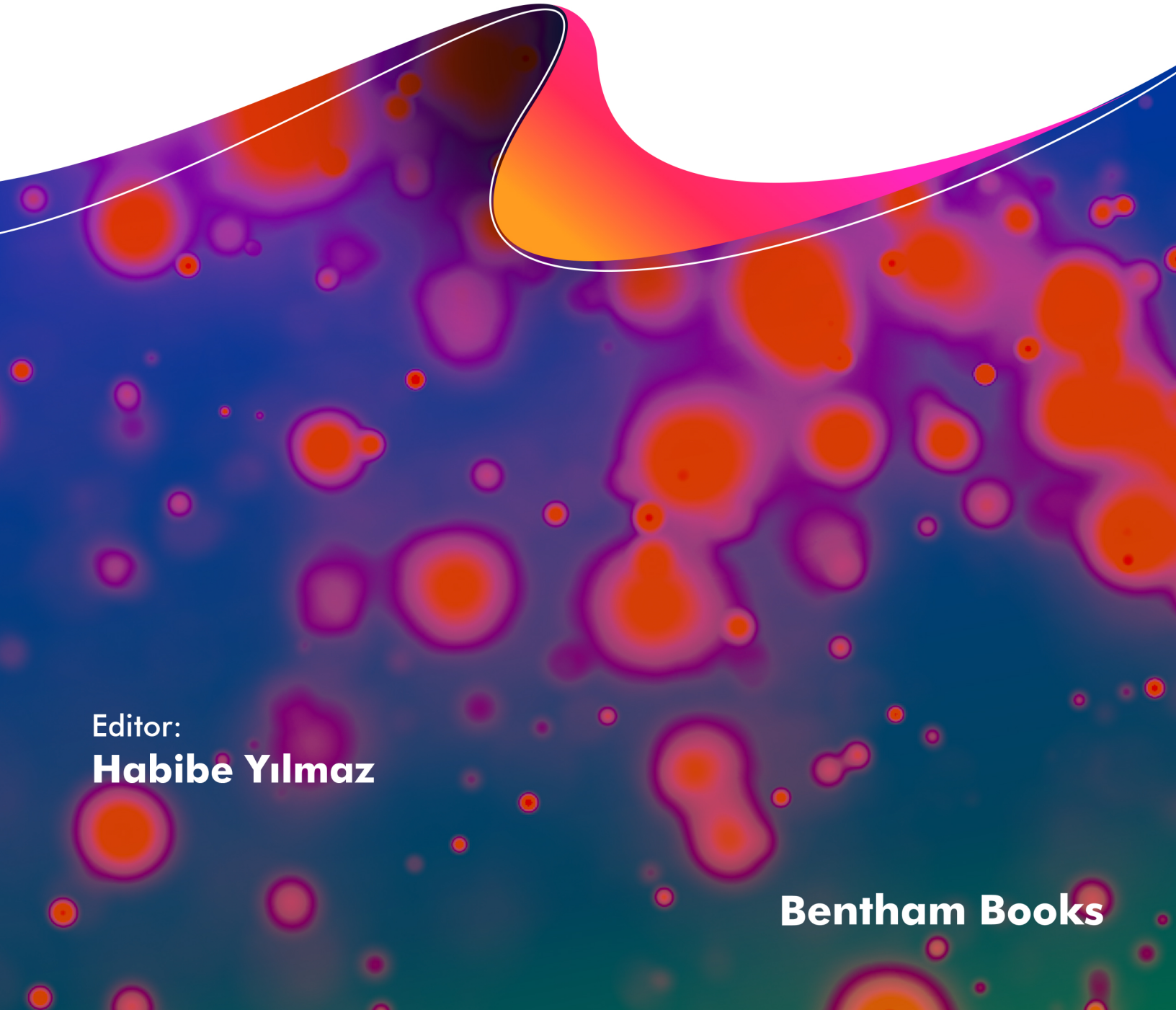
RECENT PROGRESS IN PHARMACEUTICAL NANOBIOTECHNOLOGY: A MEDICAL PERSPECTIVE



Editor:
Habibe Yılmaz

Bentham Books

Recent Advances in Biotechnology

(Volume 8)

Recent Progress in Pharmaceutical Nanobiotechnology: A Medical Perspective

Edited by

Habibe Yılmaz

Department of Pharmaceutical Biotechnology
Faculty of Pharmacy
Trakya University
Edirne
Türkiye

Recent Advances in Biotechnology

(Volume 8)

Recent Progress in Pharmaceutical Nanobiotechnology: A Medical Perspective

Editor: Habibe Yilmaz

ISSN (Online): 2468-5372

ISSN (Print): 2468-5364

ISBN (Online): 978-981-5179-42-2

ISBN (Print): 978-981-5179-43-9

ISBN (Paperback): 978-981-5179-44-6

First published in 2023.

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Pte. Ltd.
80 Robinson Road #02-00
Singapore 068898
Singapore
Email: subscriptions@benthamscience.net

CONTENTS

FOREWORD

In a world that has become global and where all kinds of technology have entered our lives rapidly with the industrial revolution, it has brought many diseases with it, even though the quality of life and duration of people have increased. The fact that we have started to use technological developments more effectively has enabled us to develop the awareness of coping with all new and old diseases that we encounter. In addition to repositioning conventional drugs, biotechnological drugs and new nano-drugs are being developed from the combination of nanotechnology with biology. It has become a necessity for every individual who wants to improve herself/himself in this field to follow the data that is revealed quickly and to follow the applications in life. Current practices and research aim to increase patient compliance and to restore patients' health as soon as possible without creating any financial and social burden.

In this context, this book focuses on recent advancements and applications of nanobiotechnology in medical application areas.

This edited book covers 14 high-quality chapters. The chapters of this book have a large variety of interesting and relevant subjects such as precision medicine, biomimetic design, exosomes, glycobiology, targeting strategies of nanomedicines, biocompatibility, *in vitro* and *in vivo* evaluation of nanomedicines as well as specific applications at different pathologies and technologies such as photodynamic therapy and biosensors. I highly recommend this book to students, academicians, researchers and industrial organizations who are interested in the new era of medicinal applications of nanobiotechnology.

Ercüment Karasulu
Department of Pharmaceutical Technology
Faculty of Pharmacy
Ege University
Bornova, 35100
İzmir
Türkiye

PREFACE

Biotechnology is a science that has many application areas such as medical, microbial, forensic, plant, agricultural, marine and food. In recent years, developments in the medical biotechnology field have accelerated. In particular, with the emergence of knowledge on the molecular and biochemical basis of diseases, biotechnological drugs have been preferred over conventional drugs. With the development of omics technologies, our increasing knowledge about diseases has revealed that diseases should be handled individually rather than in general. This, in turn, has given rise to precision medicine and the need for more specific tools for more effective treatment. Based on this information, the range of pharmaceutical products developed by medical biotechnology has expanded from recombinant proteins to nano drug delivery systems. Nanobiotechnological approaches allow many alternatives in terms of both treatment and diagnosis. There are many advantages going down to nano sizes, such as targeting, selecting, and penetrating a single cell, and providing more effective treatment with a lower drug dose.

This book aims to give the reader general information about some of the current therapeutic and diagnostic nanopharmaceutical products as well as their *in vitro* and *in vivo* applications. The reader will be able to grasp the wide possibilities and most current applications of nanobiotechnology.

Many different nanodrug systems, such as biomimetic systems, lipid and metallic nanoparticles, exosomes and glycoconjugates and photodynamic therapy, are included. In addition, information about the emergence of nanobiotechnology in personalized therapy, nanobiotechnological approaches in cancer stem cells and glioblastoma, and their use as biosensors will be conveyed. Emphasizing the importance of the biocompatibility and toxicological profile of these products, information about the relationship and modulation of bio corona, which is one of the most current issues of recent years, will also be included. Following targeting strategies, which is one of the most important advantages of nanodrugs, the book is concludes with two chapters on *in vitro* and *in vivo* studies of nanopharmaceuticals.

The application areas and tools of nanobiotechnology are so wide that it has not been possible to include them all in this book. However, we expect that readers will be informed about the most current and promising approaches to nanobiotechnology from a medical point of view.

Habibe Yılmaz
Department of Pharmaceutical Biotechnology
Faculty of Pharmacy
Trakya University
Edirne
Türkiye

List of Contributors

Abdullah Tuli	Çukurova University, Faculty of Medicine, Medical Biochemistry Department, Adana, Turkey
Aslı Sade Memişoğlu	Faculty of Education, Department of Science Education, Dokuz Eylul University, İzmir, Turkey
Ayça Erek	Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Trakya University, Edirne, Türkiye
Aysa Ostovaneh	Department of Biotechnology, Ege University, Graduate School of Natural and Applied Sciences, 35100, İzmir, Türkiye
Bakiye Göker Bağca	Department of Medical Biology, Aydin Adnan Menderes University, Aydin, Turkey
Buket Özel	Ege University, Faculty of Medicine, Department of Medical Biology, Izmir, Turkey
Ceren Sarı	Department of Medical Biology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey
Cigir Biray Avcı	Department of Medical Biology, Ege University, Izmir, Turkey
Elvan Bakar	Trakya University, Faculty of Pharmacy, Department of Basic Science, Edirne, Turkey
Emine Taşhan	Zoleant LLC, Zoeuticals, Regulatory Affairs and Quality, New York, USA
Fulya Oz Tuncay	Karadeniz Technical University, Faculty of Science, Department of Chemistry, Trabzon, Türkiye
Figen Celep Eyüpoğlu	Department of Medical Biology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey
Güliz Ak	Department of Biochemistry, Faculty of Science, Ege University, 35100, İzmir, Türkiye
Habibe Yılmaz	Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Trakya University, Edirne, Türkiye
Hande Balyapan	Department of Biochemistry, Faculty of Science, Ege University, 35100, İzmir, Türkiye
Nebiye Pelin Türker	Development Application and Research Center, Trakya University, Technology Research, 22030, Edirne, Türkiye
Nur Selvi Günel	Ege University, Faculty of Medicine, Department of Medical Biology, Izmir, Turkey
Özlem Çoban	Department of Pharmaceutical Technology, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Türkiye
Sezgi Kıpçak	Ege University, Faculty of Medicine, Department of Medical Biology, Izmir, Turkey
Sultan Eda Kuş	Faculty of Engineering, Department of Genetics and Bioengineering, Alanya Alaaddin Keykubat University, Antalya, Turkey

Şenay Hamarat Şanlıer	Ege University, Faculty of Science, Department of Biochemistry, İzmir, Turkey
Şeref Akay	Faculty of Engineering, Department of Genetics and Bioengineering, Alanya Alaaddin Keykubat University, Antalya, Turkey
Tuğba Karakayalı	Ege University, Faculty of Science, Department of Biochemistry, Izmir, Turkey
Ummuhan Cakmak	Karadeniz Technical University, Faculty of Science, Department of Chemistry, Trabzon, Türkiye
Ümmühan Fulden Aydın	Çukurova University, Faculty of Medicine, Medical Biochemistry Department, Adana, Turkey
Yakup Kolcuoğlu	Karadeniz Technical University, Faculty of Science, Department of Chemistry, Trabzon, Türkiye
Yeliz Yıldırım	Department of Chemistry, Faculty of Science, Ege University, İzmir, Türkiye Center of Drug, R&D, and Pharmacokinetic Aplications (ARGEFAR), Ege University, İzmir, Türkiye
Zehra Tavşan	Faculty of Education, Department of Science Education, Dokuz Eylul University, İzmir, Turkey
Zeynep Erim	Department of Biotechnology and Genetics, Trakya University, Institute of Natural and Applied Sciences, 22030, Edirne, Türkiye

CHAPTER 1

Bioinspired, Biomimetic Nanomedicines

Şenay Hamarat Şanlıer[1,*], **Ayça Erek**[2] and **Habibe Yılmaz**[2]

[1] *Ege University, Faculty of Science, Department of Biochemistry, İzmir, Turkey*

[2] *Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Trakya University, Edirne, Türkiye*

Abstract: Bio-inspired nanotechnology (biomimetic nanotechnology) is defined as the acquisition of nanomaterials or nanodevices and systems using the principles of biology during design or synthesis. Transferring a mechanism, an idea, or a formation from living systems to inanimate systems is an essential strategy. In this context, nanoparticles inspired by nature have many advantages, such as functionality, biocompatibility, low toxicity, diversity, and tolerability. It is known that biomimetic approaches have been used in materials science since ancient times. Today, it plays a crucial role in the development of drug delivery systems, imaging, and diagnostics in medical science. There is no doubt that interest and research in biomimetic approaches, which is an innovative approach and inspired by nature, will continue in the field of medicine and life sciences hereafter. Within the scope of this chapter, polymeric nanomedicines, monoclonal antibodies and related structures, cell and cell-membrane-derived biomimetic nanomedicines, bacteria-inspired nanomedicines, viral biomimetic nanomedicines, organelle-related nanomedicines, nanozymes, protein corona, and nanomedicine concepts and new developments will be elucidated.

Keywords: Bacteria-inspired, Bioinspired nanomedicine, Biomaterials, Biomimetic nanomedicine, Bionanotechnology, Cell membrane-derived, Cell, DNA, Monoclonal antibody, Nanobiotechnology, Nanomedicine, Nanoparticle, Nanotechnology, Nanozyme, Nature-inspired, Nature, Organelle-related nanomedicine, Protein corona, Viral, Virus-inspired.

INTRODUCTION

Before moving on to the topics that follow in the book chapter, terminology related to nanotechnology, biomimetics, and bioinspiration elaboration would be beneficial. In the last 10 years, standards related to nanotechnology or nanomaterials have been retrived, published, or withdrawn. There are 223 stan-

[*] **Corresponding author Şenay Hamarat Şanlıer:** Ege University, Faculty of Science, Department of Biochemistry, İzmir, Turkey; Tel: +90 232 311 2323; E-mail: senay.sanlier@ege.edu.tr

Habibe Yılmaz (Ed.)

dards published or under development regarding nanotechnology on the International Organization for Standardization (ISO) website.

ISO/DIS 80004-1(en) defines the nanoscale as a size in the range of approximately 1 to 100 nm. Nanomaterials, on the other hand, are defined as materials with nanoscale external dimensions or nanoscale in their internal structure or surface structure. Nanoparticles are defined as "nanoobjects with all external dimensions in the nanoscale" [1]. ISO/TS 80004-5:2011(en) defines the relationship between nanomaterials and biology. Nanobiotechnology, bionanotechnology, and bioinspired nanotechnology are defined separately. According to the standard, nanobiotechnology is the application of nanoscience or nanotechnology to biology or biotechnology, while bionanotechnology is the application of biology to nanotechnology. Bio-inspired nanotechnology (in other words, biomimetic nanotechnology) is defined as the acquisition of nanomaterials, or nanodevices, and nanosystems by using the principles of biology during design or synthesis [2].

It has been stated in a previous review that biomimetic nanomedicines exhibited minimal interaction with the biological environment when applied at the beginning of the technology development period. Interaction with the biological environment is enhanced by modifying these relatively inert nanomedicines with targeting molecules or by designing them to respond to stimuli from the biological environment. Finally, in the review, it is stated that the third generation is obtained by coating these nanoparticles on cell membranes [3].

However, bio-inspired/biomimetic nanomedicine has moved further beyond this classification. Nanoparticles are now encapsulated in organelles or can be targeted to the organelle. In addition, they can be encapsulated into bacteria, yeast, and viruses. Virosomes, or virus-like particles (VLPs), are also among the bio-inspired structures, especially in vaccines, as part of preventive therapy [4].

In addition, as will be discussed under the following headings, many other nanomedicines, such as exosomes, nanozymes, monoclonal antibodies and derivatives, and reprogramming of dendritic cells are among the bioinspired/biomimetic nanomedicines. Lab-on-a-chip or organ-on-a-chip applications are also among biomimetic/bio-inspired nanotechnological designs. However, it will not be discussed as it is out of the scope of the chapter.

Liposomes

Liposomes were discovered in England in the 1960s by Dr. Alex D. Bangham *et al.* and published in 1964. Since its discovery, much research has been done on it, and it has taken its place in the market as a liposomal drug [5].

Liposomes are nanostructures of various lipid components in the form of a lipid bilayer, like a cell. Its core offers the advantage of encapsulating hydrophilic components, while the lipid bilayer layer offers the advantage of encapsulating hydrophobic components. At the same time, it has many other advantages, such as biocompatibility, self-forming capacity, good reproducibility, derivatization of the outer surface, and suitable physicochemical behavior. Since their discovery, apart from their conventional use, liposomes have been PEGylated, derivatized with targeting molecules, and even developed as a theranostic structure [6].

In this chapter, attention is drawn to other uses of liposomes other than their conventional synthesis and derivatization. Nowadays, liposomes are used as models to understand cell membranes, and even their qualities are taken a step further by gaining features that mimic cell membranes, which are prepared as hybrid membranes that fuse with liposomes. A group of researchers in Spain carried out a study to synthesize liposomes that mimic HeLa cell membranes to facilitate liposome cell recognition and thus deliver drugs to their targets. They proved that the presence of cholesterol in the liposome structure is important for such an interaction and that it is effective as SNARE (Soluble NSF Attachment Protein Receptor) proteins [7]. They used artificial liposomes as decoy targets for toxin neutralization against infectious diseases, thus preventing the devastating effects of infection. Researchers have shown that liposomes prepared using sphingomyelin, cholesterol, phosphatidylcholine, and phosphatidylserine protect monocytes against S. *pyogenes, S. pneumoniae,* and *S. aureus* [8]. Another group prepared reconstituted high-density lipoprotein (HDL) nanostructures containing a low concentration of ganglioside monosialotetrahexosylganglioside (GM1) lipoprotein. Thus, they were able to neutralize the cholera toxin. During their study, they determined that lipoprotein configuration is essential in receptor-toxin interaction [9].

On the other hand, fusogenic liposomes are used so that the liposomes can more effectively transport drugs to the cytoplasm or to the target. Fusogenic liposomes were designed with the knowledge that cells use membrane fusion for inter- and intracellular molecule transport. Among the important factors in the preparation of fusogenic liposomes are the use of the neutral lipid dioleoylphosphatidylethanolamine (DOPE) and the cationic lipid 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP) at appropriate rates in liposome synthesis and the use of SNARE or proteins mimicking SNARE, which allows membranes to be positioned close to each other [10 - 12].

CHAPTER 2

Lipid-Based Nanocarriers and Applications in Medicine

Ümmühan Fulden Aydın[1,*] and **Abdullah Tuli**[1]

[1] *Çukurova University, Faculty of Medicine, Medical Biochemistry Department, Adana, Turkey*

Abstract: Lipid nanocarriers have recently arisen with a wide range of uses and research areas, with the advantages they offer in virtue of their unique properties. They are easily synthesized, scaled up, biodegradable, proper to transport many bioactive components, have a high loading capacity, and are convenient for various routes of administration (parenteral, oral, dermal, ocular, *etc*.). These carriers overcome the problems of bioactive substances such as low solubility, plasma half-life and bioavailability, and side effects, as well as providing controlled release, local delivery, and targeting. Lipid-based nanoparticular systems can be categorized into two basic classes, vesicular and non-vesicular. While liposomes are the most widely used vesicular structures, solid lipid nanoparticles and nano-structured lipid carriers are non-vesicular nanocarriers. These nanocarriers have many medical uses, such as cancer therapy, gene therapy, photodynamic therapy, treatment of infectious diseases and neurodegenerative diseases, vaccines, imaging, *etc*. It is essential that the synthesis method of lipid-based nanocarriers and the components from which they are composed are selected in accordance with the medical application area and characterization studies are carried out. In this article, liposomes, solid lipid nanoparticles and nano-structured lipid carriers will be discussed as lipid-based nanocarriers, synthesis and characterization methods will be emphasized and examples from medical applications will be given.

Keywords: Cancer Therapy, Characterization, Controlled Release, Drug Delivery, Emulsions, Entrapment Efficiency, High-Pressure Homogenization, Liposomes, Loading Capacity, Medical Applications, Nano-Structured Lipid Carriers, Particle Size, Polydispersity Index, Solid Lipid Nanoparticles, Solvent Evaporation, Solvent Injection, Targeted Therapy, Thin Film Hydration, Vaccines, Zeta Potential.

[*] **Corresponding author Ümmühan Fulden Aydın:** Çukurova University, Faculty of Medicine, Medical Biochemistry Department, Adana, Turkey; Tel: +90 322 338 60 60/ 3466 ; E-mail:bozkayafulden@gmail.com

Habibe Yılmaz (Ed.)

INTRODUCTION

Lipid-based nanocarriers have been in the limelight in recent years. These nanocarriers are preferred due to their dedicated physical properties as well as their unique size and shape properties. They are easily synthesized, scaled up, biodegradable, proper to transport many bioactive components, have a high loading capacity, and are convenient for various routes of administration (parenteral, oral, dermal, ocular, *etc*.). There is a need for lipid-based nano-drug carriers to increase the low biological efficiency of drugs with low plasma solubility and bioavailability due to their lipophilicity and reduce the side effects they cause by providing controlled drug release and local drug delivery. Like other nano drug carriers, lipid-based nanocarriers are designed to solve the problems of conventional therapies, such as low bioavailability, high toxicity, low plasma stability, and thus reduced half-life [1 - 3].

Lipid-based nanoparticular systems can be categorized into two basic classes, vesicular and non-vesicular. The discovery of vesicular liposomes, one of the most extensively used nanoparticles in the pharmaceutical field, was initiated in 1961 by hematologist Dr. Alec D. Bangham. Solid lipid nanoparticles (SLNs), discovered in 1991 as a choice to liposomes, and nano-structured lipid carriers (NLCs), which are superior to them, are non-vesicular lipid-based carriers [4].

In this section, the structural properties, synthesis, and characterization methods of liposomes, SLNs, and NLCs will be discussed and their medical applications will be mentioned.

LIPOSOMES

Liposomes are bilayer vesicles ranging in size from 20 nm to 2.5 μm, consisting of cholesterol and amphiphilic natural or synthetic phospholipids, allowing loading of both lipophilic and hydrophilic compounds into them. The properties of liposomes also differ according to the synthesis method, the type, composition, and ratio of lipids, and, therefore the size and surface charge that they affect [4 - 6].

Liposomes are generally classified according to their lamellarity and size. Liposomes consisting of a single bilayer are called unilamellar vesicles and are also subclassed according to their size as giant unilamellar vesicles (GUV, >1μm), large unilamellar vesicles (LUV, >100 nm) and small unilamellar vesicles (SUV, <100 nm). However, the structure in which 2-5 and >5 bilayers are sequentially intertwined is called oligolamellar vesicles (OLV, 100-1000 nm) and multilamellar vesicles (MLV, >0.5 μm) respectively, and the structure formed by the presence of bilayer vesicles in a large vesicle is called multivesicular vesicles

(MVV, >1 μm) as shown in Fig. (**1**) [7]. The sizes of liposomes can vary depending on the synthesis method, and at the same time, the area of application is an important factor in deciding the type of liposome. SUV is mostly preferred for its long circulation time, passive targeting to the desired area, and cell uptake. For example, Doxil®, one of the FDA (Food and Drug Administration)-approved liposomal drugs, is SUV, an IV-administered liposomal formulation loaded with doxorubicin used for ovarian cancer, Kaposi's sarcoma, and myeloid melanoma. However, Arikayce® is loaded with amikacin, which is used for lung diseases; its size is approximately 300 nm, and is a liposomal formulation applied with a nebulizer. Whether the liposome is unilamellar or multilamellar also affects the choice of liposome type since it is an important parameter in the release of the loaded substance. Unilamellar ones release their content more easily than multilamellar ones but have a higher loading capacity for hydrophilic agents [8 - 10].

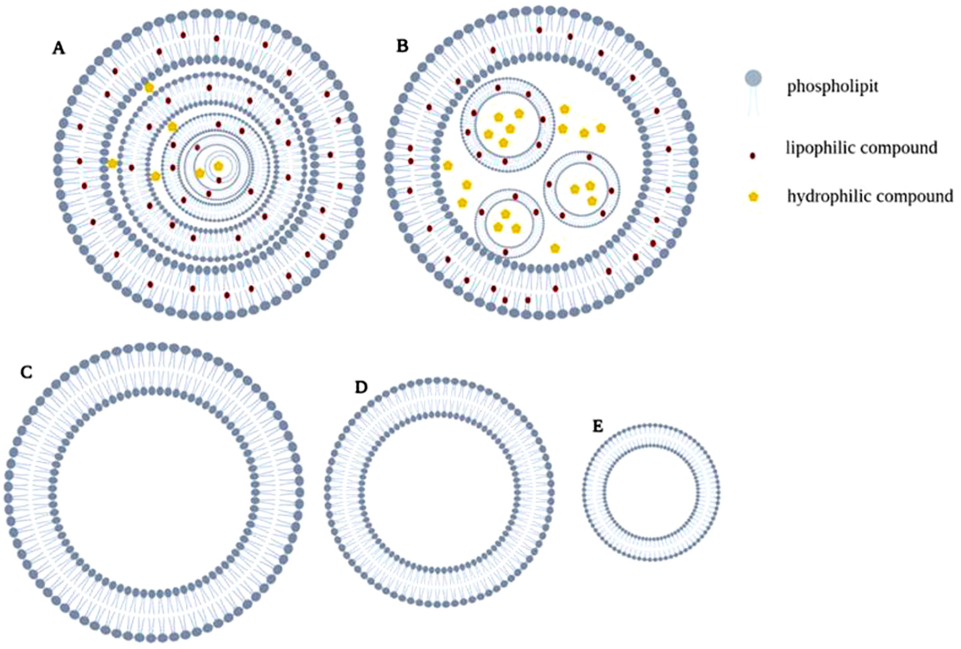


Fig. (1). Classification of liposomes according to lamelarity and size. **A**) Multilamellar liposome (MLV) **B**) Multivesicular liposome (MVV) **C**) Giant unilamellar liposome (GUV) **D**) Large unilamellar liposome (LUV) **E**) Small unilamellar liposome (SUV).

Formation and Composition of Liposomes

As it is known, phospholipids, the basic building blocks of liposomes, are amphiphilic, causing them to self-assemble in the aqueous environment due to the polar head groups and tails formed by the apolar fatty acid chains. As the polar

CHAPTER 3

Metallic Nanoparticles: Synthesis and Applications in Medicine

Şeref Akay[1,*] and **Sultan Eda Kuş**[1]

[1] *Faculty of Engineering, Department of Genetics and Bioengineering, Alanya Alaaddin Keykubat University, Antalya, Turkey*

Abstract: The progress in nanoscience and advances in the fabrication, characterization, and modification of materials at the nanoscale have paved the way for the production and use of nanoparticles with different properties. Today, the chemical agents used in many therapies cannot achieve the desired effectiveness due to dose-dependent toxicity, low solubility and bioavailability, damage to non-target organs and tissues due to non-specificity, and side effects. Nanoparticle systems produced in different forms and compositions are one of the main approaches used to eliminate the negative aspects of conventional chemical agents. Among these nanoparticle systems, metallic nanoparticles represent a promising approach. During the last two decades, metallic nanoparticles (MNPs) have drawn great attention due to their optical, electrical, and physicochemical properties as well as their size-dependent properties. The large surface to volume ratio and surface reactivity of metallic nanoparticles provide great potential for combining them with different biological/chemical agents, as well as they can also be formulated as a bioactive nanoplatform alone. In this regard, the present chapter summarizes the general aspects of metallic nanoparticles, common methods for synthesis, and various applications in the biomedical field.

Keywords: Antibacterial, Antitumor, Biogenic, Bottom-up, Cancer, Chemical reduction, Diagnostic, Drug delivery, Gold nanoparticles, Green synthesis, Chemotherapy, Iron oxide, Laser ablation, Magnetic nanoparticles metallic nanoparticles, Nanomaterial, Nanotechnology, Silver nanoparticles, Targeting, Top-down.

INTRODUCTION

The ability to detect the Earth's magnetic field and navigate through it is widespread in nature. The most well-researched organisms are *magnetotactic bacteria*, abundant aquatic microorganisms, which produce distinctive organelles called magnetosomes to accomplish *magnetotaxis* and to seek for zones that

[*] **Corresponding author Şeref Akay:** Faculty of Engineering, Department of Genetics and Bioengineering, Alanya Alaaddin Keykubat University, Antalya, Turkey; Tel: 90 242 510 61 20; E-mail: seref.akay@alanya.edu.tr

Habibe Yılmaz (Ed.)

promote growth in chemically diverse water layers and sediments [1]. *Magnetospirillum gryphiswaldense* is one of the renowned microbes representing magnetotactic bacteria.

These strains use the Earth's magnetic field for navigating owing to magnetosomes, which consist of magnetic iron nanocrystals enclosed in a membrane [2]. The nanocrystals in the magnetosomes must be aligned in a chain to create effective magnetoreception, as shown in Fig. (**1**), instead of agglomerated forms. Thus, the magnetic moments of each nanocrystal in the chain produce a dipole potent enough to line up the entire bacterium within the low geomagnetic field [1, 3]. Empty membrane capsules are formed first in the biosynthesis of magnetosomes, and large amounts of iron are transferred and magnetites (Fe_3O_4) are produced inside these vesicles [2]. These nanosized particles can work like a navigation system because they show single-field magnetic properties.

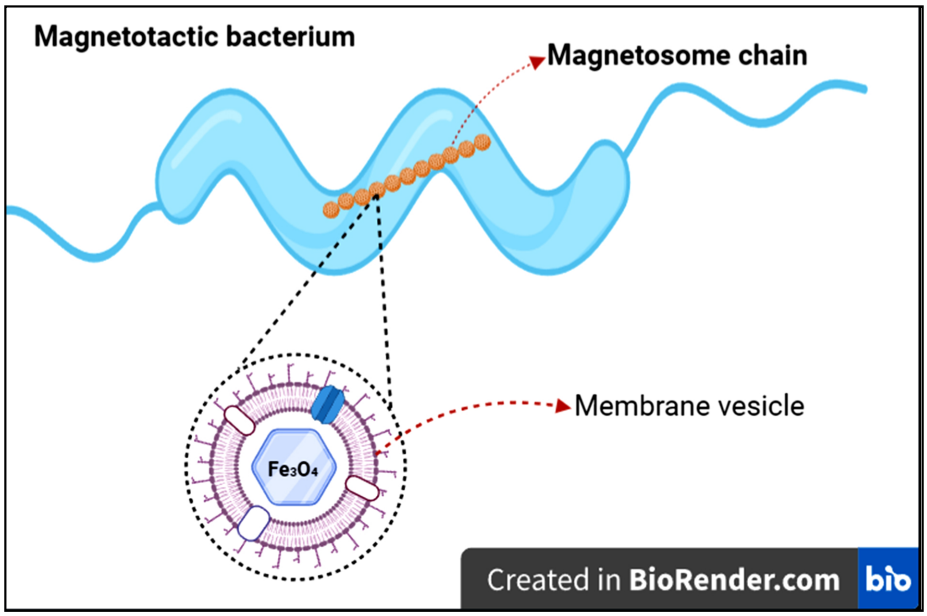


Fig. (1). Magnetosomes alignment to help bacteria floating in the water to navigate.

Instead, if the bacteria produced a single bulk material with the size of the individual particle chain, the magnetic field would become ineffective, and the bacteria would not be able to align according to the magnetic field inclination of the region [3]. This example is a very good example of the changing properties of metallic materials at the nanoscale and the benefit of nature from these changing properties.

Configurations of nanometric materials cause changes in their physical, chemical, and biological behaviors. This effect, which is defined as the quantum confinement effect, states that the properties of materials depend on their size at the nanoscale. The size-dependent properties include chemical reactivity, melting point, electrical conductivity, magnetic penetration, and alter as a function of particle size. The most substantial outcome of quantum effect is providing the ability to researchers for adjusting the properties of the material for required purposes [4]. Unpredictable alterations in surface properties due to the size at the nanoscale have drawn the attention of different fields of nanomaterials. Because the surface area and reactivity establish great potential for many applications [5].

Metallic or metal nanoparticles (MNPs) usually have a metal core consisting of an inorganic metal or metal oxide coated with a shell of organic or inorganic material. Mostly copper, gold, silver, iron, aluminum, lead, and zinc metals are used.

MNPs have emerged as a new type of nanomaterial in the field of nanotechnology in the last few years. Noble metals with useful effects on health, such as gold, silver, and platinum, are used for the production of nanoparticles. Significant advances have appeared in colloid science over the past century, including the introduction of pioneering methodologies for the production of metals, nano forms of metal oxides, and organic products. Especially with the use of MNPs as drug release and carrier systems in cancer treatment, substantial progress has been made in this area. Today, MNPs and related nanostructures are investigated more closely because of their outstanding properties which are beneficial for catalysis, material science, detection and treatment of disease, and sensor technology [5, 6].

MNPs are considered flexible and interesting nanostructures due to both the simple synthesis and the wide range of properties they present. This flexible platform provides high applicability in many fields, such as biomolecular ultrasensitive sensing, protein and cell labeling, targeted delivery of intracellular therapeutic agents, and hyperthermal therapy for cancer. In terms of comprehending their optical and electronic features, MNPs also offer an advantage over other nanoplatforms [6 - 8].

Many MNPs in commercial goods such as personal care products (cosmetics, toothpaste, *etc*.), detergents, drugs, and pharmaceuticals are in direct contact with the human body. Even with the concerns about the toxicity of some MNPs, they have been mostly proven advantageous with appropriate size and dosage. Besides the most desirable properties, such as high surface area and antimicrobial effect, it is thought that the simplicity of functionalization of MNPs can improve their biomedical capacity and enhance their therapeutic performance [9, 10].

CHAPTER 4

Photodynamic Therapy and Applications in Cancer

Ceren Sarı[1] and **Figen Celep Eyüpoğlu**[1,*]

[1] *Department of Medical Biology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey*

Abstract: The idea of using light as a therapeutic tool has been popular for thousands of years. Scientific discoveries in line with technological innovations have contributed to the advancement of photodynamic therapy as a therapeutic modality. Photodynamic therapy is based on the generation of highly reactive species that alter the molecular systematics of cells through interactions between light, photosensitizer, and molecular oxygen. It has a minimally invasive protocol that can be combined with other clinical methods or can be stand-alone. The development of photosensitizers with the integration of nanotechnological approaches has provided favorable results over the years in malignant and non-malignant diseases by facilitating target-site action, selectivity, and controllable drug release. This chapter presents a review of photodynamic therapy with its important aspects; history, mechanism of action, cellular effects, integration into nanoscale drug delivery systems, and combinational therapeutic approaches in cancer.

Keywords: Anticancer therapy, Apoptosis, Autophagy, Cancer, Tumor, Cell death, Clinical application, Combination therapy, Drug delivery systems, Immunogenic cell death, Light therapy, Nanotechnology, Necroptosis, Necrosis, Neoplasms, Photodynamic therapy, Photosensitizer, Reactive oxygen species, Singlet oxygen, Vascular endothelium.

INTRODUCTION

Photodynamic therapy (PDT) is a minimally invasive and highly selective therapeutic modality in which photosensitizer molecules, light, and molecular oxygen are combined to destroy unwanted cells and tissues. Photosensitizer molecules, which do not exert toxic effects under normal conditions, are activated by light. Following activation, they transfer energy to molecular oxygen through photochemical reactions, producing highly toxic reactive oxygen species (ROS) that lead to cell demise [1, 2].

[*] **Corresponding author Figen Celep Eyüpoğlu**: Department of Medical Biology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey; Tel: +90 462 377 7941; E-mail: fcelep@ktu.edu.tr

Habibe Yılmaz (Ed.)

PDT has yielded effective results in various clinical fields, such as dermatology, immunology, cardiology, ophthalmology, urology, and dentistry [2 - 11]. The most comprehensive field of study of PDT is cancer therapy. Antitumoral PDT studies have documented promising results in different types of cancer (breast, skin, brain, lung, head and neck, gastrointestinal, gynecological, prostate, *etc*.) [12 - 19].

PDT has various advantages compared to conventional therapeutic approaches such as surgery, chemotherapy, and radiotherapy. The only route of activation of photosensitizer is co-administration with light, resulting in minimal systemic toxicity because exposure time and site can be managed. Numerous potential applications of PDT are conceivable, as ROS can damage cells from different origins. Besides, PDT may be recommended as adjuvant therapy to overcome some of the difficulties of common therapeutic models or to support their outcomes [20 - 23].

BRIEF HISTORY OF LIGHT THERAPY

Light has a special meaning in ancient civilizations [24]. Sun worship represents many ancient religions in Asia and Europe. Ancient cultures believed the sun had a healing potential for various ailments. In the times of ancient Egypt, extracts from different plants were applied to skin lesions and exposed to sunlight as a treatment protocol. In other ancient civilizations, such as China, Greece, and India, phototherapy applications were used to treat different diseases such as vitiligo, psoriasis, skin malignancies, and even psychosis [24 - 26].

The development of modern phototherapy dates back to the early 1900s. Arnold Rikli, considered to be one of the pioneers of modern phototherapy, mentioned sunbathing as a treatment method and helped to re-emerge the therapeutic effects of sunlight. Phototherapy was popularized by Niels Finsen, who won the Nobel Prize in 1903 for his study applying carbon arc phototherapy in the treatment of lupus vulgaris. In the early 20th century, Oscar Raab, a student of Professor Herman von Tappeiner, conducted experiments to analyze the toxic effects of acridine on paramecia. He noticed that the toxic effects of acridine varied with daylight and were minimal on stormy days. Further experiments have confirmed that the toxicity of acridine is dose- and light-dependent, thus, the combination of the two has shown highly toxic effects. Continuing Raab's research, Von Tappeiner, together with dermatologist Albert Jesionek, focused on the implementation of eosin as a photosensitizer in skin cancer, skin lupus, and condylomas of the female genitals. In 1904, von Tappeiner and Albert Jodlbauer stated that oxygen is an exigency for the process of photosensitization. These studies were collected in a book in 1907, in which von Tappeiner used the term

"photodynamic action" to delineate the phenomenon of oxygen-induced photosensitization [25 - 27].

EXCITING JOURNEY OF PHOTODYNAMIC THERAPY

Von Tappeiner's clear prediction of the phototherapeutic application of photosensitizers has accelerated scientific studies in this field. The use of hematoporphyrin, a derivative of porphyrin, has greatly contributed to the development of PDT [26]. Hausmann reported that the combination of hematoporphyrin and light, which he applied to paramecium and red blood cells, significantly killed the cells. He also observed skin reactions in mice exposed to light after being treated with hematoporphyrin [25, 28]. In 1913, Friedrich Meyer-Betz self-administered hematoporphyrin to observe how it would work in humans. He was the first scientist to use porphyrins as photosensitizers in humans, observing edema and prolonged pain in light-exposed areas [28, 29].

The concept of modern PDT began in the 1960s with the studies of Samuel Schwartz and Richard Lipson. Schwartz successfully developed a hematoporphyrin derivative (HpD), which has higher phototoxicity than hematoporphyrin, and Lipson demonstrated tumoral accumulation and therapeutic effects of HpD in patients with different lesions [28 - 30]. In 1975, an important wall of PDT studies was built by Thomas Dougherty's study, which combined HpD and red light to destruct mammary tumors in mice [31]. That same year, J. F. Kelly demonstrated that HpD-PDT eradicates bladder carcinoma in mice [32]. In 1976, another breakthrough in the development of PDT occurred with the first human bladder cancer study to demonstrate the therapeutic effects of HpD-PDT by M. E. Snell and J. F. Kelly [33]. Over the years, several studies have been conducted and different types of tumors (breast, colorectal, pancreas, head and neck, brain, cholangiocarcinoma, mesothelioma, *etc*.) were treated with PDT [34 - 47].

Photofrin, one of the most common HpDs, received its first healthcare approval for use in the prophylactic treatment of bladder cancer in 1993. The Food and Drug Administration (FDA) approved Photofrin in 1995 for the treatment of esophageal cancer. Later, Photofrin was approved for usage in early-stage lung cancer in 1998 [48]. Thus, PDT has strengthened its spot in the clinical literature as a novel therapeutic modality.

DEVELOPMENT OF PHOTOSENSITIZERS

Photosensitizers are an essential part of PDT procedures. Various photosensitizers have been studied over the years, and some of them have been approved for clinical use [49]. As a result of intensive studies on PDT, it has been understood that ideal photosensitizers should have important properties such as high levels of

CHAPTER 5

Biotechnological Importance of Exosomes

Elvan Bakar[1,*], Zeynep Erim[2] and **Nebiye Pelin Türker[3]**

[1] *Trakya University, Faculty of Pharmacy, Department of Basic Science, Edirne, Turkey*

[2] *Department of Biotechnology and Genetics, Trakya University, Institute of Natural and Applied Sciences, 22030, Edirne, Türkiye*

[3] *Development Application and Research Center, Trakya University, Technology Research, 22030, Edirne, Türkiye*

Abstract: Extracellular vesicles are molecules secreted by cells, wrapped in phospholipids and carrying some types of RNA, DNA and protein in their inner region. Extracellular vesicles are classified as apoptotic bodies, microvesicles, and exosomes based on their extent and formation process. Exosomes, which have the smallest structure, have received more attention than other extracellular vesicles. Exosomes contain different types of molecules in their structures. Cell membranes comprise a lipid bilayer and contain different cargo molecules and different surface receptors, depending on the cells of origin where biogenesis takes place. The biogenesis of exosomes begins within the endosomal system. Then they mature and are released out of the cell. The biogenesis of exosomes may be associated with the ESCRT complex and may depend on many molecules other than the ESCRT complex. Exosomes excreted by the origin cells are taken up by the target cells in different ways and show their effects. The effects of exosomes on their target cells may vary according to the cargo molecules they carry. They participate in cell-to-cell communication by sending different signals to distant or nearby target cells. Exosomes have a variety of pathological and physiological effects on disease and health. They have different effects on many diseases, especially cancer. They play an active role in cancer development, tumor microenvironment, angiogenesis, drug resistance and immune system. There are many diseases that can be used as a biomarker due to increased secretion from cells of origin in pathological conditions. In addition, exosomes can be utilized as drug transportation systems due to their natural structure. In addition, they are potential candidates as effective vaccines because of their effects on immune system cells or the effects of exosomes secreted from immune system cells.

Keywords: Extracellular vesicles, Exosomes, Biogenesis, Diseases, Cancer, Metastasis, Tumor microenvironment, Biomarker, MiRNAs, Therapy, Immunity, Immune regulation, Clinical applications, Target, Vaccine, Drug resistance, Immune response, Cell metabolism, Intercellular communication, Epithelial-mesenchymal transition,.

[*] **Corresponding author Elvan Bakar:** Trakya University, Faculty of Pharmacy, Department of Basic Science, Edirne, Turkey; Tel: +905327173787; E-mail: elvanbakar@trakya.edu.tr

Habibe Yılmaz (Ed.)

INTRODUCTION

Extracellular vesicles (EVs) are identified by the International Society of Extracellular Vesicles as particles that are bounded by a bilayer lipid membrane that is intrinsically secreted from the cell and that do not proliferate, that is, do not have a nucleus [1].

Wolf discovered EVs in plasma in 1967 and defined them as "platelet dust." [2]. Vesicles are present in all biological fluids tested over time, and they are secreted by cells that have been shown to be of varying sizes. These vesicles have also been given various names over time. Nevertheless, they are now popularly referred to as extracellular vesicles [3]. Because of their potential for use in therapy and diagnosis, they are clinically significant molecules. EVs are crucial molecules in many diseases, such as cancer, neurological diseases, preeclampsia and osteoarthritis. The mechanism of action of these diseases, as well as their potential for use in treatment and diagnosis, have been investigated and are still being investigated.

EVs consist of a lipid bilayer membrane that surrounds the inner molecules. It is well understood that after being released by origin cells, recipient cells can be targeted and bound *via* EV surface proteins and thus mediate communication with different cells [4]. During development, they take an active part in several physiological processes, including morphogen transport, inflammation regulation, coagulation, and sexual behavior in all types of organisms. Furthermore, extracellular vesicles secreted by tumor cells have been demonstrated to function in cell-to-cell communication by promoting angiogenesis, altering the extracellular matrix composition and/or altering the immune response [5].

EVs are heterogeneous nanoparticles formed through various biogenesis pathways. They differ in terms of surface markers and also molecular and genetic content. Their size is highly variable, as are their biogenesis, surface markers, and molecular and genetic contents [6]. They are divided into three classes based on their sizes, biogenesis mechanisms and functions (Fig. **1**). Exosomes with sizes ranging from 30nm to 100nm are the smallest of the EVs. Macrovesicles are released from the cell membrane by burgeoning directly outward, and their dimensions range from 100nm to 1μm. Apoptotic bodies are EVs that form from surface bubbles and are especially released by apoptotic cells. Their dimensions range from 50nm to 2μm [7]. Apoptotic bodies differ significantly from exosomes and microvesicles. The primary difference is that apoptotic bodies have organelles, whereas microvesicles and exosomes do not. Therefore, the proteomic properties of apoptotic bodies are extremely similar to the cell's lysate. On the

other hand, exosomes have certain differences between their cells of origin. Exosomes are interesting for a variety of reasons [8].

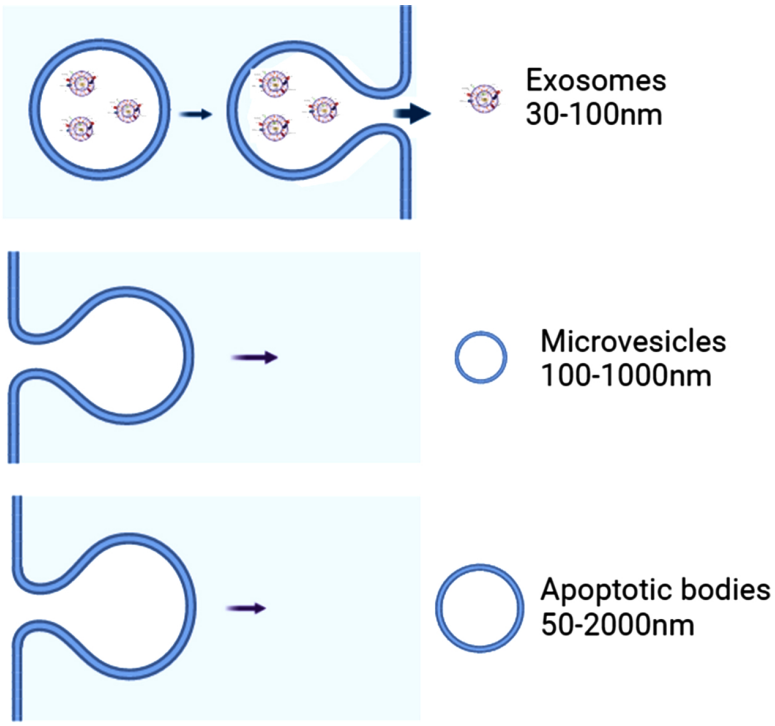


Fig. (1). Extracellular vesicles of various sizes.

Exosomes are the main topic of this chapter, and in the following topics, their structures, biogenesis, functions, effects on various diseases, particularly cancer, use for treatment and diagnosis, and potential applications will be mentioned in detail.

STRUCTURE AND BIOGENESIS OF EXOSOMES

Exosomes, one type of EV, have received more attention than microvesicles and apoptotic bodies. Therefore, data obtained in order to comprehend their structure, biogenesis, and release from the cell membrane are more abundant than for other types of extracellular vesicles. However, research is ongoing to fully comprehend them.

“Exosome” term was used first time in 1987 by Johnstone in a study of vesicle formation in sheep reticulocytes [9]. In membrane-bound structures, it has been named exo-some because it is a process that generally involves the outward release of internal vesicular contents, as opposed to endocytosis which is the

CHAPTER 6

Biosynthesis and Function of Glycoconjugates

Elvan Bakar[1,*], **Nebiye Pelin Türker**[2] and **Zeynep Erim**[3]

[1] *Trakya University, Faculty of Pharmacy, Department of Basic Science, Edirne, Turkey*

[2] *Development Application and Research Center, Trakya University, Technology Research, 22030, Edirne, Türkiye*

[3] *Department of Biotechnology and Genetics, Trakya University, Institute of Natural and Applied Sciences, 22030, Edirne, Türkiye*

Abstract: Investigations to ascertain the physiological roles of carbohydrates in biological systems are being given more importance each day. Basically, carbohydrates are biomolecules with a wide range of biological functions, although they represent the primary energy source for metabolic processes. Carbohydrates are found as structural components in connective tissue in animal organisms. They also act as structural elements in both plant and bacterial cell walls. In the cell, they bind to lipids and proteins to form glycoconjugates called glycolipids, glycopeptides, glycoproteins and peptidoglycans. By binding to lipids and proteins on the cell surface, they perform as molecules that support intercellular adhesion and intercellular communication. Glycobiology is the science that investigates the structure, biosynthesis, and impacts of glycans on biological functions. In biology, glycoconjugates serve a variety of key roles. In mammalian cells, the majority of proteins are glycosylated, and this explains how proteins perform their various functions. In the future, these techniques will be crucial for the identification and treatment of specific diseases. The most major area of progress in glycobiology is the development of carbohydrate-based medicines.

Some diseases, including cancer, can be diagnosed *via* altered cell surface glycosylation pathways as a biomarker. Therefore, regulating glycosylation mechanisms and understanding the phenotypic characteristics of glycoconjugates are crucial steps in the design of novel strategies.

This chapter discusses the biosynthesis of glycoconjugates, their wide range of biological functions, and their significance for therapy.

Keywords: Biomarker, Biosynthesis, Carbohydrate, Cancer, Diagnosis, Function, Glycan, Glycosylation, Glycoconjugate, Glycoprotein, Glycolipid, Glycosidases, Membrane, *N*-Glycan, *O*-Glycan, Oligosaccharide, Protein, Proteoglycan, Transferases, Therapeutic Effects.

INTRODUCTION

Glycobiology studies the biosynthesis, structural and biological functions of glycoconjugates. It is an important sub-branch of biology that has been deve-

[*] **Corresponding author Elvan Bakar**: Trakya University, Faculty of Pharmacy, Department of Basic Science, Edirne, Turkey; Tel: +905327173787; E-mail: elvanbakar@trakya.edu.tr

Habibe Yılmaz (Ed.)

loping rapidly in the last 80 years. Glycans and oligosaccharides, which are covalently bonded to proteins and lipids, surround all cells. In complex multicellular organisms, the biological functions of these glycans are crucial for interactions between cells, tissues, and molecules. Depending on the biomolecules in the cell membrane, there are numerous distinct glycans Fig. (**1**). These glycans regulate cell signaling and cell-cell adhesion [1]. Glycans that are bound to proteins are also prevalent in cells' cytoplasm and nucleus. The sugar components of glycoconjugates also modulate various functions in physiological and pathophysiological processes in addition to forming structural properties [2].

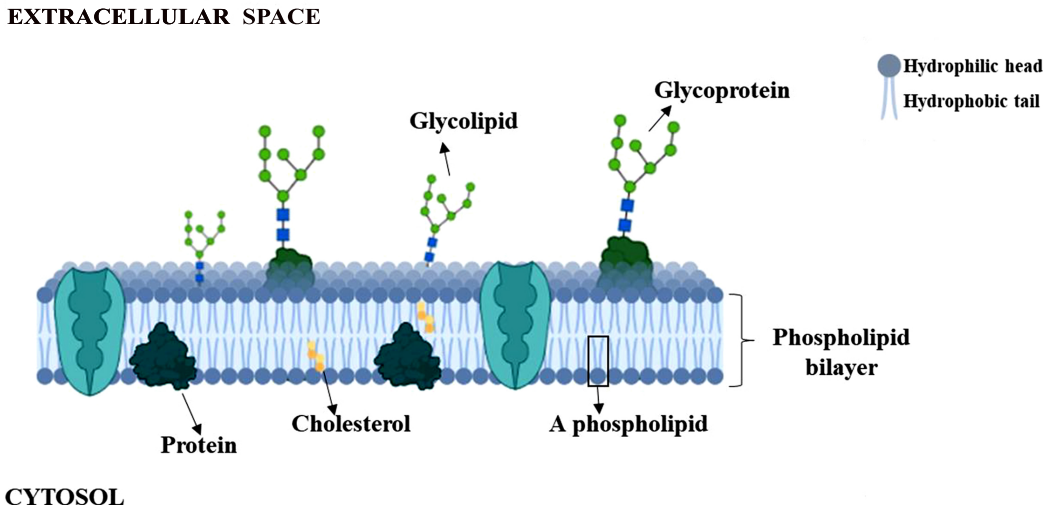


Fig. (1). Glycans on the cell surface.

The chemistry and metabolism of carbohydrates were among the most significant issues in the first part of the 20th century. However, it has lagged far behind other molecular biology studies due to the inability to predict the biosynthesis of glycans directly from a DNA template and its structural complexity [1, 3]. Glycobiology has been a broad field of research formed by the merger of many sciences, such as basic research, biotechnology, and biomedicine. This field includes the formation of glycans, the roles of glycans in complex biosystems, their chemistry and metabolism, enzymology and their analysis by various techniques. Therefore glycans glycobiology isn't based only on chemical synthesis, terminology, structure, biosynthesis, and function but also on cell biology, molecular genetics, medicine, physiology, and developmental biology, such as required by interdisciplinary work (3).

Monosaccharides are found in alpha (α) and beta (β) forms. Different connection types, positions, numbers, attachment points, and functional group differences of monosaccharides are important in polymer structures [4]. Each glycoform has a different function as a biological ligand. Many studies conducted in the field of glycobiology are on the determination of glycosylation mechanisms, elucidation of their molecular structure, investigation of biological control mechanisms in different cell types, and their use in therapy [5].

Complex carbohydrate polymers covalently bound to proteins and lipids serve as signals that determine the position and function of hybrid molecules, and these compounds are called glycoconjugates. Glycoconjugates are formed by a biochemical process called glycosylation. Glycosylation starts in the endoplasmic reticulum of eukaryotes and ends in the Golgi cistern. Glycoconjugates are also necessary for long-term immune protection. They participate in detoxification, cell-cell communication, and interactions with the cell matrix because of this [6, 7]. The most varied molecules in nature in terms of structure and function are glycoconjugates. Covalent bonds between carbohydrates and proteins and lipids result in three different forms of glycoconjugates. Proteoglycans function chemically like polysaccharides despite sharing the same types of linkages as glycoproteins. Moreover, mucins composed of glycoproteins and glycos-phingolipids are monosaccharide-modified glycoconjugates [7]. Some of them are structures on a single polypeptide with more than 100 distinct saccharide side chains, which are possibly the most complicated molecules in life [8]. In many studies using cationic dyes such as alcian blue and ruthenium red, it has been shown that almost all cells are surrounded by glycocalyx, known as carbohydrate sheath [6, 9]. Oligosaccharides and polysaccharides that are linked to proteins or lipids in the membrane make up glycocalyx. Even though erythrocytes are small eukaryotic cells, they feature a relatively extensive and complicated glycocalyx [10, 11]. This structure, which is present in all cells, consists of several glycoconjugates, which are quite complex. This structure is critical to cell biology as it specifically mediates and modulates interactions of the cell with small molecules, macromolecules, the extracellular matrix, and other cells. From this point of view, the glycocalyx is much more complex and selective in terms of its molecular interactions, although it has the physical properties of both ion exchange resins and gel filtration. In addition to serving as recognition molecules in multicellular interactions, saccharides of glycocalyx linked to proteins and lipids also act as binding sites for bacterial and viral pathogens [12, 13]. Laminin, collagen, and fibronectin, among other secreted glycoproteins, fill the gaps between eukaryotic cells. Additionally, proteoglycans and glycosaminoglycans contribute substantially to the extracellular matrix's structure. For instance, the three different types of corneal cells secrete proteoglycans and collagens that are highly ordered to preserve the tissue's uniqueness [14 - 18]. Likewise, the

CHAPTER 7

Nanoparticle Targeting Strategies In Cancer Therapy

Hande Balyapan[1] and **Güliz Ak**[1,*]

[1] *Department of Biochemistry, Faculty of Science, Ege University, 35100, İzmir, Türkiye*

Abstract: This review outlines major cancer targeting strategies for nanoparticle systems. Targeted therapies have superiority over conventional chemotherapy or radiotherapy methods. Nanoparticles as drug nanocarriers enable drug delivery to the tumoral regions. For targeted drug delivery, nanoparticles are designed and tailored depending on the cancer and the purpose of the targeting mechanism. In this review, nanoparticle targeting for cancer therapy was summarized into three sections: passive, active, and physical targeting. Each issue was described and discussed with recent nanoparticular studies and their findings. In addition, a combination of targeting with diagnostics and theranostics was also presented.

Keywords: Active targeting, Antibody targeted delivery, Aptamer targeted delivery, Cancer, Cancer electrotherapy mediated drug delivery, Drug delivery systems, Epr effect, Lectin targeted delivery, Magnetic targeted delivery, Nanomedicine, Nanoparticle, Nir-triggered drug delivery, Passive targeting, Physical targeting, Receptor targeted delivery, Stimuli responsive nanoparticle, Targeted nanoparticle, Theranostic, Thermoliposome, Ultrasound sensitive/ mediated drug delivery.

INTRODUCTION

One of the main challenges of cancer treatment is to treat the disease site without damaging healthy tissues. Drug targeting basically refers to the delivery of a drug to a specific disease site in order to achieve safer and more efficient therapeutic results. A smart practice for drug transportation can be enabled using nanotechnology. Thanks to this technology, nanocarriers can escape from the reticuloendothelial framework, prevent the drug from degradation despite biological obstructions, and deliver the drug to the target cancerous area [1 - 3]. There are several nanoparticle targeting strategies for cancer treatment.

* **Corresponding author Güliz Ak:** Department of Biochemistry, Faculty of Science, Ege University, 35100, İzmir, Türkiye; Tel: +902323111711; E-mail: guliz.ak@ege.edu.tr

Habibe Yılmaz (Ed.)

Types of Targeting

Passive Targeting

With passive targeting, the behavior of drug delivery systems in the body is controlled by using body defense mechanisms such as metabolism, excretion, opsonization, and phagocytosis. The high permeability of the vasculature and the immaturity of the lymphatic drainage system allow the cytotoxic agent to accumulate in the tumor mass. For targeting, the modification and design of the surface charge of the system are carried out by taking into account the molecular weight, size, and surface hydrophobicity of the system. Thus, by allowing the long-term circulation of drugs in the blood, it is ensured that the desired areas are targeted. Uncontrolled drug release with passive targeting can be met with negative consequences such as off-target drug delivery and, as a result, multidrug resistance. Furthermore, the permeability of vessels can be heterogeneous throughout a single tumor. These two situations limit passive targeting [4].

Increased Permeability and Retention Effect (EPR)

When solid tumors reach a certain size, the vascular system surrounding them is not sufficient to provide the oxygen supply necessary for the tumor to proliferate. Cells start to die from a lack of oxygen. As a result, they release growth factors from the surrounding capillaries that trigger the budding of new blood vessels. In this process, called angiogenesis, due to rapid growth, blood vessels are irregular with discontinuous epithelium and have no basement membrane, forming a leaky vasculature with a fenestration of 200 to 2000 nm. This allows better penetration of blood components as it reduces resistance to extravasation into the tumor interstitium. This indicates the improved permeability portion of the EPR effect [5].

The extracellular fluid of healthy tissues is continuously emptied into the lymph vessels at an average flow rate of 0.1-2 μm/s. This allows for the continuous replenishment of interstitial fluid as well as the return of extravasated solutes and colloids to the circulation. Because the lymphatic functions are defective in tumor cells, the outflow of interstitial fluid is blocked. Molecules smaller than 4 nm diffuse back into the bloodstream and are reabsorbed, while the diffusion of macromolecules and nanoparticles are hindered by their large hydrodynamic size. Therefore, nanoparticles reaching the perivascular space are not cleaned sufficiently and concentrate in the tumor interstitium. This aspect represents the enhanced retention part of the EPR effect [6].

Due to the abnormal and leaky vasculature and EPR effect in tumor tissues, nanoparticles accumulate more in this region than in other tissues. Moreover, the

nanoparticle system increases the half-life of the drug owing to the escape of the drug from renal clearance. Factors such as protein binding and nanoparticle aggregation have an impact on the EPR effect by increasing the size of the nanoparticle system based on complex formation. It is known that the reticuloendothelial system in the liver and spleen reduces the effect of EPR [7]. Wang *et al.* developed ultrafine iron oxide nanoparticles and studied a breast tumor model. Nanoparticles were accumulated in the tumor interstitial space due to their size, which was formed by self-assembling in the acidic area. Thus, tumor targeting occurs with EPR driven passive targeting [8].

Localized Delivery

The physiological barriers faced by passively and actively targeted nanoparticles need to be overcome. Administering drugs directly to the disease area helps to overcome such obstacles as it does not interfere with the systemic circulation barrier. This method is very effective because it is easy to administer drugs to certain parts of the body, such as the lungs, bladder, brain, peritoneum, and eyes. Topical application, which is one of the local delivery methods, can significantly increase the pharmacological action in the disease area and reduce systemic toxicity since it does not enter the circulation and can take effect in a short time. A variety of drug carrier systems such as liposomes, microparticles, polymeric films, and hydrogels have been prepared for localized delivery. However, while *in vitro* and preclinical studies have been reported for a number of nanoparticles for local delivery in cancer, just a few have reached the clinical stage.

This targeting method is particularly effective when localized chemotherapy of non-metastatic primary tumors and surgical resection are contraindicated. Moreover, for debulking surgeries requiring adjuvant or neoadjuvant chemotherapy to minimize local regional recurrence, local delivery of chemotherapeutics may lead to better therapeutic results with lower toxicity. However, the use of a local delivery strategies is limited in cancer types that are difficult to reach [9].

Tumor Microenvironment

There are some parameters that distinguish the tumor microenvironment from healthy cells. Compared to blood vessels in healthy tissues, the vasculature at the tumor site exhibits varying structural and functional properties. The tumor microenvironment is highly acidic as a result of anaerobic glycolysis and lactic acid production of cancer cells, and this microenvironment is characterized by hypoxia and restricted nutrient supply. As a result of the hypoxia state the gene expression of tumor cells can be altered, thereby cell survival can be increased. The variations in vascular networks generate a distinct tumor microenvironment and ultimately affect therapeutic efficacy. By targeting these physical variables,

CHAPTER 8

Nanomedicine Based Therapies Against Cancer Stem Cells

Aslı Sade Memişoğlu[1,*] and **Zehra Tavşan**[1]

[1] *Faculty of Education, Department of Science Education, Dokuz Eylul University, Izmir, Turkey*

Abstract: A tumor consists of not only cancer cells but also an ecosystem including different subpopulations. Cancer stem cells (CSCs) are a rare subpopulation in the tumor cell population. Traditional therapies, such as chemotherapy and radiotherapy target cancer cells except for CSCs. Therefore, the self-renewal and colony formation capacity of CSCs provides the recurrence of tumors as well as drug resistance. Different strategies are used to eradicate CSCs with the knowledge of CSC properties. The recent technologic revolution gives a chance to design nanoscale medicines for the effective treatment of CSCs. Nanoparticle-based delivery systems improve the transport of traditional therapeutic drugs across biological barriers with maximum bioavailability, less toxicity, and side effects, and take advantage in combination with specific CSC targets, controlled and site-specific release. This chapter summarizes the current models of CSCs, the molecular mechanisms leading to metastases and drug resistance of CSCs, strategies to target CSCs, examples of currently approved nanomedicine drugs and future perspectives.

Keywords: Biodistribution, Cancer stem cell, Chemosensitivity, Chemotherapy, Differentiation, Drug delivery system, Drug resistance, Epithelial-to-mesenchymal transition, Metastasis, Nanomedicine, Nanoparticle, Nanotechnology, Radiotherapy, Regenerative medicine, Self-renewal, Stability, Solubility, Traditional therapy, Tumor-initiating cell, Tumor relapse.

INTRODUCTION

Although there are improvements in cancer therapy, many patients continue to have therapy failure, which causes the illness to grow, return, and lower overall survival. Recent advances in screening tumors revealed that a tumor is not only a collection of uniform cancerous cells, instead, a tumor is an ecosystem that includes heterogeneous tumor cells as well as a microenvironment that can affect how the tumor functions as a whole [1]. Individual tumor cells within a tumor also differ from each other through both genetic and non-genetic mechanisms, result-

[*] **Corresponding author Aslı Sade Memişoğlu**: Faculty of Education, Department of Science Education, Dokuz Eylul University, Izmir, Turkey; Tel: +90542374464; E-mail: asli.memisoglu@deu.edu.tr

Habibe Yılmaz (Ed.)

ing in variance in the "hallmarks of cancer" and the formation of different tumor cell populations. In addition, the heterogeneity within a tumor seems not constant but plastic in nature, which adds another level of complexity to the process of tumorigenesis [2].

Cancer stem cells (CSCs) are defined as a separate population of cells in the tumor and have been demonstrated to exhibit long-term clonal repopulation and self-renewal capacity in many cancers [3, 4]. The defining features of this stemness have also been shown to drive therapy resistance. Evidence from both clinical studies and experimental models suggests that CSCs endure many of the cancer therapies that are frequently used and contribute to metastasis and recurrence of the disease. Additionally, CSC-specific characteristics and transcriptional patterns are significantly prognostic of overall patient survival, demonstrating their therapeutic importance [5, 6].

Traditional therapies, such as chemotherapy, radiotherapy, and drugs that target tumors, frequently lead to a selection of the CSC population, increasing their likelihood of survival and dissemination. To specifically determine CSC response to treatment, additional preclinical and clinical research is required. Additionally, effective therapeutic techniques against CSCs must be developed in order to boost the efficacy of cancer therapy. Over the past few years, a growing number of therapeutic drugs that can destroy CSCs have been evaluated or developed [5]. Unfortunately, most such agents share traits with other anticancer medications, such as small peptides and molecule drugs, that restrict their clinical applications. These include side effects, inadequate biodistribution, low solubility, insufficient circulation time, instability and low therapeutic effects.

The ultimate goal of nanomedicine is to enhance the quality of life by using nanoscale instruments for disease detection, prevention, and therapy as well as to gain insights into the intricate pathophysiology of disease. The limitations of traditional pharmaceutical delivery methods make them less suitable for delivering bioactive compounds into distant tissues [7]. The use of biosensors for diagnostic purposes and biocompatible nanomaterials as delivery systems for therapy, such as nanocapsules for the treatment of cancer, are among the main research areas of nanomedicine. One of the main goals of nanomedicine is to use nanotechnology to find treatment for diseases, such as cancer, and to use targeted drug delivery for more efficient treatment with fewer adverse effects. Targeted delivery methods include liposomes, polymers, micelles, conjugates, nanoparticles, and conjugates of this nanopharmaceuticals [8].

WHAT ARE CANCER STEM CELLS?

As early as the 19th century, some striking parallels between embryonic development and tumor growth were recognized. Malignant tumors were shown to contain rare cell populations that are capable of self-renewal and proliferation. Several decades ago, the first evidence supporting the CSC hypothesis was produced [9]. This study revealed that a limited number of cells harbored the potential to initiate leukemia in mice. These cells also exhibited high similarities with hematopoietic stem cells (HSCs). In addition to expressing typical HSC markers (CD34+/CD38−), these cells could also renew themselves and differentiate. Notably, tumors that developed in mice after *in vivo* transplantation of CSCs, formed a very comparable cell population to the primary tumor. Therefore, the same process may be repeated at every stage of the development of cancer: CSCs produce cells that are the same as the transplanted ones, as well as additional differentiated cells undergoing a process of differentiation and losing their tumorigenic potential. CSCs have now been seen in a variety of malignancies, including those that affect solid tissues. For example, very few CSCs (*e.g.*, 100 cells) could form breast cancer tumors in mice, in contrast to thousands of different phenotype cells that could not start tumors [10]. Later research on colon, brain, head and neck, and prostate cancers has determined other small populations of uncommon cells that can grow *in vivo* tumors [6].

The existence of a distinct subset of tumor cells with specific proliferation, self-renewal, and differentiation abilities - often referred to as cancer stem (-like) cells or tumor-initiating cells (TICs) - has been confirmed by a growing body of research on the heterogeneity of tumors and relevant mechanisms [4, 11]. Later studies focused on the origin of CSCs as well as their methods of eradication. CSCs are thought to have self-renewal and differentiation capacity. They can produce differentiated cells, which make up the majority of tumor tissue. CSCs within solid tumors have an unknown origin; nevertheless, some studies suggest they might come from normal stem cells and others argue that they may also originate from differentiated cells [12]. The critical role of epithelial-to-mesenchymal transition (EMT) programs in the development of CSC-like cells in a variety of cancers has also been pointed out in several studies [13]. Therefore, the CSC theory can be summarized in three different models according to the proposed origin of how they emerge.

Hierarchical Model

The hierarchical model was the original CSC model, in which the rare stem cells residing in a tumor give rise to a stem cell and a differentiated cancer cell (DCC), providing the continuity of the stem cell population in a tumor mass. Accordingly,

CHAPTER 9

Novel Nanotechnological Therapy Approaches to Glioblastoma

Bakiye Goker Bagca[1] and **Cigir Biray Avci**[2,*]

[1] *Department of Medical Biology, Aydin Adnan Menderes University, Aydin, Turkey*

[2] *Department of Medical Biology, Ege University, Izmir, Turkey*

Abstract: Glioblastoma is one of the most aggressive and deadly types of cancer. The blood-brain barrier is the biggest obstacle to overcome in glioblastoma treatment. Nanomedicine, which describes the use of nanostructures in medicine, has significant potential for glioblastoma. Nanomedicine provides advantages in crossing the blood-brain barrier, increasing the amount and effectiveness of drugs reaching the cancer site, monitoring diagnosis and treatment through imaging agents, and increasing the effectiveness of treatments in combination applications. This chapter reviews current nanotechnology research in glioblastoma over the past few years.

Keywords: Blood-brain barrier, Cancer, Chemotherapy, Cubosome, Dendrimers, Glioblastoma, *In vitro*, *In vivo*, Lipid nanoparticle, Liposomes, Micelles, Nanodiamonds, Nanomedicine, Nanoparticles, Nanostructures, Nanotubes, PAMAM, Polymersomes, Quantum dots, Radiotherapy.

INTRODUCTION

The grade IV cancer of the brain, glioblastoma, is the most aggressive and lethal subtype of the primary brain tumor. It is responsible for approximately half of all malignant central nervous system cancers. Its mean incidence varies between 3.19 and 4.17 per person per 100,000 a year. With a median survival of 15 months and a 5-year survival of approximately 5%, glioblastoma has a poor prognosis [1].

The fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System (CNS5) defines glioblastoma under The "Gliomas, glioneuronal tumors, and neuronal tumors, Adult-type diffuse gliomas" group. According to the classification, glioblastoma is genetically defined as IDH-wildtype and occurs *de novo* at approximately 60 years of age [2].

* **Corresponding author Cigir Biray Avci:** Department of Medical Biology, Ege University, Izmir, Turkey; Tel: +90 (232) 390 22 62; E-mail: cigir.biray@ege.edu.tr

Habibe Yılmaz (Ed.)

Various risk factors have been identified, including tobacco, smoking, nitrosamines, inflammation, ionizing radiation, electromagnetic radiation, obesity, metal ions, nutritional factors, chemical exposure, and genetic factors [1].

Glioblastoma treatment includes surgical resection, radiotherapy, and chemotherapy. An alkylating agent, temozolomide, is the major chemotherapeutic in glioblastoma treatment. This lipophilic molecule crosses the blood-brain barrier (BBB) and causes irreversible mutations that trigger cancer cell apoptosis. In addition to temozolomide, FDA-approved glioblastoma chemotherapy contains lomustine, carmustine, and bevacizumab agents [3].

However, the BBB is the primary hurdle to chemotherapy. It limits chemotherapeutic options to only BBB-crossing agents and forces dose increase. Increased chemotherapy doses cause systemic toxicity and drug resistance in cancer cells over time. Therefore, enhancing the quantity and effectiveness of the drug reaching the cancer site by overcoming BBB constitutes one of the main focus points in glioblastoma research [4].

Nanotechnology utilization has enormous power to break down the therapy limitations originating from the BBB.

CURRENT NANOMEDICINE PLATFORMS RESEARCHED IN GLIOBLASTOMA

Nanotechnology utilizes the surface, electrical, magnetic, and optical properties of nanosized materials (<100 nm) to produce designable, controllable, and smarter nanodevices. Nanomedicine defines the development of new-generation diagnostic, imaging, and treatment approaches using nanotechnology tools [5]. The widespread nanoparticles in nanomedicine originate from inert and low-toxicity molecules such as gold, silica, iron, and zinc. Biocompatible molecules such as chitosan are also among the potential nanomaterials in glioblastoma. Nanoparticle shapes are variable, such as cubes, spheres, plates, stars, and tubes. They are powerful drugs delivered through encapsulation or conjugation mechanisms. Encapsulation or conjugation allows drugs to pass the BBB or to increase the amount and effectiveness of agents reaching glioblastoma tissue. Likewise, imaging agent transport enables online monitoring of diagnosis and treatment. It also empowers the genetic regulation of glioblastoma by nucleic acid delivery. The ability of nanoparticles to directly target glioblastoma cells or tumor tissue through surface modifications minimizes toxicity in healthy cells [6 - 12]. In addition to nanoparticles, a wide variety of nanostructures are evaluated in glioblastoma according to usage purposes Fig. (**1**).

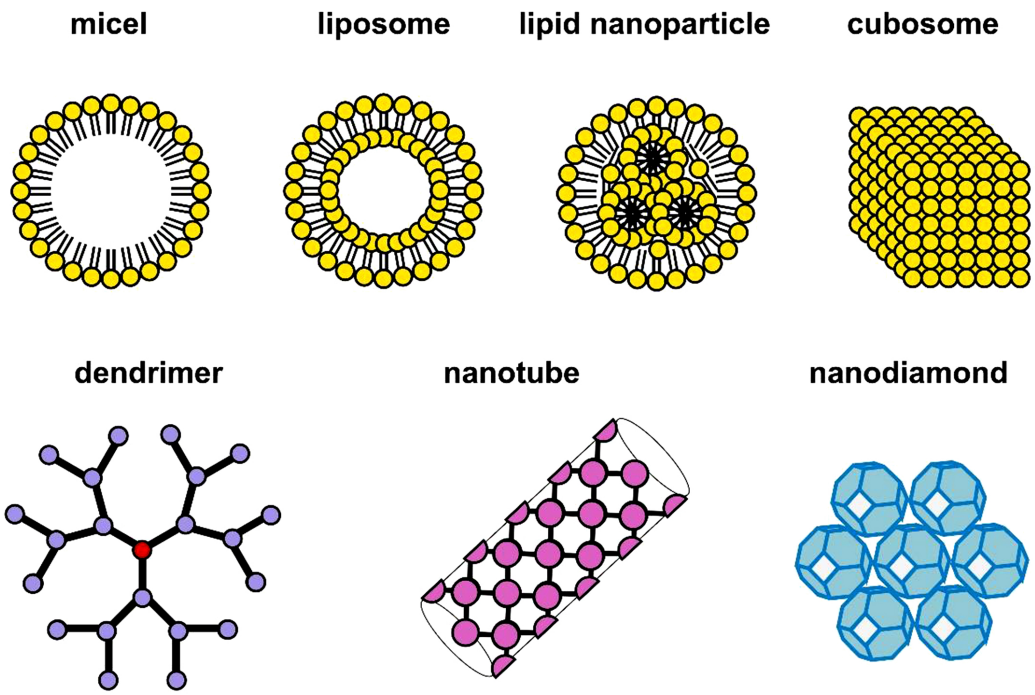


Fig. (1). Examples of nanostructures commonly used in nanomedicine.

Micelles

Micelles are single-layered vesicles composed mainly of amphiphilic molecules, especially proteins or lipids. These molecules form compartments by clustering in the aqueous medium such that their hydrophilic surfaces remain outside and their hydrophobic surfaces remain in the lumen. The vesicular structure allows the safe delivery of high-dose chemotherapeutic agents that may cause systemic toxicity in routine practice, efficient delivery of hydrophobic drugs or nucleic acids, targeting glioblastoma cells directly through surface modifications, crossing the BBB, and simultaneous regulation of multiple signaling pathways by dual drug loading. Micelles with conventional applications can increase the effectiveness of chemoradiotherapy and immunotherapy in glioblastoma (Table **1**). They can also increase photothermal, photodynamic, and sonodynamic therapy effectiveness [13, 29].

CHAPTER 10

Biocompatibility of Nanomedicines and Relation with Protein Corona

Yakup Kolcuoglu[1,*], **Fulya Oz Tuncay**[1] and **Ummuhan Cakmak**[1]

[1] *Karadeniz Technical University, Faculty of Science, Department of Chemistry, Trabzon, Türkiye*

Abstract: When NPs are included in a Biological environment, they associate with a large number of circulating proteins. As a result, they interact dynamically with each other. This structure, which is defined as PC, affects the physical parameters of NPs and causes positive or negative effects on them. PC composition is affected by many properties of NPs, such as size, shape, and surface charge. Therefore, various surface modifications on NPs directly affect PC formation and nature. Although many studies have been carried out to understand the formation and composition of the resulting PC structure, this area still maintains its popularity as a research topic. This review aims to briefly give an idea about the effect of proteins in metabolism on NPs designed as carrier molecules, the determination of these protein structures and the final fate of NPs after PC formation.

Keywords: Atomic force microscopy, Biocompatibility, Differential centrifugal sedimentation, Dynamic light scattering, Fluorescence correlation spectroscopy, Hard corona, Isothermal titration calorimetry, Nanomaterial shape, Nanoparticle, Nanoparticle application, Protein adsorption, Protein corona, Protein corona characterization, Protein corona dispersion, Protein corona stability, Protein corona formation, Small-angle X-ray scattering, Soft corona, Transmission electron microscopy, UV-visible spectroscopy.

INTRODUCTION

Nanomaterials are defined as substances up to several hundred nanometers in size. With these features, nanoparticles (NPs) have a similar size range with the structures in the cell, and therefore they have many applications in nanomedicine. Because of their physical and structural properties, well-designed nanomaterials have the power to significantly improve the treatment and diagnosis of diseases [1, 2]. In fact, humanity began to produce nano-structured materials since the time of the Lycurgus Cup during the Roman Empire and was impressed by their unique

* **Corresponding author Yakup Kolcuoglu:** Karadeniz Technical University, Faculty of Science, Department of Chemistry, Trabzon, Turkey; Tel: +90 462 377 2495; E-mail: yakupkolcuoglu@ktu.edu.tr

Habibe Yılmaz (Ed.)

nature [3]. NP have attracted increasing attention in the last 20 years, due to the development of new synthesis, characterization and analysis methods and the increase in investments in this field [4 - 6]. Due to the increasing use of nanoparticles, it has become inevitable for scientists to investigate how nanoparticles are involved in human health, their effects on the environment, and how they are involved in metabolism. As a result of the researches, it is reported that besides the positive aspects of nanoparticles, they can cause toxic effects on living things as a result of excessive misuse [7, 8]. At the point reached today, the knowledge we have about the factors affecting the safety of nanoparticles is not enough for the development of reliable and effective nanomedicine. Due to the ability of nanoparticles to penetrate different cells and tissues after entering the body, the need for further investigation of the risks of nanoparticles on health arises [9]. Biocompatibility is defined as the ability of a used material to produce a host response under certain conditions. Metabolism reacts to NPs, as it does to all foreign substances, in order to clean the NPs it sees as foreign matter. The response of the living organism is determined by the level of interaction of the NP with various biological substances in the environment. Biocompatibility is achieved when a substance involved in metabolism enters the circulation without causing carcinogenic, immunogenic, thrombogenic or toxic responses. However, toxicity or bio-incompatibility occurs if there are undesirable responses in biological processes.

The current applications of nanomaterials in medicine as drug carriers are increasing day by day and, at the same time, gaining importance [10]. The main purpose of nanodrug carriers in oncological applications is to prevent serious side effects of toxic compounds used in treatments. As nano-sized biomaterials have high free energy when incorporated into the system, due to large surface areas, they tend to interact dynamically with the surrounding molecules due to these energies [11]. When nanomaterials are included in the biological environment, proteins are the main molecules that bind tightly with the surface of these NPs, and as a result of this interaction, the proteins form a layer surrounding the surface. Although the formation of this layer (about 30 sec) is thought to be tight, the process is reversible. In this case, there is a dynamic protein exchange with the microenvironment. This protein structure is called protein corona (PC). The PC layer consists of two different structures characterized by slow change (hard corona) and fast protein exchange (soft corona). PC formation has numerous biological effects, such as cell interaction control of NPs, induction of their cytotoxicity, optimal targeting, and possible modulation of drug pharmacokinetics [12 - 14]. Remarkably, only a fraction (some of them are defined as opsonins and some others are dysopsonins) of the approximately 3,400 proteins of human plasma could be detected to interact directly with nanoparticles. Although small in number, these proteins produce impressive results in terms of cellular uptake [15 -

18]. For example, the adsorption of some proteins on the surface of nanoparticles may lead to the deterioration of the protein structure depending on the surface charge of this material [19]. For similar reasons, the physiological folding process of nanomolecules by proteins is the main part of the complex that determines their cellular uptake [20].

Protein adsorption has been extensively investigated in biomedical NPs to determine the modification and cellular uptake of nano-sized drug carriers [21]. The binding of proteins to the surface of NPs is an unpredictable complex process that alters the toxicological properties and efficacy of nanomaterials [22]. In the protein adsorption of nanomaterials, the type, geometry and conformation of this material are significantly effective [23]. Numerous studies have been carried out to modify the molecular surfaces of such molecules so that they do not interact with the protein. Examples of these studies are PEGylation [24, 25], colloidal silica nanoparticle production by adding PEG and Pluronic-F127 with different molecular weights [26] and silver nanoparticle production [27]. However, PEGylation remains an important standard for modifications of nanocarriers designed for drug delivery [28].

FACTORS AFFECTING PC FORMATION

After being metabolized, NPs interact with physiological biomolecules as a result of combining with blood and other biological fluids. As a result, the PC layer comes into existence. PC formation is a dynamic process described as the "Vroman effect" involving different forces (such as Van Der Waals forces, π–π stacking bond, H-bonds, electrostatic and hydrophobic interactions) between nanomaterials and proteins [29, 30]. Initially, proteins with high abundance but low affinity in the medium of NP inclusion are rapidly adsorbed on the surface of these molecules; they are then replaced by low amount and high affinity proteins [31]. As a result of all these formations, two different layers are formed as hard corona (HC) and soft corona (SC). HC-type binding, which is responsible for the behavior of NPs in metabolism, affects the membrane interaction and biodistribution of these molecules. Proteins in HC bind directly and with high affinity to the surface of the molecule. As a result, they form a stable layer [32, 33]. The proteins forming the SC layer are a replaceable layer depending on the environmental conditions and they are indirectly located on the surface of the NPs as they interact with the proteins in the HC layer [34]. The process of formation of PC and, consequently, the cellular behavior of NPs is highly dependent on factors such as size, morphology, surface properties, the type and composition of the biological fluid (cytoplasm, blood, extracellular matrix) in which the nanomaterials are contained, pH, and temperature [35].

CHAPTER 11

Role of Nanoparticular/Nanovesicular Systems as Biosensors

Özlem Çoban[1,*] and **Emine Taşhan**[2]

[1] *Department of Pharmaceutical Technology, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Türkiye*

[2] *Zoleant LLC, Zoeuticals, Regulatory Affairs and Quality, New York, USA*

Abstract: Biosensors are analytical apparatus utilized for the qualitative and quantitative detection of various biological or non-biological analytes. Early diagnosis of diseases (cancer, infectious disease), monitoring environmental pollution, and ensuring food safety are very important in terms of individual and public health. Therefore, it is also crucial to detect these markers sensitively and accurately, with cheap and simple methods, especially despite limited resources. Nanoparticles, thanks to their nano size, provide wide areas of biosensing and amplify signals. In most of the works, it was observed that the limit of detection (LOD) value decreased and the selectivity improved in biosensors prepared using nanosystems compared to conventional sensors. In this respect, the results give us hope for the use of nanosystems in biosensors. In this section, the subject of biosensors is briefly mentioned and mainly studies on the use of nanoparticular/nanovesicular systems in the field of biosensors are included.

Keywords: Analyte, Bioassay, Biosensor, Bioreceptor, Biosensing, Conjugate, Detection, Genosensor, Gold nanoparticle, Immunosensor, Limit of detection, Liposome, Nanobiosensor, Nanomaterials, Nanosystems, Nanotechnology, Polymeric nanoparticle, Quantum dots, Signal amplification, Transducer.

INTRODUCTION

Biosensors basically consist of two components as bioreceptor and transducer [1]. More recently, it has been stated that biosensors consist of three components: The sensor, which is a membrane with varied biological structures, the transducer and the electronic system that magnifies and saves the signal for data presentation (Fig. **1**) [2]. A bioreceptor is a biomolecule that recognizes and can selectively bind to target analytes. The transducer, on the other hand, converts the binding

[*] **Corresponding author Özlem Çoban:** Department of Pharmaceutical Technology, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Türkiye; Tel: +905057642367; E-mail: o.coban88@gmail.com

Habibe Yılmaz (Ed.)

event into electrical or optical signals, usually *via* electrochemical or fluorescent techniques [1].

It is very important to follow biological or biochemical processes for medical and biological applications [3]. In addition, the detection of microorganisms, dangerous chemicals and other hazardous wastes in water and soil, drugs and toxic substances in food, trace gases in mining regions, and the decrease in the ozone layer become more of an issue for environmental health [4]. There is great attention in the work of sensitive, selective and economical biosensors because of their contribution to the realization of high-precision diagnostics and personalized medicine. Various biosensors have been extensively investigated since the development of the first generation biosensors, in which glucose oxidase is immobilized on an amperometric oxygen electrode for glucose sensing, which was developed by Clark and Lyons in 1962 [3, 5].

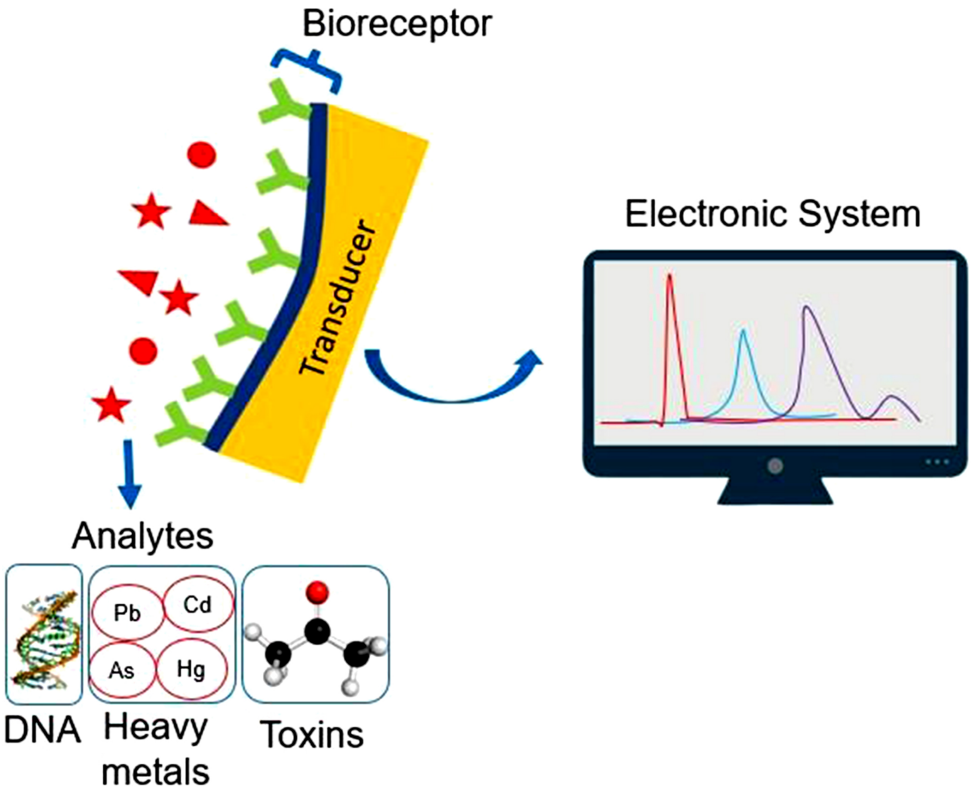


Fig. (1). Schematic image of the biosensor and its components.

Biosensors have many advantages over other biological sensing methods, such as high test speeds and flexibility. For example, they provide information that can be

useful in patient care planning to healthcare providers thanks to their ability to perform fast and real-time analysis. They can do multi-target analysis. The system is suitable for automation and thus testing costs can be reduced. They make contributions to the betimes detection of cancer and other diseases due to their fast, selective and high sensitivity properties and thus to the improvement of the prognosis. It may be useful for communities where healthcare delivery is inadequate since its ease of use and portability [6].

Classifications of biosensors are based on the physicochemical transduction mode used for the detection and analysis of signals, or type of biorecognition material [4, 5].

According to the transducer mode, biosensors are divided into 4 groups:

• Electrochemical biosensor

• Optical biosensor

• Thermal biosensor

• Piezoelectric biosensor [5]

Electrochemical biosensors: They measure fluctuations in current, potential, conductivity or impedance in the test sample induced by the interaction among the biological material and the analyte. In this case, we can classify electrochemical biosensors into amperometric, potentiometric, conductometric or impedimetric biosensors [4]. Amperometric biosensors meter the current arised during the oxidation or reduction of the material, which is electrically active, whereas potentiometric biosensors quantify the potential of the biosensor electrode relative to the reference one. On the other hand, conductometric biosensors gauge the change in conductivity resulting from the biochemical reaction [5], and impedimetric biosensors meter variances in charge conductivity and impedance on the sensor surface resulting from specific binding to the target [7]. There are many studies on the detection of glucose, cholesterol, amino acids, urea, alcohol, neurotransmitters, carbon dioxide, pesticides, heavy metals, chemicals, possible markers and endocrine disrupting hormones, polychlorinated biphenyls and milk toxins in biological or environmental media using ampoterimetric, potentiometric, conductometric or impedimetric biosensors [4].

Optical biosensors: As a result of the interaction between the biocatalyst and the sample, the optical properties of the sample may change, resulting in an alteration in the intensity of the absorbed or emitted light [4, 5]. Optical biosensors rest on several methods, namely absorption, fluorescence, luminescence, and surface

CHAPTER 12

Role of Nano and Biopharmaceutics in Precision Medicine

Habibe Yılmaz[1,*] and **Ayça Erek**[1]

[1] *Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Trakya University, Edirne, Türkiye*

Abstract: As our knowledge of developing technology and human biology increases, the need for changes in our perspectives on diseases and treatment modalities has emerged. The individual variation of diseases at the molecular level has long led to the abandonment of the one-fits-to-all approach. These changes at the molecular level are illuminated using -omics technologies and are among the most powerful tools in precision medicine. The discovery of new drug targets and biomarkers results in the structural elucidation of targets. Thus, it has been possible to develop new drug molecules as well as to select the appropriate drug for the target, the appropriate dose, and, when necessary, the appropriate drug combination. Awareness of the changes in diseases at the molecular level has also updated clinical research designs to make precision medicine applicable. In this section, information and examples of developments in precision medicine, diagnosis and treatment in precision medicine, as well as -omics technologies and other technologies are presented.

Keywords: Basket design, Biobank, Biopharmaceutics, Biosensors, Diagnostics, Epigenomics, Genomics, Metabolomics, Nanomedicine, Omic technologies, Pharmaceutics, Precision medicine, Precision medicine tools, Personalized medicine, Proteomics, Transcriptomics, Targeted therapy, Theranostic, Therapeutics, Umbrella design.

INTRODUCTION

Precision medicine, which is formerly known as personalized medicine, has attracted many researchers in recent years. This chapter provides an overview of precisions medicine's history, tools, diagnosis, therapeutics, recent updates, and the future. Traditional medicine focused on diseases and epidemics, and it was thought that the treatment methods applied would be suitable for all patients. However, some patients can tolerate a certain drug without any side effects while the same dose can be toxic to other patients. With the development of medical

[*] **Corresponding author Habibe Yılmaz:** Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Trakya University, Edirne, Türkiye; Tel: +90 284 2350180 - 1181; E-mail: habibeyilmaz@trakya.edu.tr

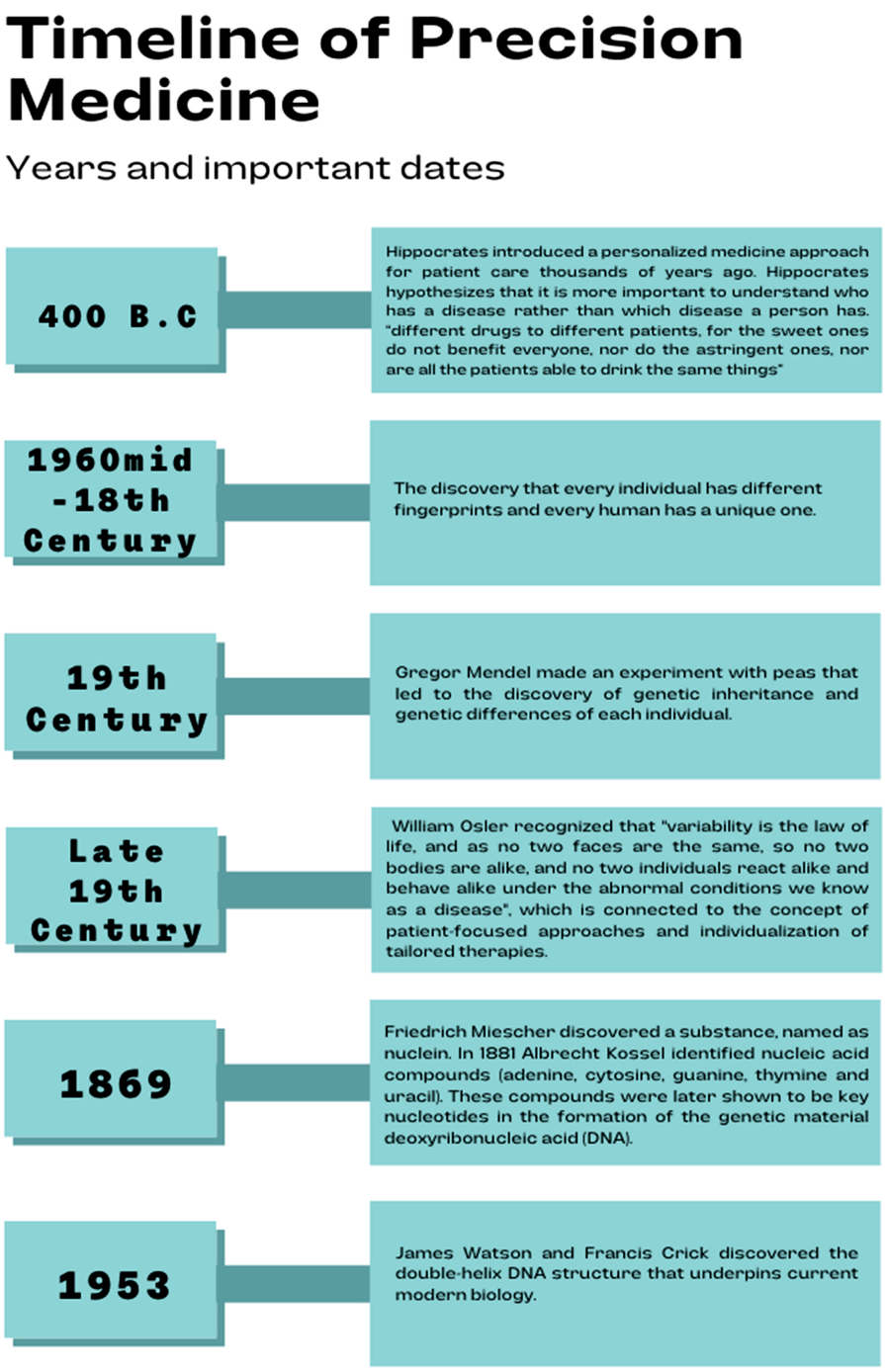
Habibe Yılmaz (Ed.)

science, it has emerged that the same disease progresses differently in different individuals and the same treatment cannot be applied [1]. Today, the concepts of personalized medicine and precision medicine, in which patients are at the forefront rather than diseases, have entered our lives. According to the National Institutes of Health (NIH), precision medicine is "an emerging approach to disease treatment and prevention that takes into account the individual variability of genes, the environment, and each person's lifestyles." This approach allows to more accurately predict which treatment or measure for a disease will be beneficial for which patient or group of patients. This concept is quite different from the "one size fits all" approach in traditional medicine, which focuses on therapeutic strategies for the average patient [2]. This difference between inter individuals could be caused by genetic factors, age, gender, ethnicity, race, habits, environmental factors reasons may originate. Therefore, despite the differences in patients, the understanding of disease-oriented treatment has led to the waste of drugs, increased costs, and poor patient and physician satisfaction [3].

Throughout the chapter, before establishing the connection between precision medicine and nanotechnology, information about precision medicine and its tools will be given, and then its relationship with nanomedicine will be discussed.

PRECISION MEDICINE HISTORY

Although the concept of precision medicine is not a new concept, it appears even in the hypotheses of Hippocrates, who is considered the father of traditional medicine, centuries ago. According to Sir William Osler, it is more important to know which patient has the disease than to know what type of disease the patient has. Advances in genomics and medicine have accelerated the development of precision medicine. Examples of these are the discovery of the double helix structure of DNA in 1953, the development of Sanger sequencing in 1977, and most importantly, the launch of the Human Genome Project in 1990. The dates and events important to the development of precision medicine are shown in detail in Fig. (**1**). The concept of precision medicine was first used and accepted by the US National Research Council (NRC) in 2011 after the publication of a report titled "*Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease*" [4]. In this report, it was recommended to classify diseases according to genetic or genomic basis, not symptoms. Then, with the speech of US President Barack Obama in January 2015, the concept of precision medicine was heard by the masses for the first time, and after this speech, there was a great increase in interest and research in precision medicine techniques. While there were approximately 4000 articles in the National Library of Medicine before 2007, it is seen that this number has reached 91,947 in 2022 [5, 6].


Timeline of Precision Medicine
Years and important dates
400 B.C
Hippocrates introduced a personalized medicine approach for patient care thousands of years ago. Hippocrates hypothesizes that it is more important to understand who has a disease rather than which disease a person has. "different drugs to different patients, for the sweet ones do not benefit everyone, nor do the astringent ones, nor are all the patients able to drink the same things"
1960mid -18th Century
The discovery that every individual has different fingerprints and every human has a unique one.
19th Century
Gregor Mendel made an experiment with peas that led to the discovery of genetic inheritance and genetic differences of each individual.
Late 19th Century
William Osler recognized that "variability is the law of life, and as no two faces are the same, so no two bodies are alike, and no two individuals react alike and behave alike under the abnormal conditions we know as a disease", which is connected to the concept of patient-focused approaches and individualization of tailored therapies.
1869
Friedrich Miescher discovered a substance, named as nuclein. In 1881 Albrecht Kossel identified nucleic acid compounds (adenine, cytosine, guanine, thymine and uracil). These compounds were later shown to be key nucleotides in the formation of the genetic material deoxyribonucleic acid (DNA).
1953
James Watson and Francis Crick discovered the double-helix DNA structure that underpins current modern biology.

(Fig. 1) contd.....

CHAPTER 13

In Vitro Applications of Drug-carrying Nanoparticle Systems in Cell Culture Studies

Nur Selvi Günel[1,*], Tuğba Karakayalı[2], Buket Özel[1] and **Sezgi Kıpçak[1]**

[1] *Ege University, Faculty of Medicine, Department of Medical Biology, Izmir, Turkey*

[2] *Ege University, Faculty of Science, Department of Biochemistry, Izmir, Turkey*

Abstract: The safety and efficacy of each drug candidate, including nanomedicine considered for pharmaceutical use, primarily must be determined *in vitro*. In this context, the most widely used method is cytotoxicity tests, which include cell culture studies. It examines the parameters of membrane integrity, metabolite incorporation, structural alteration, survival and growth in tissue culture, enzyme assays, and the capacity for transplantation within the scope of viability tests. Within the scope of cell culture studies, tests related to apoptosis, which are effective in proper cell cycle, immune system and embryonic development, are also included. Another way to detect cell viability is to detect the biomolecules it expresses. Determination of protein expression is one of the preferred methods in this sense. Within the scope of this chapter, there is information about cell culture-based methods under these main subjects, which are applied to nanomedicines.

Keywords: Annexin V, Apoptosis assay, Capture antibody, Cell culture, Colorimetric assay, Comet assay, Cytotoxicity, DNA Fragmentation, Enzyme-linked Immunosorbent Assay, Flow cytometry, Fluorescence-activated cell sorting, Fluorometric assays, Luminometric assay, Microtiter plate, Multiplex ELISA Polyvinylidene difluoride, Propidium iodide, Real-time viability assay, SDS-PAGE Electrophoresis, Western Blot.

INTRODUCTION

Nanoparticles have currently been used in the analysis and treatment of many illnesses. This means that nanoparticles possess great physical and chemical properties, but need to be biocompatible [1, 2] in order to be safely introduced into the body and have a positive effect on tissues and cells.

[*] **Corresponding author Nur Selvi Günel:** Ege University, Faculty of Medicine, Department of Medical Biology, Izmir, Turkey; Tel: 902323902262; Fax:+902323420542; E-mail: nur.selvi.gunel@ege.edu.tr

Habibe Yılmaz (Ed.)

In this chapter, we discussed about the methods that are frequently used in cell culture studies. These methods used to determine the cytotoxic and apoptotic effects of nanoparticles on the cell lines of the targeted disease are described. In addition, the protein expression analysis methods used to determine the molecular changes caused by the nanoparticles used are discussed in detail.

CYTOTOXICITY ASSAYS

The cytotoxicity assays are types of biological assessment and concealing test that looks at how nanoparticles influence cell growth, reproduction, and morphology by using tissue cells *in vitro*. There is no better predictor of a nanoparticle's toxicity than its cytotoxicity. Additionally, it is easy to use, fast, sensitive and can prevent toxicity in animals [3, 4]. Definitions of cell viability often center on the concept of survival. Viability assays are also commonly employed to study cell proliferation over time within a population. To determine cell viability, researchers might use either a population-based or a single-cell approach. Population analysis is more efficient than single-cell viability assays, but the resulting data is less specific [5].

Assessment of cell viability following potentially damaging treatments is a common requirement in biological research (such as radiation, heat, chemicals, *etc*.). The major parameters in viability research are membrane integrity, metabolite incorporation, structural alteration, survival and growth in tissue culture, enzyme assays, and the capacity for transplantation. High correlations can be expected between the many indices of viability that can be obtained from these various criteria. However, the criterion for cell viability must be adjusted for the specific aims of each study. For instance, if the membranes of the cells are still in good shape, a viability test that relies on this property will consider them to be alive even though they can't divide any longer [6].

Tests for cytotoxicity and cell viability are put into different groups.

• Trypan blue, eosin, and erythrosin B assay cannot be used with these dyes.

• Colorimetric assays include the MTT, MTS, XTT, WST-1, WST-8, Lactate dehyrogenase, Sulforhodamine B, Neutral red uptake, and Crystal violet assays.

• Fluorometric assays include the alamarBLue and 5-Carboxyfluorescein Diacetate, Acetoxymethyl Ester (CFDA-AM) assays.

• ATP assays and Real-time viability assays are both luminometric assays.

Dye Exclusions

Trypan Blue Assay

The trypan blue assay was one of the oldest techniques to determine whether or not a cell is viable, and it is still frequently employed nowadays. It is predicated on the notion that live cells have an entire cell membrane, allowing the trypan blue dye to pass through. As a result of their membrane's inability to regulate the movement of macromolecules, dead cells absorb trypan blue and appear blue. For the experiment, the cells must be in a single cell suspension, and they are then counted under a microscope using a defined volume haemocytometer or recently developed automated counting equipment. Using these counts, it is reasonably easy to calculate the total number of cells and the proportion of viable cells within a population [5].

It is problematic that cell membrane integrity is used to indirectly measure viability. Even though a cell's membrane integrity has been preserved, its viability may have been impaired. Alternatively, a cell's membrane integrity may be compromised, but the cell may still be able to repair and recover normal viability [7].

Because the amount of dye absorption is evaluated subjectively, there is also the possibility that even minute levels of dye uptake that suggest cell damage will not be identified. This is yet another potential problem. In this context, more non-viable cells with dye uptake are found in examinations with a fluorescence microscope and a fluorescent dye than in examinations with a transmission microscope and trypan blue [7].

Eosin Assay

Measurements of an organism's potential for growth should not be the sole basis for determining whether or not a unicellular entity is alive or dead; rather, the distinction should be made using criteria that are more fundamental and applicable. It has been demonstrated that the ion (eosin) exclusion principle is a straightforward and efficient method for achieving this aim. In cell and tissue cultures, the conditions necessary for making accurate observations have been specified in terms of the concentrations of eosin, serum, and electrolytes. These simplified methods have replaced culturing procedures in studies of the effects of exposure of sensitive cells to pancreatin, desiccation, and tuberculin; storage of cell suspensions without renewal of the medium; and use of such methods to study nutritional or metabolic needs.These methods have also been used to find out what nutritional or metabolic requirements are needed [8].

CHAPTER 14

An Overview of *In Vivo* Imaging Techniques

Aysa Ostovaneh[1] and **Yeliz Yildirim**[2,3,*]

[1] *Department of Biotechnology, Ege University, Graduate School of Natural and Applied Sciences, 35100, İzmir, Türkiye*

[2] *Department of Chemistry, Faculty of Science, Ege University, İzmir, Türkiye*

[3] *Center of Drug, R&D, and Pharmacokinetic Aplications (ARGEFAR), Ege University, İzmir, Türkiye*

Abstract: Imaging is developing very quickly in various study bases. Nowadays, due to the desire for the technology coming to imaging, it is widely used to detect molecular and structural targets in *in vivo* studies. The aim of developing new non-invasive imaging methods is to provide affordable, high-resolution images with minimal known side effects for studying the biological processes of living organisms. For this purpose, X-ray-based computed tomography (CT), magnetic resonance imaging (MRI), ultrasound (UI), Nuclear imaging methods (positron emission tomography (PET), single-photon emission computed tomography (SPECT)), and optical imaging, are techniques widely used in imaging. Each of these has unique advantages and drawbacks. The background of imaging techniques and their developments have been shown in this chapter and we discuss in detail the use of optical imaging through bioluminescence, fluorescence, and Cerenkov luminescence techniques in various diseases for preclinical applications, early clinical diagnosis, treatment, and clinical studies.

Keywords: Bioluminescence, Biological processes, Computed tomography, Clinical diagnosis, Cerenkov luminescence, Fluorescence, High-resolution images, *in vivo* imaging, Imaging technique, Living organisms, Molecular imaging, Magnetic resonance imaging, Nuclear method, Non-invasive method, Optical imaging, Positron emission tomography, Single-photon emission computed tomography, Technology, Ultrasound and X-ray.

INTRODUCTION

In vivo imaging is an indispensable imaging technique that could be used in research and clinical trials. The visual display capacity and possibilities have attracted great attention and have been developed over the years. This allows researchers and clinicians to delve deep into living systems to display their anatomical features, search for specific biomolecules, and illuminate the varieties

[1] **Corresponding author Yeliz Yildirim:** Department of Chemistry, Faculty of Science, Ege University, İzmir, Türkiye and Center of Drug, R&D, and Pharmacokinetic Aplications (ARGEFAR), Ege University, İzmir, Türkiye;
E-mail: yeliz.yildirim@ege.edu.tr

Habibe Yılmaz (Ed.)

of a complex array of components and ingredients that underlie a wide variety of repositories [1]. Imaging uses signals with different mechanisms and sources, making it possible to visualize critical cellular and molecular processes [2]. Various techniques are developed for *in vivo* imaging: from computerized tomography (CT) [3] to photon-based *in vivo* fluorescence imaging (FLI), *in vivo* bioluminescence imaging (BLI) [4] and Cerenkov luminescence imaging (CLI), magnetic resonance imaging (MRI) [5] and sound wave-based ultrasound imaging (UI) [6], as well as radionuclide-based single photon emission computed tomography (SPECT) [7] and positron emission tomography (PET) [8]. In this chapter, we will first discuss the background, advantages, and drawbacks of all the above imaging techniques. Then we will delve into the detail of the optical imaging technique details and the utilization of these techniques in the diagnosis and treatment of diseases in preclinical *in vivo* studies.

Computerized Tomography (CT) imaging, also called "CAT" (Computed Axial Tomography). The root of tomography is generated from the Greek word "tomos" meaning "slice" and "graphia" which means "describing". British engineer Godfrey Hounsfield and physicist Allan Cormack concocted CT in 1971. It was publicly announced in 1972 [9, 10]. In 1979, Nobel Peace Prize was awarded to Hounsfield and Cormack in two different fields of medicine and science [11]. A CT scan is based on an X-ray. This imaging technique takes a series of X-ray slices from different angles of various parts of the body (bones and blood vessels, *etc*.) and is collected all data from the detector and is transferred to the computer for analysis. Whenever the machine starts turning around, computerized information is obtained comparing conventional X-rays with CT scans. CT images provide more detailed information about a particular area in a cross-section, eliminating image overlap [12].

Magnetic Resonance Imaging (MRI) is a type of non-invasive/non-ionizing scan that utilizes powerful magnetic fields and radio waves that force the proton in the body to arrange with that field to generate images of any part of the body. In this imaging technique, the hydrogen nucleus (a single proton) is used due to its abundance in body fluids and fat [13]. Bloch and Purcell described nuclear magnetic resonance (NMR) in 1946. They won the Nobel Prize for Physics in 1952. Magnetic resonance images were introduced clinically in Nottingham and Aberdeen in 1980 for the first time [14, 15]. MR imaging can assist in the diagnosis of disorders and treatments as well. It is increasingly being used and demanded as several new indications have been created in the last few years [16]. By bypassing the radiofrequency flow through the body, the protons are stimulated, and it causes unsteady protons, which str*etc*h against the pull of the magnetic field. MRI sensors detect the energy released from realigned protons when the magnetic field is switched off. The chemical structure of the molecules

can affect the amount of energy that is released from protons. Contrast agents (*e.g.*: element Gadolinium, Manganese, Europium) may be given to a patient intravenously, orally, or intra-articularly before or during the MRI to increase the speed at which protons realign with the magnetic field for more specific types of imaging. Although all metallic properties, whether inside or outside the body, are better not to be used in MRI because of being in a magnetic field, MRI has no known biological hazards. MRI uses radiation which does not cause any significant damage to the tissue as it passes through.

Ultrasound (UI) is a non-invasive technique, used widely in medicine as both a diagnostic and therapeutic tool to image the inside of the body. UI was discovered approximately 10 years before the X-ray (1883), but it found application long after it started to be used in medicine. The first use of this technique refers to the investigation of submarines in the First World War. After that, it was used in the medical field for the first time in 1950. The sound waves are generated by transducers (Ultrasound probes) which work at frequencies much higher than the human hearing range (in the megahertz (MHz) range) [17]. Its mechanism is based on the detection and conversion of ultrasound waves, which are reflected from different tissues of different natures [18]. Because of using non-ionizing radiation, UI is a safe technique even for pregnant. Ultrasound is not only among the most accurate imaging technologies, but they are also the most cost-effective and accessible imaging technique. Its is said that one of the important drawbacks of UI is being dependent on the technician's experts.

Types of Nuclear Imaging Techniques: SPECT and PET determine by accessing the basis of nuclear technology for today's usages in the 1990s. The definition of uranium radiation was described by Henri Becquerel in 1896 and Marie and Pierre Curie in 1898 won the Nobel Prize in Physics for the introduction of "radioactivity" terms. Nowadays, there are various important techniques in nuclear medicine imaging, such as Positron Emission Tomography (PET) imaging or Single-Photon Emission Computed Tomography (SPECT). The first operation of SPECT and PET started simultaneously in the middle of the 20th century. PET imaging was described and conceptualized first by Brownell and Sweet in 1950 [19].

Single-Photon Emission Computed Tomography (SPECT) is defined as a nuclear medicine, a tomographic imaging technique which uses gamma rays for detection. In SPECT, single photon emissions from an introduced radioactive tracer (also known as a probe) are detected to produce a computer-generated image of the local radioactivity distribution in tissues [20]. These probes generally are equipped with a detectable radioactive isotope gamma camera, providing images depending on the type of scan being carried out. A Gamma Camera rotates

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

R

S

T

U

V

W

Z

Habibe Yılmaz

Asst. Prof. Dr. Habibe Yılmaz, is currently working as a lecturer and head of the Pharmaceutical Biotechnology Department in Trakya University Faculty of Pharmacy for 3 years. Her scientific interests are focused on the development of novel nanomedicines mainly on the axis of cancer. However, she also works on the nanoantibiotics systems in order to develop new controlled drug delivery systems to overcome antibiotic resistance due to patient misuse. She also has hands-on experience on the quality control analysis of biopharmaceuticals, such as monoclonal antibodies and antibody fragments as well as small molecule drugs. She has also worked as Contracted Research Organisation (CRO) Unit manager for one and a half years, and has improved her perspective on diseases and treatments. She has 4 patent applications pending and a few international publications on nanomedicines and biomimetic design.