Technological Applications in Pharmaceutical Sciences

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ÖZGÜR PRESS

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Published by
Özgür Yayın-Dağıtım Co. Ltd.
Certificate Number: 45503

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Editor: Pakize Cantürk

Language: English
Publication Date: 2025
Cover design by Mehmet Çakır
Cover design and image licensed under CC BY-NC 4.0
Print and digital versions typeset by Çizgi Medya Co. Ltd.

ISBN (PDF): 978-625-8554-79-3

DOI: https://doi.org/10.58830/ozgur.pub1094

Suggested citation:
Cantürk, P. (ed) (2025). *Technological Applications in Pharmaceutical Sciences.* Özgür Publications. DOI: https://doi.org/10.58830/ozgur.pub1094. License: CC-BY-NC 4.0

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Preface

Our book aims to bridge the gap between foundational research and the future of pharmaceutical innovation. The chapters curated here open new horizons across several high-impact domains. From the biochemical sophistication of Organ-on-a-Chip models described by Burcin Gungor to the precision of nanoparticle-mediated oncology addressed by Sumeyye Idil Celikkaya, the diversity of these topics reflects the complexity of modern drug discovery. The analytical core of the book is strengthened by the contributions of Bekir Caglar Celikkaya, and John A. Parkinson on NMR Spectroscopy, and Bekir Caglar Celikkaya on the modernization of DNA gel electrophoresis. Concluding with a forward-looking perspective, Pakize Canturk reviews the trajectory of Biotechnological Therapeutics.

I would like to extend my deepest gratitude to all the contributing authors for their scholarly rigor and dedication. Their expertise has been instrumental in shaping this volume into a comprehensive resource that reflects the multi-faceted nature of modern pharmaceutical research.

We respectfully present this work as a testament to the ongoing evolution and excitement within the pharmaceutical sciences.

Contents

Chapter 1

Organ on a Chip

Burcin Gungor[1]

Abstract

Traditional drug development methods have relied on 2D cell cultures and animal models, which have not been able to adequately represent human biology. Due to genetic and physiological variances in human biology, animal models cannot yield accurate results, whereas 2D cell cultures are insufficient in simulating tissue architecture and cell-to-cell interactions. These limitations have led to the development of organoid technology, which is made from stem cells and imitates the three-dimensional structure of organs. Organoids offer a strong platform for drug testing, disease mechanism modeling, and the creation of individualized treatment plans, but they have drawbacks, including long-term stability and microenvironment control. By incorporating organoids onto microchips, organ-on-a-chip technology has been created, improving biological realism and allowing us to evaluate pharmacological effects more precisely. Organ-on-chip technology replicates the microphysiological characteristics of organs on a chip by using bioengineering methods and microfluidic devices. The study of cancer is among the most notable applications of this technology. By accurately simulating the tumor microenvironment and the interactions between cancer cells and their surroundings, cancer-on-a-chip models help us comprehend the intricate nature of cancer biology. They thus offer a potent platform for the creation of fresh approaches to treatment as well as the assessment of the efficacy of current ones. These models can be used to precisely and thoroughly examine the efficacy of treatment regimens and drug combinations, especially for aggressive disease types like glioblastoma and breast cancer.

Organ-on-chip technologies have revolutionary promise for advancing tailored treatment plans, understanding the mechanisms underlying complicated diseases like cancer, and speeding up medication development. These creative methods are ground-breaking instruments that will influence contemporary medicine and improve human health in the future.

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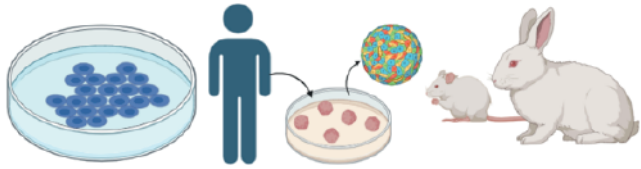
https://doi.org/10.58830/ozgur.pub1094.c4352

1. Organoids

Organoids are three-dimensional (3D) biological entities that are produced in vitro from stem cells. These small, organ-like structures may replicate organogenesis processes, exhibit tissue-specific cell variety, and replicate organ functions-all of which have the potential to mimic human biology (Lehmann et al., 2019). Because they lack cellular connections and natural tissue structure, traditional animal models and two-dimensional (2D) cell cultures have hindered study in this area. However, organoids have given science a new lease on life. These organoids have great promise for a variety of applications, including drug screening, tailored therapy, and the modeling of genetic disorders (Clevers, 2016).

2D cell cultures and conventional animal models have been for years crucial for understanding cellular processes and drug development research; however, it is well known that these systems fall short of accurately representing human biology. The transferability of data collected in animal models to therapeutic applications is limited by interspecies genetic, physiological, and metabolic variations resulting from human biology. Results from animal models do not always translate to human outcomes, particularly in drug development processes. The associated 3D structure of tissues and the intricacy of cellular interactions cannot be adequately reflected by 2D cell cultures, despite the fact that they offer an appropriate platform for studying cellular processes in a more controlled environment. The microenvironment created when cells grow on a flat surface lacks cell-matrix interaction, giving rise to a structure that is very different from the physiology of natural tissues.



	2D Cell Cultures	3D Organoid Cultures	Animal Models
Vascularization	Limited	Limited	Feasible
Biobanking	Feasible	Feasible	Feasible
High-througput screening	Applicable	Applicable	Not Applicable
Modeling organogenesis	Not Applicable	Suitable	Not Suitable
Modeling patient-derived organoids	Not Applicable	Feasible	Poorly Feasibel
Manipulation	Feasible	Feasible	Limited
Modeling for human physiology	Limited	Feasible	Feasible
Reproducibility	High	Low	Low
Heterogeneity	Low	High	High
Modelling cellular communications	Feasible	Feasible	Limited

Figure 1. Advantages and limitations of 2D cell cultures, animal models, and 3D organoid cultures. Organoids offer significant advantages over traditional 2D cultures and animal models. This makes them an ideal platform for performing a variety of experiments, modeling human diseases, and performing high-throughput drug screens.

Three-dimensional (3D) organoid models developed to overcome these limitations stand out as innovative systems that complement the shortcomings of traditional modeling approaches (Heydari et al., 2021), (Kim et al., 2020) (Figure 1). These innovative models are becoming increasingly important in fields such as organogenesis, disease modeling and medicine. development that offers a higher level of physiological realism at both the cellular and tissue level.

1.1. Historical development of organoids

Innovations in technology and biological research have affected the evolution of organoids over time. Henry Van Peters Wilson showed the cells' ability to self-organize in 1907 when he found that isolated sponge cells could self-organize and regenerate a whole organism. Malcolm Steinberg proposed the "differential adhesion hypothesis" in 1964, contending that various surface adhesions may account for cell organization.

The isolation of pluripotent stem cells (PSCs) from mouse embryos in the 1980s and the discovery of human embryonic stem cells in 1998 gave impetus to organoid research. The development of iPSC technology in 2006 created a major revolution in mimicking embryonic development processes. During the same period, three-dimensional culture media such as ECM and Matrigel enabled cells to organize similarly to their natural environment.

In 2009, the foundations of modern organoid studies were laid when Hans Clevers and his team derived organoids from mouse intestinal stem cells. This work created a growth media that allowed stem cells to self-organize, and other organoid types were subsequently able to use this technique. A new age in biomedical research has been made possible by organoid models made from human stem cells, such as the brain, liver, and pancreas (Zhu et al., 2024), (Corrò et al., 2020), (Han et al., 2022) (Figure 2).

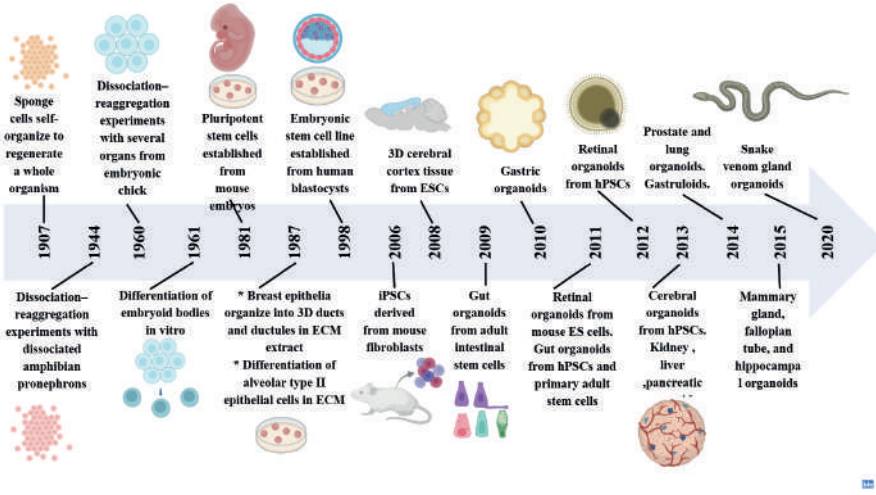


Figure 2. Timeline of organoid technology development. It provides a summary of important breakthroughs and fundamental studies in this field.

1.2. Derivatization of organoids

The mechanism by which stem cells self-organize and create 3D structures under specific circumstances is the foundation for the derivatization of organoids. The characteristics of the organoid are determined by the cellular resources, the signals given, and the culture techniques employed in this procedure. Because organoids mimic how tissues form in nature, they are a valuable tool for understanding disease causes, medication testing, and tissue engineering.

Different culture methods are used in organoid derivation. 3D matrix culture allows organoids to grow in a supportive environment. Natural extracellular matrix (ECM) proteins or synthetic matrices can be used for this purpose. Intestinal and gastric organoids, in particular, are often derived successfully in this method. Suspension cultures, on the other hand, allow cells to grow in free suspension without or with ECM proteins. Optic cup and cerebellar organoids can be derived by this method. Additionally, structures such as kidney organoids can be obtained from cell pellets using the air-liquid interface culture method (Rossi et al., 2018). The development of organoids depends critically on the balance of endogenous and external signals. While some organoids need exogenous cues to self-organize, others can do it solely through endogenous signals. For instance, human stomach organoids first differentiate by foreign cues and then self-organize by endogenous mechanisms, but mouse optic cup organoids only organize by endogenous signals. Kidney organoids and other structures need continuous external stimulation. These signals enable cells to construct the ultimate organoid structure by transitioning from a homogeneous population to an asymmetric organization (Rossi et al., 2018).

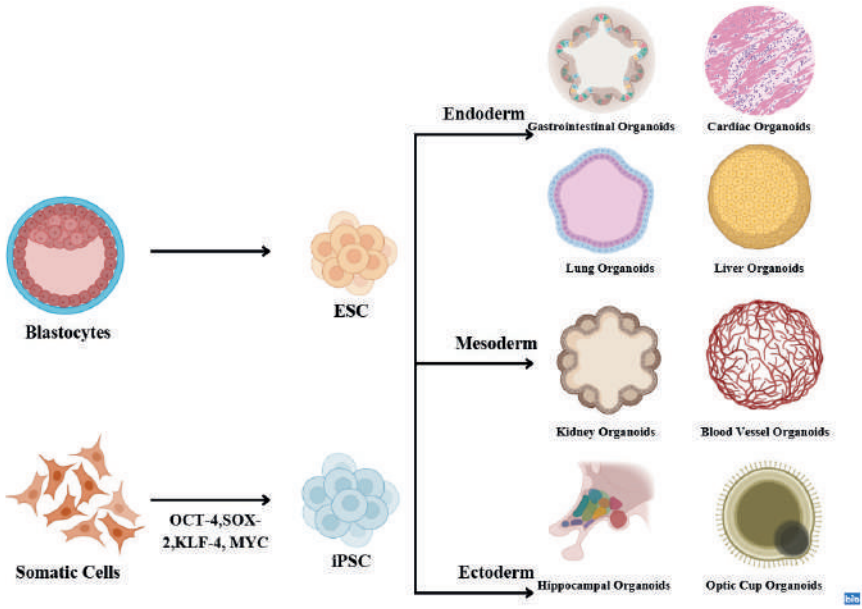


Figure 3. Organoids derived from pluripotent stem cells (PSCs) are created using embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Somatic cells can be reprogrammed into iPSCs with the help of transcription factors. Blastocysts differentiate into ESCs and form three germ layers (endoderm, mesoderm, ectoderm).

The starting cell type is an important factor in organoid derivation. The process, which begins with a single cell, involves cells organizing over time from a homogeneous population into complex structures (Rossi et al., 2018). For example, intestinal organoids are derived this way, starting from adult stem cells. Alternatively, the method of starting from a homogeneous aggregate of cells employs self-patterning mechanisms, as in optic cup organoids. Culturing different cell types together allows complex structures such as liver organoids.

There are several different sources of stem cell-derived organoids, including adult stem cells (AdSCs), induced pluripotent stem cells (iPSCs), and embryonic stem cells (ESCs). The benefits and drawbacks of each kind of stem cell in organoid formation vary.

The blastocyst contains embryonic stem cells (ESCs), which can develop into the endoderm, mesoderm, and ectoderm germ layers (Thomson et al., 1998). The development of diverse organoids, including the lung, liver, kidney, blood arteries, etc., is the result of these differentiation processes. Early organogenesis processes are particularly studied using ESC-derived

organoids, which are crucial to comprehending human developmental biology. The process of genetically reprogramming cells to become pluripotent again is necessary to produce iPSCs from somatic cells. Usually, reprogramming factors like Oct4, Sox2, Klf4, and c-Myc are used to carry out this process (Teshigawara et al., 2015), (Tang et al., 2022), (Balistreri et al., 2020). These factors cause the cells to revert to their embryonic stem cell state and lose their differentiated identity. By cultivating reprogrammed cells under the right circumstances, they can develop into distinct germ layers and, consequently, different organoids (Figure 3).

Because they can be produced using quick and easy procedures, organoids grown from adult stem cells (AdSCs) provide a useful substitute. These organoids have characteristics that are similar to those of adult tissues and are useful in research on viral infections, tissue regeneration, and repair. They are useful for both fundamental research and therapeutic applications due to their long-term genetic stability. By adding certain growth factors, AdSC-derived organoids may be grown in vitro for extended periods of time (Kim et al., 2020), (Tang et al., 2022).

However, AdSCs' restricted ability to differentiate results in these organoids often concentrating on a single cell type (Figure 4). Although this could lessen the variety of organoids, these structures' resemblance to adult tissues offers a significant benefit, particularly when simulating adult tissue regeneration and illness (Loya, 2014), (Hammond-Browning, 2012). AdSCs derived organoids are a valuable tool for therapeutic applications and medical research because of these features.

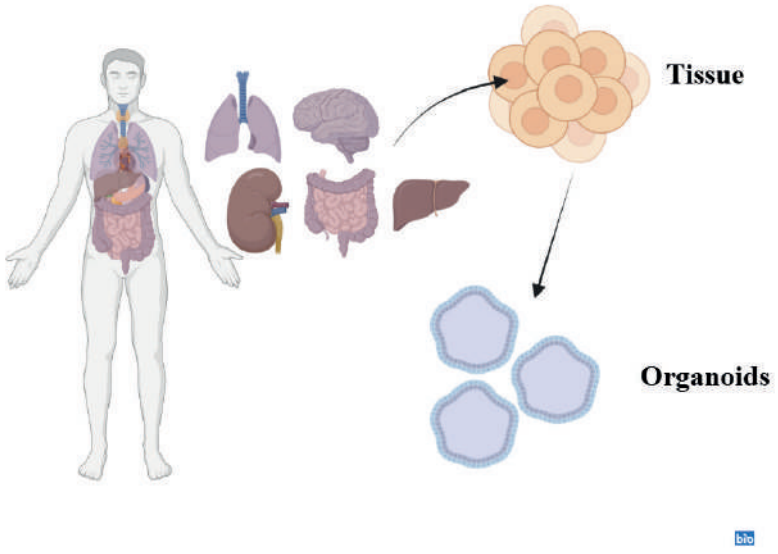


Figure 4. The process of deriving organoids from adult stem cells (AdSCs) begins with undifferentiated cells obtained from specific adult organs. AdSCs found in organs such as lung, brain, liver, intestine and kidney can form organoids by showing self-organization ability in vitro when appropriate culture conditions are provided.

1.3. Establishment of Organoid Cultures

By forming miniature models of biological systems, organoid cultures are an important instrument for researching disease mechanisms and comprehending human biology. Various cell sources are significant in this process. Organoids can be tailored to represent unique biodiversity using tissue-derived cells obtained from human or animal tissue and organ biopsies. Furthermore, organoids can differentiate into any desired cell type thanks to induced pluripotent stem cells, which are cultivated in a lab setting and have an infinite capacity for proliferation (Clevers, 2016). Particularly, cancer stem cells are favored for use in cancer research and for modeling tumor biology.

Successful development of organoids requires the use of soluble factors that promote the growth and differentiation of cells. These factors consist of growth factors and small molecules that direct the transformation of cells into a particular phenotype. While signaling molecules such as Wnt, EGF, HGF, BMP and TGF are important for adult stem cells, factors such as Activin-A, BMP4 and VEGF are used in pluripotent stem cells. These

biochemical signals provide the microenvironment required for cells to form complex organoid structures (Yi et al., 2021) , (Zhao et al., 2022).

The matrix is another key component utilized in organoid cultivation. These matrices are typically composed of natural or synthetic materials and offer a supportive physical environment for cells to build three-dimensional structures. Extracellular matrix components like collagen and Matrigel are examples of natural materials; yet, because of their adaptable topologies, synthetic hydrogels are frequently utilized in organoid creation (Aisenbrey & Murphy, 2020). With characteristics like rigidity and biochemical content, the matrix can control cell behavior in addition to ensuring cell adherence and organization.

Physical clues are also of critical importance for organoids to achieve a functional structure. Extracellular matrix support provides cells with both a mechanical structure and biochemical signals. At the same time, the physical structure of the matrix allows nutrients and wastes to move freely between cells, which supports the healthy development of organoids. Creating a dynamically tunable microenvironment enables organoids to mimic and thrive in their natural environments.

Using integrative cues to improve the biological functionality and structural integrity of organoids is the last stage of organoid engineering. By arranging organoids in a particular order, bioprinting technologies make it possible to create functioning tissues (Murphy & Atala, 2014), (Brassard et al., 2021). Additionally, by putting organoids on a microchip, organ-on-a-chip devices can simulate particular organ systems. By merging several organoids, these systems provide ground-breaking potential in fields like drug development and disease modeling and allow the study of intricate biological processes.

The combination of all these processes requires a holistic approach to establishing organoid-based cultures. Cell sources, biochemical and physical factors, supporting matrix and advanced engineering techniques enable the creation of small yet powerful models to understand and simulate human biology (Figure 5). These technologies are becoming increasingly important in modern biomedical research and contribute to the development of new solutions for human health.

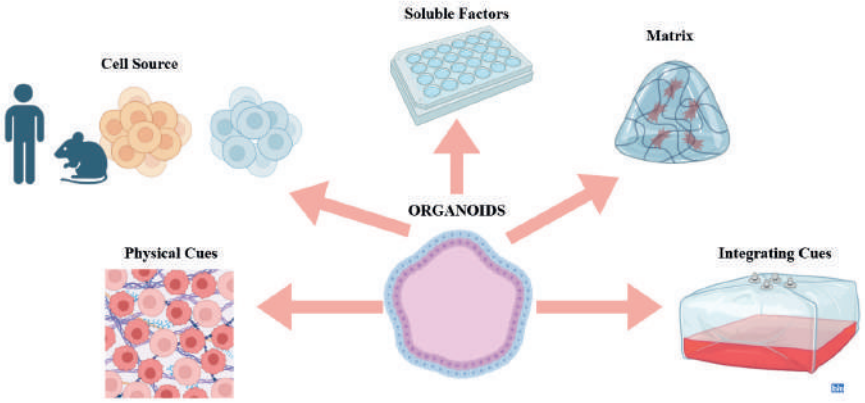


Figure 5. Organoid engineering components. Establishing organoid-based cultures relies on the successful integration of cell sources, soluble factors, matrix, and physical cues.

1.4. Applications of Organoid Technology

3D cell cultures known as organoids that replicate the composition and capabilities of human organs may now be produced in the lab thanks to developments in stem cell culture. Numerous biomedical research fields can benefit from this novel technique, including disease modeling and mechanism study, drug development and toxicity testing, genetic illnesses and personalized medicine, infectious diseases and microbiological research, cancer research, and gene repair (Figure 6).

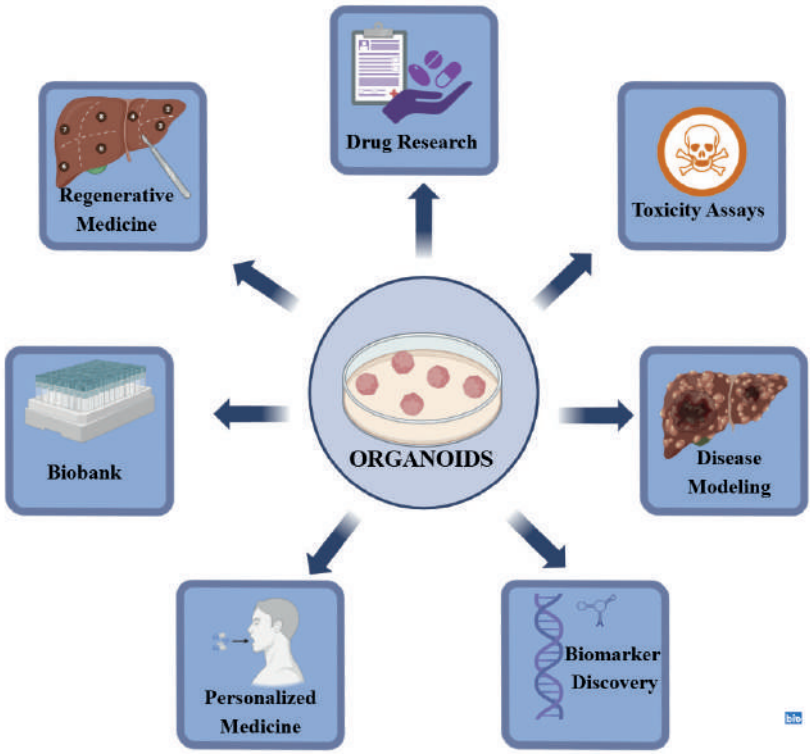


Figure 6. Various applications of Organoid technology

1.4.1. Drug discovery

The drug discovery process suffers from by limitations like patient diversity, uncertain results, and time-consuming drug testing. Organoids now more accurately depict human tissues and physiology, providing a significant substitute in the processes of drug development and toxicity evaluation. The benefits of this technology allow for more accurate testing of new medications' efficacy and safety.

Organoids, which mimic the genetic features of tumors, are used to more precisely assess the effects of certain medications, especially in cancer research. Organoids made from the cells of patients with genetic abnormalities, such colon cancer, for instance, provide effective resources for finding novel medications to treat cancer (van de Wetering et al., 2015), (Lancaster & Huch, 2019). Similarly, antiviral medications for the treatment of such illnesses can be quickly investigated by modeling infections like the Zika virus using brain organoids (Lancaster & Huch, 2019).

Organoids also make it easier to develop personalized medications. Individualized therapy options can be rapidly assessed by modeling diseases based on a person's genetic makeup. Organoids of genetically distinct people can be used to create more specialized and efficient treatment plans. This has a lot of promise, particularly for people with genetic abnormalities and unusual disorders. Organoids therefore provide significant promise for improving drug discovery speed, accuracy, and efficiency.

1.4.2. Toxicity assessment

Organoids have improved drug safety evaluations in toxicology research by offering microenvironments that are more similar to human physiology. Due to their crucial roles in drug metabolism and excretion, the liver and kidney are susceptible to damage from drugs. Due to limited cellular interaction or biological differences between species, traditional models, such as 2D cell lines and animal models, may not accurately represent human physiology (Tang et al., 2022), (S. Yang et al., 2023). This reduces the preclinical test results' confidence.

Organoids address these shortcomings and provide useful substitutes in toxicological research. For instance, high levels of CYP enzyme expression in liver organoids improve hepatotoxicity tests. An essential technique for evaluating hepatotoxicity is liver organoids. Key aspects of liver metabolism, including the production of CYP enzymes, are represented by intrahepatic cholangiocyte organoids (ICOs) generated from human AdSCs that were produced under circumstances of differentiation into the hepatic lineage. Shi et al. showed that ICOs are a practical ex vivo model for evaluating bile cytotoxicity. In this study, the relationships between necroptosis and biliary tract disease were defined with the help of ICOs (Shi et al., 2022). In nephrotoxicity evaluations, kidney organoids are perfect for tracking changes in cell viability and gene expression. Gu et al. tested the possible nephrotoxic effects of Esculentoside A using kidney organoids generated from iPSCs. This study showed that exposure to Esculentoside A results in morphological abnormalities, altered gene expression patterns, and decreased cell viability. These findings support the notion that kidney organoids are a viable method for assessing a compound's harmful effects (Gu et al., 2023).

In addition, 3D skins models provide an innovative approach to toxicity testing in the pharmaceutical and cosmetics sectors. Reconstructed epidermis and full-thickness skin are examples of artificial models that accurately represent both healthy and pathological skin situations. These models are widely used and are continuously being refined, adding new layers like

immune cells, despite the limitations on animal testing (Caipa Garcia et al., 2022).

1.4.3. Cancer studies

Organoid technology offers a significant innovation in understanding cancer biology and developing treatments. Organoids derived from patients overcome the limitations of traditional models by accurately mimicking the genetic characteristics and microenvironment of tumors. Animal cancer models, human cancer cell lines, or primary patient-derived tumor xenografts (PDXs) are tools used in cancer research. However, these methods were insufficient to accurately represent the biology and pathophysiology of the host tumor (H. Xu et al., 2018). Cancer cell lines include primary (originating from patients) and immortalized tumor cells. While primary cancer cells retain the characteristics of the original tumor to a limited extent, their short lifespan and slow growth limit research capacity. Immortalized cell lines, despite the advantage of unlimited proliferation, may fail in phenotypic representation by losing genetic diversity in long-term cultures. 2D culture systems are also inadequate to mimic in vivo conditions and cannot accurately reflect tumor heterogeneity. PDX models are created by transferring patient-derived tumors to animals, but have limited use due to interspecies biological differences and high cost (S. Yang et al., 2023), (Fan et al., 2019).

Organoid technology provides a potent substitute to get beyond such barriers. Cells from surgical samples or biopsy material are cultivated in a three-dimensional matrix to create a model that replicates the natural tumor microenvironment. By reflecting the tumor's genetic makeup and drug response in a patient-specific way, this technique makes individualized treatment methods possible. Especially in examining metastasis, the mechanisms of spread of tumor cells to other tissues can be analyzed through organoids (Lo et al., 2020). Ultimately, organoid technology makes it possible to better understand tumor biology in cancer research and develop more effective, personalized treatment strategies.

2. Development of Organ on a Chip

The drug development process is considered one of the most complex and challenging areas of biomedical research as it takes a long time and requires high costs. The failure rate in the procedure is more than 80%, even though only a small percentage of medication candidates investigated in preclinical stages pass clinical trials. The failure of medication candidates owing to toxicity (30%) and ineffectiveness (60%) are the primary causes of

this state of affairs (X. Xu et al., 2024). Conventional modeling techniques are insufficient to address these issues. 2D cell cultures are inadequate for simulating the natural microenvironment and tissue architecture of cells, yet animal models cannot accurately represent human biology because of biological variations between species. Organ on a chip (OoC) technology, developed as a solution to these problems, has the potential to model human biology more accurately.

OoC technology are microengineering platforms that can mimic the functional and structural features of human organs in a laboratory environment. Combining fields like microfluidic systems, bioengineering, stem cell technologies, and bioprinting produced this ground-breaking breakthrough. OoCs are small-scale biomimetic devices that incorporate human cells and are usually made of biocompatible and flexible materials (such as poly(dimethylsiloxane)). These systems offer a unique platform that mimics the physiological circumstances of actual organs by simulating the microenvironment and functioning of one or more organs in vitro. Microfluidic channels that replicate the natural milieu of cells and tissue organizations are employed in the creation of OoC. These channels are made to resemble biological functions like blood flow and the movement of nutrients and oxygen. Oxygen and nutrients are continuously supplied by the fluid flow, which also eliminates waste products and metabolites from the cells. Physical stimuli like fluid shear force also encourage the histochemical differentiation of several cell types, including endothelial and epithelial cells (Whitesides, 2006), (Driver & Mishra, 2023). The main biological elements utilized in OoCs are immortalized cell lines and human-derived stem cells (Park et al., 2019). More accurate and trustworthy outcomes in drug development, toxicity testing, disease modeling, and precision medicine have been made possible by OoC models (H. Wang et al., 2024). This technology has become a ground-breaking tool in biomedical research because it more accurately replicates human biology.

2.1. Application areas of Organ-on-a-Chip

One particularly noteworthy instrument in the drug research and discovery process is OoC technology. Accurate modeling of drug absorption, distribution, metabolism, and excretion (pharmacokinetics) and their effects on target organs (pharmacodynamics) is made possible by these systems, which replicate the biological, chemical, and mechanical processes of human organs at the microscale. In addition to being a potent platform for personalized medicine, genetic disease research, and cancer studies, OoC offers quick and accurate results in crucial procedures including

toxicity assessment and efficacy analysis (Figure 7). This method saves time and money in preclinical testing, which improves the efficiency of drug development procedures (Joseph et al., 2022).

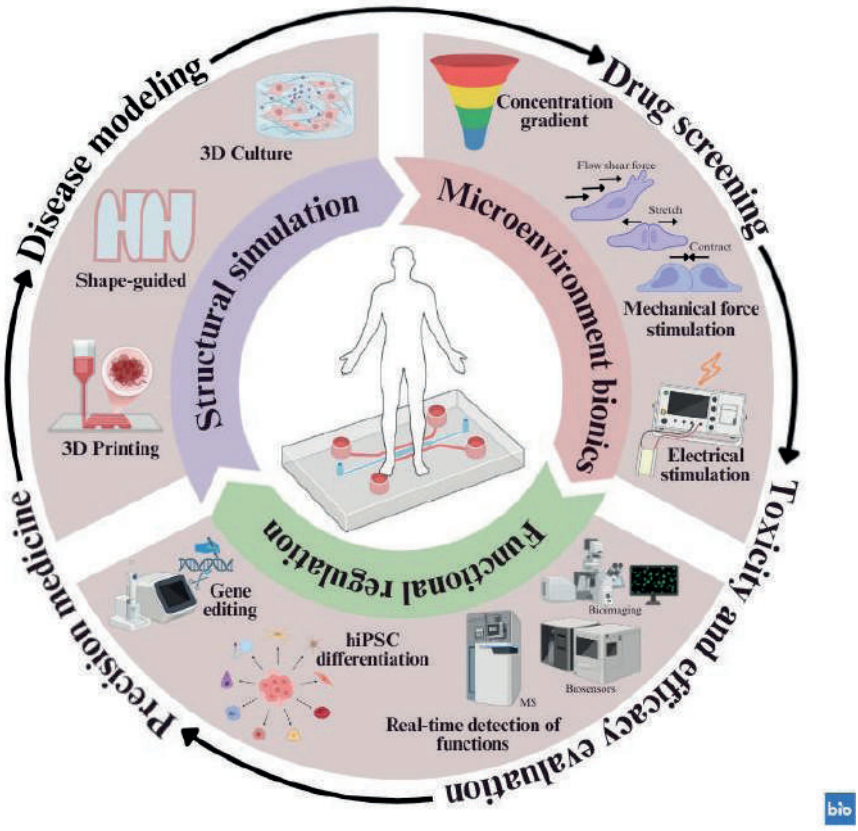


Figure 7. Scheme of application areas of Organ-on-Chip technology

2.1.1. Drug discovery

From the discovery stage to the drug's release onto the market, the development of new medications necessitates a very high time and resource commitment. About 90% of medication candidates that make it to clinical trials fail, which costs the industry a lot of money and delays patients' access to new therapies (Zhai et al., 2019). Among the most important processes in the drug development process is the selection of the best compounds based on factors including toxicity, efficacy, and safety. Prior to human

clinical trials, one of the most important strategies to expedite the process and cut expenses is to have a thorough and accurate screening procedure (Y. Wang et al., 2023).

Traditional drug screening methods generally rely on animal-based in vivo models and cell-based in vitro systems. However, these methods offer limited predictive ability because they cannot adequately mimic human biology. Recent advances in microfabrication and tissue engineering have enabled the development of OoC technology as an innovative solution to overcome these limitations. OoC mimic the in vivo structure and function of human organs, eliminating interspecies differences and providing higher accuracy in preclinical assessments such as drug metabolism, toxicity, and efficacy (Liu et al., 2019). For example, chips that mimic specific organs, such as the liver or kidney, can be used to study the effects of drugs at the organ level, while by integrating multiple organ chips, the systemic effects and interorgan interactions of drugs can be comprehensively analyzed.

2.1.2. Toxicity assessment

Accurately predicting drug-induced toxicity is one of the most difficult tasks in the drug development process. Interspecies differences limit the ability of traditional animal-based models to forecast toxicity, which sparks ethical debates. By utilizing human cells and avoiding interspecies variations, OoC systems improve the ability to anticipate toxicity. Particularly useful for analyzing the harmful effects of medication metabolic byproducts are liver and kidney chips. For example, in 2009, Toh and his team developed a microfluidic liver cell chip to evaluate drug toxicity in a laboratory setting. This device incorporated multiple cell culture chips and a linear concentration gradient generator. Its structure, designed with microcolumns, allowed hepatocytes to settle in a central cell chamber and perform their metabolic activities. The researchers tested five hepatotoxic drugs, including paracetamol, on this chip and successfully obtained in vitro toxicity data consistent with in vivo findings. Such innovative approaches make the drug safety assessment process in preclinical testing more precise while reducing reliance on animal models (Toh et al., 2009), (Z. Li et al., 2022).

2.1.3. Cancer

OoC systems offer a significant benefit for comprehending the intricacy of cancer biology and creating novel therapeutic strategies. An excellent platform for assessing the efficacy and adverse effects of cancer medications is provided by tumor-on-a-chip models, which replicate the cancer microenvironment and the interactions of tumor cells with surrounding tissues. Furthermore,

these models offer a deeper comprehension of intricate biological processes as immunotherapies, tumor microenvironment, and cancer metastasis. This is seen as a major development, particularly for applications in customized treatment (Sontheimer-Phelps et al., 2019).

For instance, Ingber and friends created a chip for lung tumors in order to study cancer. This chip demonstrated that tumor cells were restricted to smaller regions during simulated lung breathing movements, but that the cells spread when the movements were stopped. Furthermore, orthotopic non-small cell lung cancer growth, treatment responses, and tumor dormancy mechanisms were investigated in vitro using the model built on a microfluidic platform. According to research, tumor cells occupy the alveolar space, decreasing the lung's respiratory motions. This creates a positive feedback loop that encourages tumor development and invasion. Additionally, co-culturing tumor cells and alveolar epithelial cells has been shown to enhance cell-cell interactions; however, endothelial cells may counteract this impact. These results clearly demonstrate the promise of OoC systems in cancer research by improving our knowledge of the dynamics of the tumor microenvironment (Hassell et al., 2017) (Liu et al., 2021).

2.1.3.1. Cancer on a Chip

Being one of the diseases with the highest death rates in the world, cancer places a significant strain on healthcare systems. Uncontrolled cell growth, proliferation, and invasion of surrounding tissues as a result of genetic abnormalities and epigenetic modifications are hallmarks of cancer. Through a process known as metastasis, tumors can spread to other parts of the body during this unchecked growth, making therapy considerably more challenging. Even with the recent development of novel techniques including immunotherapies, targeted treatments, and integrated therapy approaches, comprehending and managing the intricate molecular makeup of cancer remains a significant scientific problem. It is known that the complex biochemical and physical environment surrounding tumor cells, called the tumor microenvironment (TME), plays a major role in the development of cancer, as well as genetic mutations. TME is a dynamic structure containing immune cells, fibroblasts, vascular structures and extracellular matrix (ECM) and not only supports tumor growth but also directs the processes of drug resistance and metastasis. However, traditional in vitro and in vivo models have limitations in understanding the molecular mechanisms of cancer and its interactions with the TME. The intricacy and physiological characteristics of the human tissue microenvironment are not reflected in current systems, despite the fact that transgenic mice and immunodeficient animal models

have been utilized to research some forms of cancer. The creation of novel cancer treatments is severely limited by this deficit (Sontheimer-Phelps et al., 2019), (C. Li et al., 2023).

To overcome these limitations, one of the most innovative approaches that has attracted the attention of the scientific community in recent years is "organ-on-a-chip" technology. OoC has the capacity to mimic the biological and mechanical dynamics of complex microenvironments by bringing together different cell types. The biggest advantage of this technology is that it can successfully model biochemical and mechanical microenvironments that traditional cell culture methods cannot reproduce. Thus, it more accurately reflects the complexity of the tumor microenvironment, providing more realistic data about the behavior of cancer cells. Numerous applications for cancer biology and therapeutic research are available using OoC technology. For instance, the intricate relationships between breast cancer cells and the surrounding tissues are being studied using breast tumor models-on-a-chip. These models are an effective way to assess how medications affect the tumor and create more targeted treatment plans. In a similar vein, glioblastoma-on-a-chip models serve as a valuable resource for comprehending the intricate characteristics of this aggressive brain tumor as well as its resistance to treatment. These applications show how flexible OoC platforms are for various cancer kinds and how useful they can be for research procedures. (Sontheimer-Phelps et al., 2019), (C. Li et al., 2023), (Nejati et al., 2025).

Breast tumor on a Chip

One of the most prevalent cancers in women globally, breast cancer accounts for a sizeable portion of cancer-related fatalities. Genetic and environmental variables interact to cause breast cancer, which is a complicated process with a high degree of molecular and morphological variety. There are various subtypes of breast cancer. Hormone receptor positive (HR+), HER2 positive (HER2+), and triple negative breast cancer (TNBC) are the most prevalent subtypes of these (Firatligil-Yildirir et al., 2023). The treatment process might be difficult because each subtype has unique biological characteristics and therapy responses. Comprehending intricate processes such tumor invasion, metastasis, and medication modes of action is necessary to comprehend the course of breast cancer. These processes have traditionally been modeled using animal models and 2D and 3D cell cultures. These methods, however, are unable to forecast the actual effects of medications in humans and cannot accurately capture the biochemical and physical characteristics of the natural TME. As of right now, a solution that can accurately replicate the tumor microenvironment is breast tumor on a

chip (Figure 8). These models offer a perfect platform for researching how cancer cells proliferate, spread, and react to medications. Additionally, these systems provide a distinct advantage over other conventional techniques due to the controllability of fluid flow, shear stress, media and gas supply, and biochemical gradients (Firatligil-Yildirir et al., 2023), (Subia et al., 2021) (Moccia & Haase, 2021).

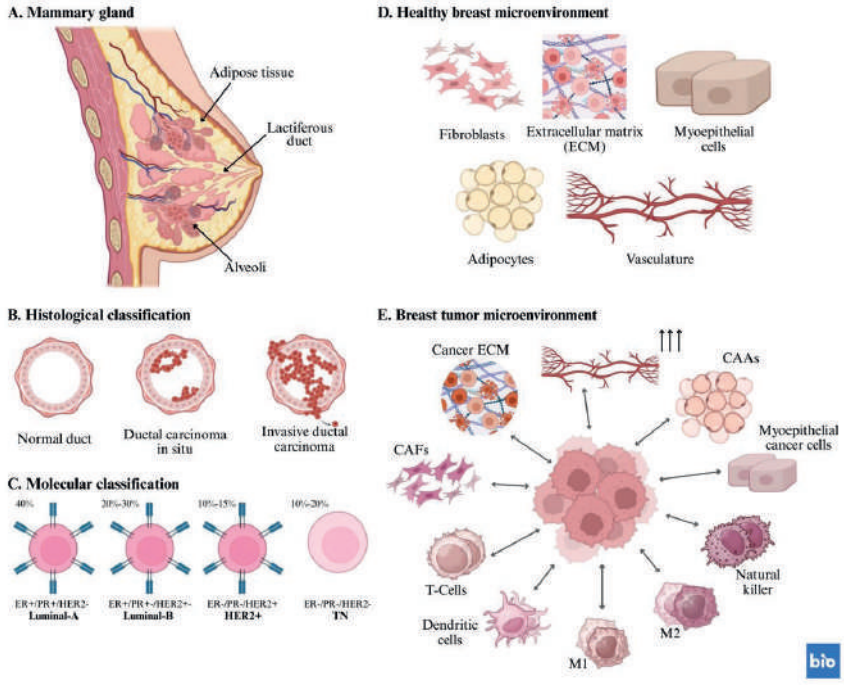


Figure 8. Breast cancer classification and breast-specific tumor microenvironment. (A) Graphical representation of the mammary gland. (B) Histological classification of the breast cancer subtypes. Magenta represents cancerous cells. (C) Molecular classification of the breast cancer subtypes demonstrating their frequency and commonly associated markers. (D) Schematic representation of components of the most abundant healthy mammary gland microenvironment. (E) Components often transformed in the breast tumor microenvironment (modified from Moccia & Haase, 2021).

Glioblastoma on a Chip

One of the most lethal and aggressive brain tumors in the central nervous system is glioblastoma multiforme (GBM). About 17% of all brain tumors are this type of malignant tumor that develops from astrocytes. Due to its extremely invasive nature, GBM spreads quickly to nearby tissues, limiting the effectiveness of conventional therapeutic methods and surgical

procedures. Patients often only live for 16–21 months following diagnosis, and recurrence rates are rather high. Treatment for GBM is made even more challenging by treatment resistance, the intricate complexity of the tumor microenvironment, and the blood-brain barrier's (BBB) ability to block medications from entering the brain (Silvani et al., 2021).

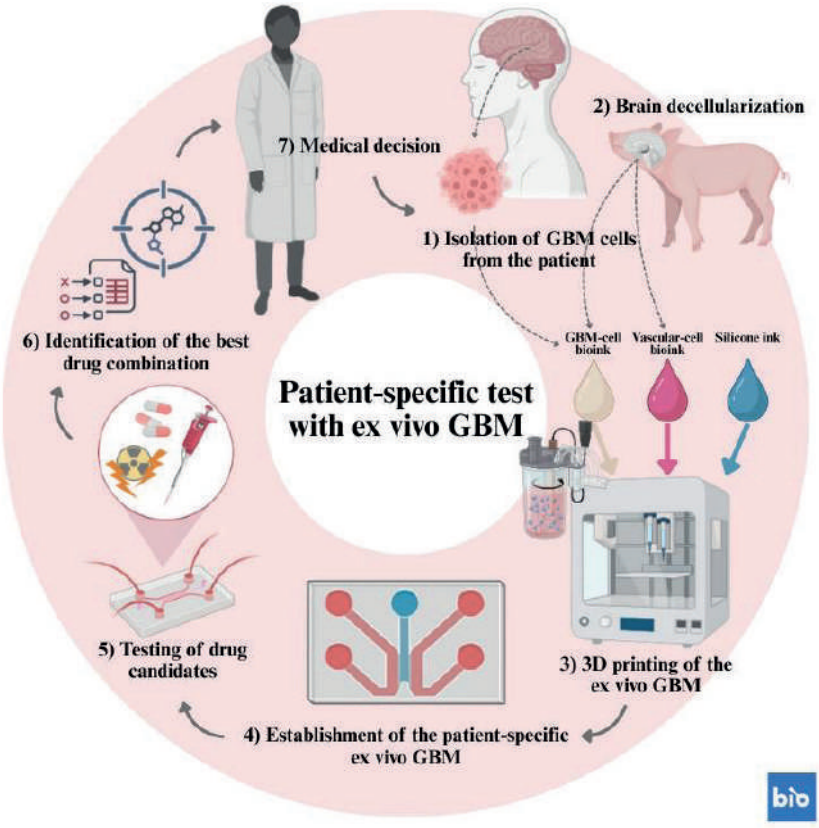


Figure 9. Patient-derived GBM cells are used to produce a GBM-on-a-chip model using pig BdECM bioink. The cells are then cultivated for one to two weeks, and the effectiveness of drug combinations is tested to design a personalized treatment plan (modified from H.-G. Yi et al., 2019).

A glioblastoma-on-a-chip model that can accurately replicate the biochemical and biophysical characteristics of GBM has been created in order to get around these difficulties. First, GBM cells are isolated from the tumor tissue surgically removed from the patient (Figure 9, 1). These cells form the basic building block of the individualized model. The decellularized extracellular matrix (BdECM) bioink from pig brain is then made (Figure 9,

2). Because BdECM replicates the mechanical and metabolic characteristics of the natural tumor microenvironment, it improves the biological fidelity of the model. 3D bioprinters are used to print this bioink once it has been mixed with patient-derived cells. Additional materials like silicone-based inks and bioinks with vascular cells are used in the printing. process to mimic biological processes like oxygen gradients and heterogeneous structure in the tumor microenvironment (Figure 9, 3). The tumor model made on the chip is cultivated for one to two weeks following printing (Figure 9, 4). GBM cells develop in an environment that closely resembles the tumor's clinical characteristics during this phase, and microenvironmental interactions become clearly visible. The cultured model is used to test different combinations of potential drugs (Figure 9, 5). The purpose of these tests is to assess how each treatment option affects the tumor. The most effective combinations are identified as a consequence of the investigation (Figure 9, 6). In particular, the responses of patient-derived tumors to clinical treatments such as temozolomide (TMZ) and concurrent chemoradiation (CCRT) can be modeled on a chip to obtain data compatible with clinical results (H.-G. Yi et al., 2019).

Chip-based glioblastoma models offer a special tool for comprehending tumor biology as well as creating novel medications and therapeutic approaches. Clinical decision-making and the development of individualized treatment plans can both benefit greatly from these models. These modern platforms are regarded as a revolutionary approach in patient-centered treatment procedures and scientific research for cancers that are resistant and lethal, like GBM (H.-G. Yi et al., 2019).

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Chapter 2

Combined Approaches of NMR Spectroscopy in Pharmaceutical Sciences

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John A Parkinson[2]

Abstract

NMR spectroscopy is extensively utilized across various disciplines of pharmaceutical research. The advancement of magnetic resonance technology has enabled diverse and high-resolution analyses. The importance of data from different sources in pharmaceutical research has increased. NMR spectroscopy is an effective pharmaceutical method with easy application and routine applicability. Another advantage of this method, which allows drug research not only from the chemical but also from the biological and biophysical aspects, is that it is marker-free. Initially utilized at a single level and primarily focused on specific elements such as chemical synthesis and structure-activity elucidation, NMR methodology has evolved to produce multi-level biomolecular and clinically relevant data, including metabolomics and 3D modelling, by integrating it with other instrumental techniques used in pharmaceutical sciences. From this perspective, NMR spectroscopy has found broader applications in pharmaceutical sciences. This section discusses pharmaceutical instrumentation methods combined with NMR technology, their importance, and current publications.

1. Single-level NMR Applications in Pharmaceutical Sciences

Over the decades, Nuclear Magnetic Resonance (NMR) Spectroscopy has been used in the pharmaceutical sciences as a "gold" standard and a helpful tool in a broader range between compound characterization and clinical data (Daly and Cohen 1989, Forseth and Schroeder 2011, Breton and Reynolds 2013, Emwas, Szczepski et al. 2020). There are various advantages to the

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https://doi.org/10.58830/ozgur.pub1094.c4353

usage of NMR spectroscopy in pharmaceutics research, and these are the reasons why researchers choose this method in every step of the research, which will be mentioned later in this section.

One of the most powerful ways of using NMR is by utilizing various types of research material, such as liquid-state and solid-state samples (Webb 2020). Therefore, this instrumentation can efficiently employ a wide range of compounds and biomaterials related to drug activity and delivery. Another strong side of the NMR method in pharmaceutical research is the function of different types of atoms (^{1}H, ^{13}C, ^{15}N, ^{17}O, ^{31}P, etc.) (Rehman and Akash 2020) present in the drug and target molecules. This feature can help to selectively identify molecular interactions and provide information on the target-ligand approaches. One of the other advantages of the NMR approach is that it is not necessary to label[3] the target or ligand molecules (Gossert and Jahnke 2016). Therefore, the ligand and target do not need to be chemically modified, and there is no need to add an extra synthesis or characterization step. The significance of label-free spectroscopy is related to reaction monitoring using NMR in various steps. Another excellent value of this method is its adaptability when used with different magnetization and relaxation parameters. By modifying (increasing) the magnetic field strengthand probe types, the sample will be able to be investigated in depth and more accurately (Conradi 2022).

Despite its widespread use and the possibility of label-free analysis, NMR spectroscopy does not provide detailed data on functional pharmaceutical aspects with a single methodology alone. The main reason for this is that proton NMR, which is the most commonly used, reflects every proton in the sample in the spectrum. This results in a highly complex NMR spectrum, making spectrum interpretation challenging. Complexity of NMR spectrum for samples containing biological material with high molecular weight (protein, peptide, long DNA chains, cell cultures, etc.) is a disadvantage for interpreting the biomolecular NMR data (Ardenkjaer-Larsen, et al. 2015, Selenko 2019). Another issue is the type of solvent and its applicability in pharmaceutical research (Laszlo 1967). Samples containing protons must be replaced with deuterium, and this modification must be made at every stage of the research. Deuteriation is a very costly method that requires additional process optimization (Di Martino, et al. 2023). In addition, although it

3 Except in situ labeling. In this technique, the label (isotope) is not required for spectrum acquisition, but it helps to understand the interaction mechanism or reaction footprinting, by the subsequential changes on label atom resonances. For details, please see Nishida, N., Y. Ito and I. Shimada (2020). *"In situ structural biology using in-cell NMR."* *Biochim Biophys Acta Gen Subj* ***1864****(2): 129364.*

is easy to apply, the NMR methodology must be expertly designed and optimized to perform more qualified research. This optimization requires qualified experts, new protocols using different probes, and detailed information. Finally, interpreting the optimized spectrum requires complex software and a detailed interpretation protocol (Johnson and Blevins 1994, Vranken, et al. 2005, Elyashberg, et al. 2008, Ellinger, et al. 2013, Lee, et al. 2015, Maciejewski, et al. 2017).

From synthesis to in-cell NMR, from clinical sample analysis to biological imaging techniques, NMR spectroscopy is used in pharmaceutical research for a wide range of purposes. In this section, examples of this usage, single-level applications, and multiple-level methods in pharmaceutical research will be discussed (Shapiro and Wareing 1998). In addition, not only NMR spectroscopy but also the advantages of NMR techniques combined with other instrumental methods in the pharmaceutical field will be discussed and a brief discussion horizon for future studies will be presented.

1.1. Chemical Characterizations

1.1.1. Small-molecule synthesis characterization

Over the past several decades, more than 150,000 distinct publications[4] have established a strong correlation between NMR spectroscopy and chemical synthesis in the fields of drug and pharmaceutical discovery. NMR strategies have been confirmed to be invaluable for elucidating and characterising the structural compositions of both reactants and products, determining functional and side groups, studying reaction monitoring progress, assessing solubility, measuring pH, assessing purity, and fulfilling miscellaneous experimental requirements (Figure 1.1). Accurately determining the chemical structures of compounds synthesised as potential drug candidates is crucial for drug research, as well as for investigating structure-activity relationships and ADME (Absorption, Distribution, Metabolism, and Excretion) properties. Furthermore, the pharmaceutical industry relies on NMR as an essential tool for routine quality assurance testing in drug manufacturing workflows, in addition to its application in research and development studies.

4 According to the PubMed listing.

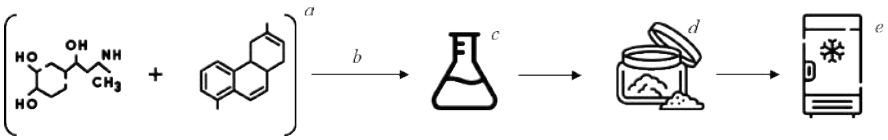

Figure 1.1. The scheme of chemical synthesis of small-molecule drug candidate/ pharmaceuticals (*a*: reactants, *b*: reaction, *c*: product (in liquid form), *d*: lyophilized powder, *e*: stability)

As illustrated in the small-molecule synthesis scheme (Figure 1.1), NMR can be utilised as the sole analytical method at various stages of pharmaceutical sciences. Most chemical syntheses in pharmaceutics begin with the acquisition of 1D-^{1}H spectra to identify impurities and confirm the intended structural composition of the target compound. Reaction monitoring is employed to determine whether a reaction has occurred (using 1D-^{1}H NMR) and to observe time-scale structural modifications through Diffusion NMR. The NMR spectra also reveal changes in the synthesis media and catalysts, referred to as reaction spectra. These reaction spectra are essential for optimising synthesis processes to enhance reaction efficiency, allowing for modifications to accommodate different reactions based on reference synthesis data.

The analysis of products can be performed using 1D-^{1}H spectra, as well as homonuclear or heteronuclear spectroscopy[5]. Product evaluation includes structural analysis, yield assessment, solubility testing, and pH measurement[6]. Once the liquid product is obtained at the conclusion of synthesis, a lyophilisation process is implemented to ensure the product's stability over time. Additionally, lyophilisation serves to standardise the concentration in the solvent (molarity) for subsequent pharmaceutical research steps. For these purposes, the lyophilised powder is tested using methodologies akin to those applied to liquid-form products. Stability studies can also be conducted using NMR spectroscopy to quantify the molecules and assess long-term structural modifications in small-molecule compounds.

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5 Homonuclear spectroscopy refers to only one type of spectra that includes a single type of atom, and heteronuclear spectroscopy refers to combining resonance effects from two different types of atoms (^{1}H-^{31}P, ^{1}H-^{13}C, ^{1}H-^{15}N etc.). Therefore, NMR spectroscopy gives an opportunity to illustrate the interactions of different atom types in the drug molecule.

6 These tests are not only used in novel drug research, but also in routine pharmaceutical drug quality processes.

1.1.2. Natural products characterization

Natural-sourced drug raw materials undergo a variety of analytical processes after being extracted from plant and animal sources. NMR spectroscopy plays a crucial role at every stage of the characterization and quantification of these compounds (Figure 1.2). It is particularly effective for both qualitative and quantitative analysis of the analyte.

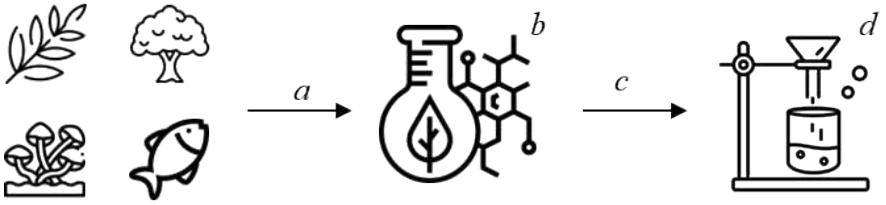

Figure 1.2. The scheme of naturally sourced drug candidate/pharmaceuticals (a: source extraction process (plant, fungi, animal, marine etc.), b: extracted material, c: purification process, d: purified compound/semi-synthesis material)

In addition to one-dimensional NMR spectroscopy, two-dimensional NMR techniques are utilized to monitor the extraction outcomes of raw materials from plant-or-animal-derived drug precursors, as well as to optimize the extraction process. Furthermore, in the semi-synthesis method employed for obtaining these drug raw materials, the characterized pre-pharmaceutical compounds sourced from plants or animals are subsequently transformed into full pharmaceutical drug candidates through semi-synthesis. NMR continues to play an essential role at this stage as well.

1.1.3. Drug delivery system characterization

Drug delivery systems are specialized pharmaceutical formulations designed to facilitate the effective delivery of active drug ingredients to targeted tissues and organs while minimizing drug toxicity. The stages of synthesis, characterization, and purification of these materials often employ NMR spectroscopy techniques. The methodologies used vary based on the chemical classification of the drug delivery system. In addition to the delivery systems themselves, drug-loaded formulations are also analyzed using NMR spectroscopy. Typically, one-dimensional methods such as DOSY and COSY, along with other NMR techniques, are applied for the characterization of drug delivery systems. Long-term stability assessments are conducted by measuring the stability of micellar nanoparticles and nano-delivery systems using quantitative NMR methods (Figure 1.3.).

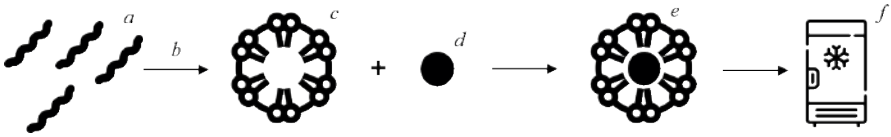

Figure 1.3. The scheme of synthesizing micellar nanoparticles (a: amphiphilic alkyl chains, b: micelle formation process, c: micelles, d: drug active ingredients, e: drug-loaded micelles, f: stability)

1.1.3. Biopharmaceutical characterization

The phrase 'biopharmaceutical' was first created to refer to therapeutic proteins generated through genetic engineering, as opposed to those obtained from typical biological sources (LeVine 2013). Over time, its definition has expanded, and the term now includes nucleic acids along with proteins, vaccines alongside therapeutic products, and even therapies based on cells (Figure 1.4.). Although these biologically based therapeutic approaches are considered to be areas where NMR methodology is not widely used due to their complex structures, it is possible to come across examples of NMR spectroscopy at different steps within the relevant methods.

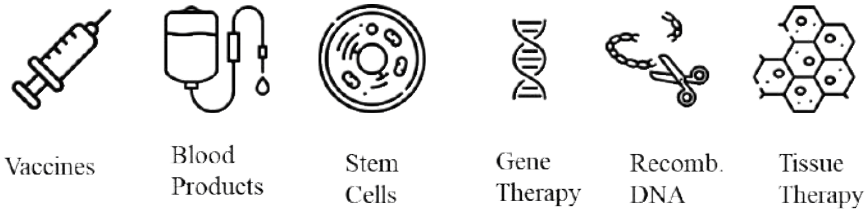

Figure 1.4. Examples of various biopharmaceuticals that are in use within pharmaceutical applications.

Structural changes (Wishart 2013, Ma, et al. 2023) and enzyme-substrate complexation (Scott 1986, Palmer 2015, Vang, et al. 2022) of biopharmaceuticals can be tracked with the NMR method. Saturation Transfer Difference spectra can be employed to identify the on/off states for protein-ligand interaction widely. Thus, it provides an idea about the therapeutic target binding mechanism. In addition, the NMR metabolomics technique can be used to show metabolic changes of biopharmaceuticals that will be mentioned later.

1.2. Structure-Activity Relationship (SAR) Applications

NMR methodology is widely used in drug discovery studies to determine structure-activity relationships. Among the basic principles of NMR, chemical shift and related principles are widely used. These principles are prominent, such as electron density, chemical anisotropy, mesomerism, and steric effects (Diehl 2008). Using these principles, pre- and post-treated structure-activity and binding mechanics are elucidated, and suitable drug candidate compounds can be effectively selected (Dias and Ciulli 2014). Binding titration and K_D values can be determined with concentration-dependent sample studies, which are informative about drug efficacy and safety from a pharmacological perspective (Dalvit, et al. 2023).

Structure-based drug design (SBDD) employs computational chemistry tools that utilize the structural information of a protein to identify or create new chemical compounds capable of binding to the target, thereby inhibiting the target protein. This method helps determine how compounds should be arranged, called the "docking pose." Scoring methods predict the most stable interactions, guiding the choice of compounds for testing against the target protein. NMR data of target-ligand interaction can be employed to support the SBDD computational docking studies to guide the binding interaction residues (Sugiki, et al. 2018). In each step, SBDD data can be checked with single-level NMR methods (Figure 1.5.).

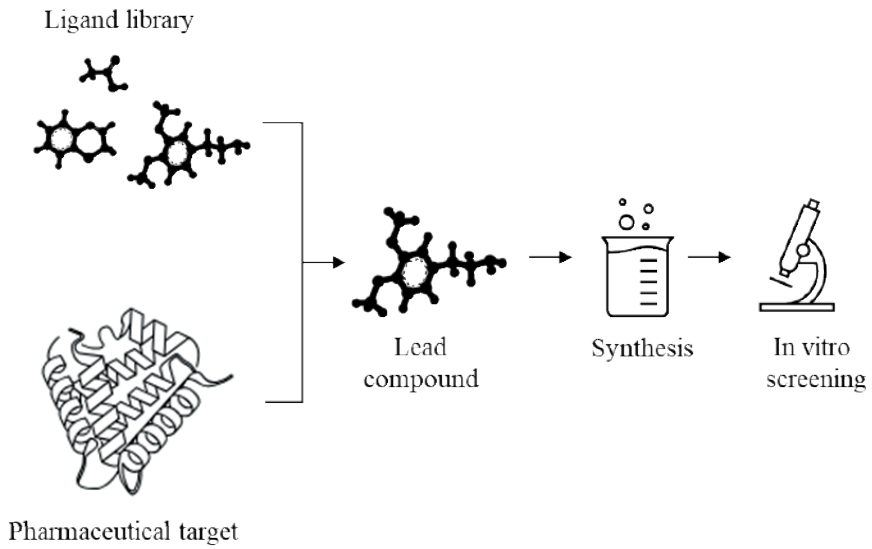

Figure 1.5. The workflow of structure-based drug design (SBDD) process in general.

At the single-level NMR spectroscopy aspect, the SBDD data is not generated adequaetly to conclude the lead compound alone. In this step, other major approaches should be employed to better understand target-ligand interaction from a ligand library source. Moreover, most of the SBDD approaches need to modify the structure, for optimizing the efficacy and safety limits of lead compounds. In this case, NMR data should be combined with other in vitro approaches (Stark and Powers 2012).

Pharmacological analogs are used as scaffolds in new generation drug design, allowing more effective and target-selective drug design through pharmacophore interactions. New drugs from the same group are designed with different modifications using the skeleton of the chemical analog. NMR spectroscopy is used in scaffold-based drug design studies using on/off binding affinity (Figure 1.6.).

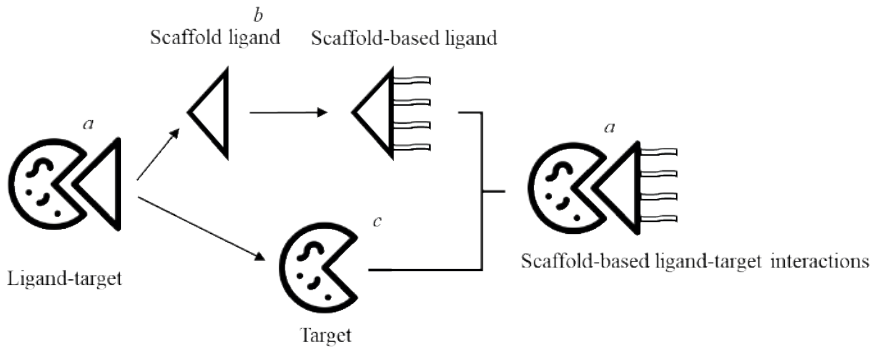

Figure 1.6. The illustration of the process for scaffold-based drug design. NMR spectroscopy is used in a) complex 2D correlation spectra, b) ligand characterization spectra and c) target identification spectra.

Not only scaffolds but also chemical fragments are effectively used in the design of new drugs (Figure 1.7.). The main difference here is that, apart from pharmacophore analogs, related chemical functional group fragments are used (Prosser, Kohlbrand et al. 2021). NMR spectroscopy is used for the structure-activity elucidation of compounds obtained by combining fragment combinations (Li and Kang 2024).

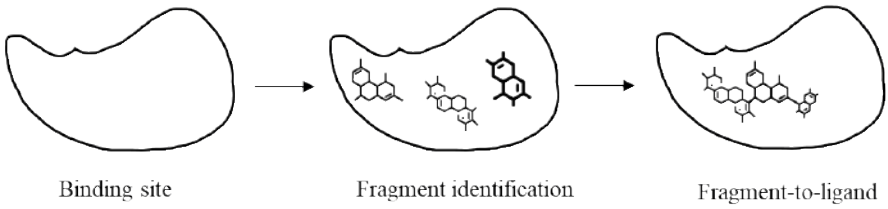

Figure 1.7. The scheme of fragment-based drug design.

The usage of alone NMR is quite common in drug design and discovery stages, which are the most essential pillars of pharmaceutical research. However, NMR data alone cannot elucidate binding and action mechanisms. When NMR data is integrated with various methods, it yields a precise structure-activity profile and serves as an effective tool for standardizing drug functionality.

1.3. Drug Metabolism Monitoring

The metabolism of drugs is crucial in establishing the boundaries of efficacy and safety within pharmaceutical research. These investigations are also employed for optimizing drugs and updating clinical practices. Metabolomics is the scientific field that explores metabolism-related data in an organism and its environment (Idle and Gonzalez 2007). Generally, metabolomics data can be monitored from cells, tissues, and organisms following drug activity. This monitoring allows for an examination of metabolism pathways and drug toxicity at the cellular level.

NMR spectroscopy is commonly utilized in studies involving metabolomics (Nagana Gowda and Raftery 2023). While it is typically applied in titration and time-dependent multiple spectra analysis, NMR correlation spectroscopy, which allows for the tracking of metabolites in two dimensions, is also practically applicable (Figure 1.8.). This technique isolates the metabolite of interest from the spectrum by sampling from the extracellular fluid. Consequently, both known and unknown metabolites can be structurally identified (Nagana Gowda and Raftery 2021). In addition to extracellular fluid, some methods focus on directly observing active cellular metabolism. These techniques fall under the category of in-cell NMR. This approach aims to obtain an instantaneous NMR spectrum from the cellular environment in real time through an automated system. Thus, it enables the monitoring of pathways where the drug exerts its effects. A significant challenge associated with this technique is the complexity of the spectrum, which requires a strong magnetic field; otherwise, high-resolution resonance monitoring becomes quite challenging (Singh, et al. 2023).

Another method to analyze metabolites is through ADMET studies. Data from metabolomics provides insight into the drug's ADMET profile and pharmacokinetic effects (Phapale 2021, Nath and Chetia 2023). This information helps in anticipating potential issues that may arise during clinical applications of the drug. Additionally, it supports the development of drug candidates that are metabolized into less toxic byproducts in the context of new drug development.

Besides in vitro metabolomics techniques, NMR spectroscopy is also applied in vivo within metabolomics studies. Pharmacodynamic and pharmacokinetic profiling can be conducted by analyzing samples taken from living subjects using various NMR methodologies.

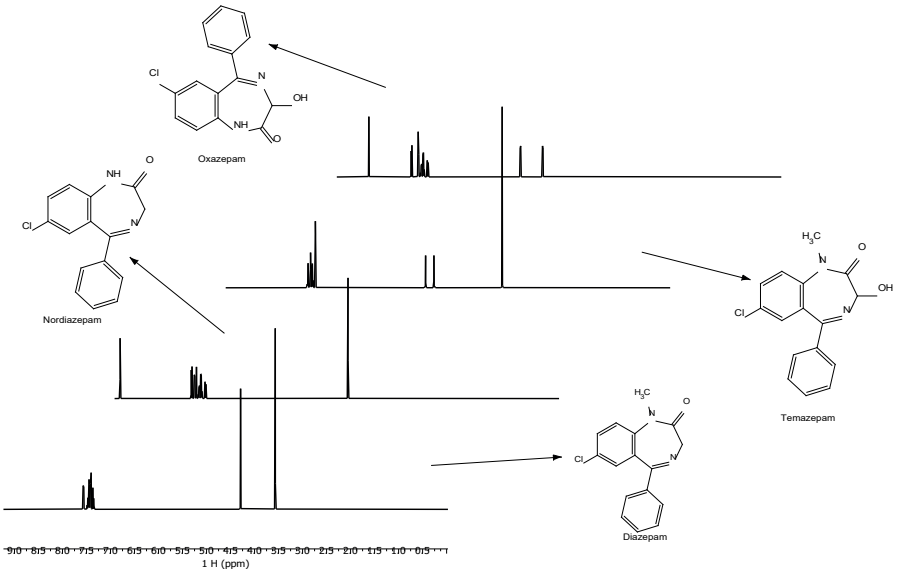

Figure 1.8. The predicted NMR spectra (MestreNova™) of different metabolites of diazepam (Langman and Jannetto 2020).

1.4. Drug Formulation and Delivery Research

Drug formulations and delivery can be effectively analyzed using a range of NMR techniques, along with various other instrumental analysis methods (Li, et al. 2021, Mousa, et al. 2023). One fundamental application is the determination of drug titrations collected from intracellular or extracellular fluids before and after drug delivery. NMR can also be utilized to characterize different drug delivery systems, while routinely assessing

the structural integrity and stability of these systems. Furthermore, NMR technology plays a key role in optimizing delivery systems by elucidating the pharmacological activities of these systems at the molecular level through techniques such as 2D homonuclear or heteronuclear correlations (Wang, et al. 2022, Zhao, et al. 2022).

The physicochemical properties of drug formulations can be clarified using NMR methodology. This technique allows for a detailed examination of the interactions between the carrier system and the active pharmaceutical ingredients through various NMR spectra. Consequently, NMR methodology is widely employed in the optimization of drug carrier formulations and in general preclinical formulation processes.

In addition to enhancing the transport system within the body, NMR can also shed light on cellular uptake mechanisms, as well as on drug efficacy and resistance mechanisms (Figure 1.9.). Moreover, intracellular uptake models developed with isolated cellular carrier components can be quantitatively assessed using NMR through concentration gradient testing (Diffusion NMR) (Nicolay, et al. 2001, Claridge 2009).

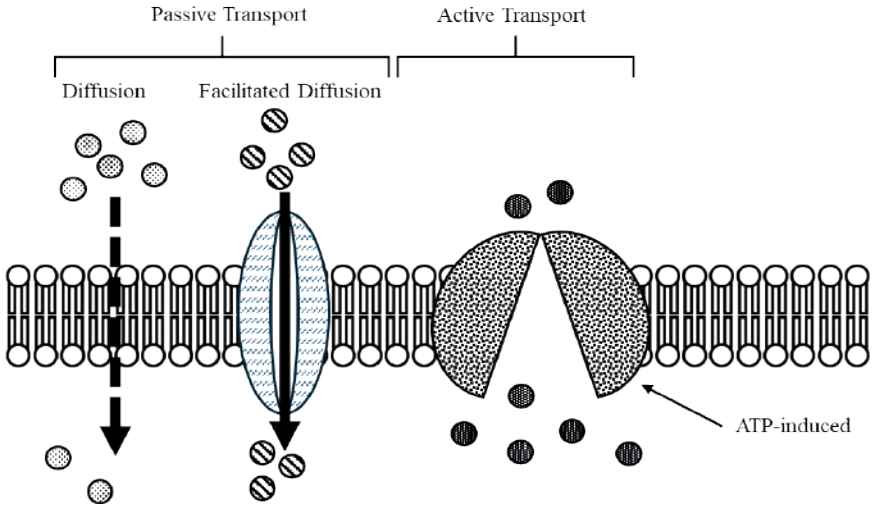

Figure 1.9. The illustration of cellular membrane and key mechanism of cellular transport.

1.5. Clinical Pharmacology

Clinical pharmacology applies various biology-based assays to evaluate the effects of drug activity on living organisms (Huang, Lertora et al. 2012). In clinical studies, the efficacy and reliability of a drug are assessed through the profiling of its metabolism in specific body fluid samples, along with

the biochemical changes observed in these samples. NMR methodology, similar to the NMR metabolomics approach, serves as an auxiliary tool for monitoring clinical phase studies and determining both the activity and dosage limits of a drug, whether qualitatively or quantitatively (Bydder, et al. 1982, Buonanno, et al. 1983, Garcia, et al. 2017).

Another notable application of NMR methods is in the analysis of clinical samples during repurposing studies. These studies enable the identification of new indications for approved drugs. In this context, NMR is utilized to investigate structure-activity relationships and to conduct drug-target binding titrations in clinical samples (Khan, et al. 2023, Rahman, et al. 2023, Atif, et al. 2024).

2. Joint (Combined) NMR Spectroscopy Data on Multi-method Pharmaceutical Research

The NMR method is commonly used in pharmaceutical research because it provides high-quality data from a single viewpoint. Improvements in NMR technology and the creation of new probes make it even more effective. However, using only NMR spectra does not provide a complete picture for pharmaceutical research. In fact, as highlighted in the previous section, more sophisticated and detailed chemical and biological testing setups are required across various research areas. Consequently, it becomes evident that multi-method approaches in NMR are more advantageous than single-method techniques, as they improve the validation of NMR data and facilitate a meaningful assessment of biological responses in model organisms or cells (Dijkstra, et al. 1989, Wang and Jardetzky 2002, Paul, et al. 2018).

Particularly for target biomolecules with high molecular weights, NMR spectra can yield complex results. To obtain clearer insights, diverse spectroscopic and chromatographic methods are employed, especially in studies focusing on drug structure-activity relationships. The challenge of molecular weight also brings forth issues related to the homogeneity and solubility of liquid-phase samples, which are addressed using various physicochemical techniques. Furthermore, the necessity for using multiple methods arises in relation to cellular function. In research involving chemotherapeutic approaches focused on targeted cellular mechanism, it is essential to relate cellular metabolite information with biological indicators like cell viability and growth. Moreover, assessing the drug's effects directly on the organism through in vivo strategies is essential for comprehensive evaluation.

Currently, numerous drug studies have effectively integrated NMR methods into their research frameworks. As a result, NMR has established itself in the field of pharmaceutical sciences not only as a "gold standard" but also as a "complementary methodology" applicable at nearly every stage of drug development and in various innovative treatments (Kotar, et al. 2020). In many advanced studies, NMR data has been successfully combined with other foundational methods that underpin the research (Figure 1.10).

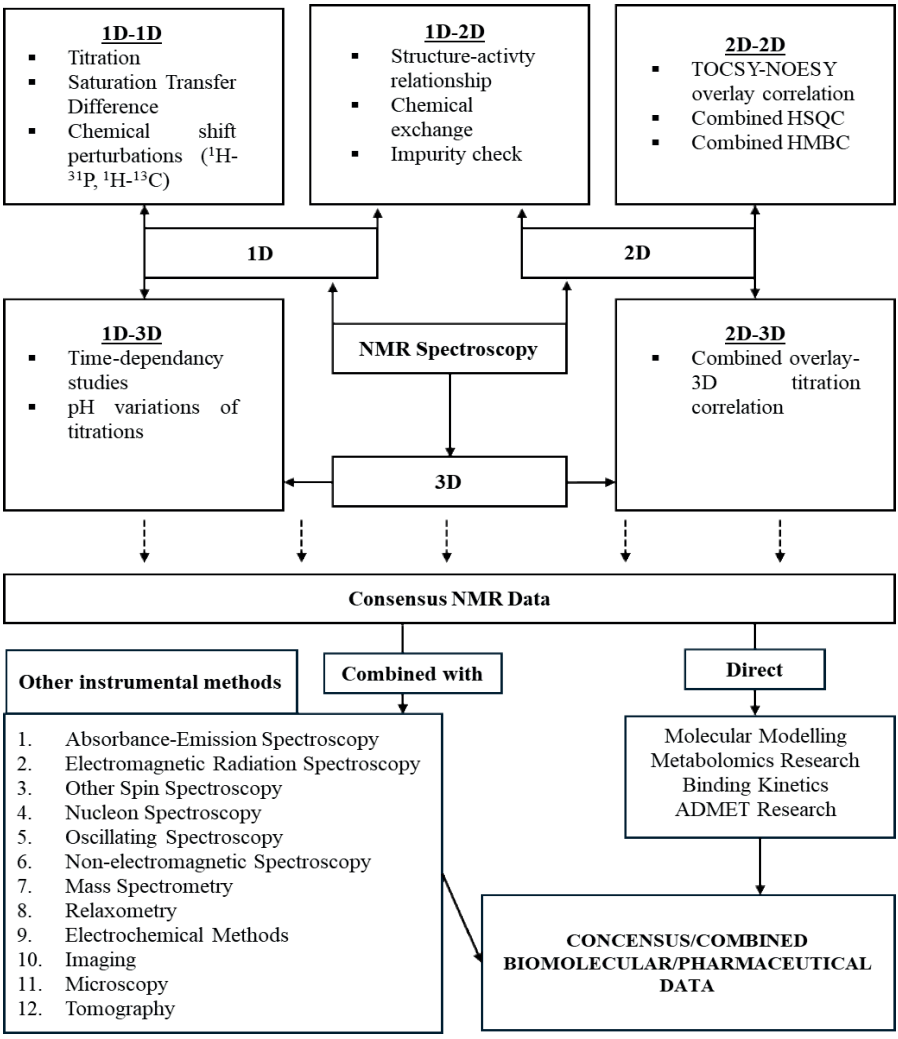

Figure 1.10. The schematic illustration of combined methologies of NMR. The data can be combined from various NMR techniques, and also combined with other external experimental methdologies (Topcu and Ulubelen 2007, Malet-Martino and Holzgrabe 2011, Farag, et al. 2012, Guennec, et al. 2014, Maggio, et al. 2014, Thamizhanban, et al. 2016, Lansdown, et al. 2019)

2.1. NMR combined with other spectroscopic approaches

Concentration-based pharmaceutical studies are primarily conducted using absorbance-emission spectroscopy. A key aspect of these studies is the validation of quantitative drug values obtained by integrating the absorbance spectrum with the quantitative NMR (qNMR) method. Additionally, diffusion-based membrane permeation studies for drugs are performed utilizing diffusion NMR spectroscopy. In this context, qNMR data is combined with the findings from these diffusion NMR studies.

Infrared spectroscopy (IR) has been a key technique for characterizing chemical structures and examining various chemical processes for quite some time (Günzler and Gremlich 2002). It is often used in conjunction with other methods. This technique is primarily utilized for the determination, detection, and analysis of small molecule drug formulations. For many years, it has played a crucial role in structure characterization and chemical structure determination when coupled with NMR spectroscopy. Today, IR spectroscopy remains widely utilized in pharmaceutical sciences across various applications wherever IR technology is accessible, frequently in tandem with NMR technology (Brittain, et al. 1993). The main reason is that both methods are very effective, especially in determining functional groups found in small-molecule drugs. The other is that IR spectroscopy, like one-dimensional NMR, is accessible and easy to apply.

Raman spectroscopy applications, particularly concerning Raman-tagged drug candidates synthesized through specific tagging techniques, are valuable in pharmaceutical sciences (Vankeirsbilck, et al. 2002). This approach evaluates drug efficacy, distribution, and solubility using Raman spectroscopy. Unlike NMR, which does not require an alkyne tag, Raman spectroscopy is instrumental in studying drug interactions and cellular entry. The necessity of using an alkyne tag can present challenges in analyzing NMR resonances that include alkyne groups. Nevertheless, the in vitro applications of both Raman and NMR spectroscopies are highly compatible, especially when the two techniques are employed in conjunction (Trefi, et al. 2008).

Mass spectrometry is one of the most effective methods used with NMR in determining the profile of target-ligand interactions (Loos, et al. 2016). NMR and mass spectrometry provide practical information at the molecular level in determining chemical and structural changes. Mass spectrometry, which is used in the pharmaceutical field by mapping drug and target complexes in detail and revealing mass changes of possible interactions, is also used to utilise synthesis monitoring in drug chemistry effectively. When

the NMR and MS methods are performed using similar samples, the results are compared on the same ground; in this case, the data can be correlated. This combination data is later used in drug optimisations and in determining structure-activity analyses. Different types of mass spectrum methods (such as MALDI-TOF and ESI-MS) can be combined with advanced 2D NMR correlation techniques to obtain detailed results about the mechanism of drug activity (Letertre, et al. 2020).

2.2. NMR combined with chromatographic approaches

Chromatographic methods are frequently used in pharmaceutical research to ensure the synthesis of drug molecules, assess their purity, and observe chemical reactions (Albert 1999, Hoffman, et al. 2008). Although multiple chromatographic techniques are available, the core principle revolves around separating components in the sample according to their molecular weight. NMR spectroscopy is often used alongside techniques such as TLC, HPLC, and GC in developing small molecule drug candidates, particularly for structure elucidation and reaction monitoring. Furthermore, NMR is selected using chromatographic methods for data validation in routine quality analysis and stability studies.

Combined HPLC-NMR methods can also examine the type of salt and crystal structure of a mixture sample alongside a defined molecular weight range identified through HPLC (Lindon, et al. 2000). This technique offers important consensus data during pre-formulation research. Additionally, while HPLC is effective, it may not suffice for conformational structure analysis, making NMR spectra essential for comprehensive evaluation.

2.3. NMR combined with chemical approaches

Chemical methods have long been employed in drug research for various purposes. While these methods typically evaluate samples containing drugs from a single perspective, they are particularly effective in determining physicochemical properties. Techniques such as electrophoresis are utilized not only for the analysis of small molecule active drug substances but also for assessing large molecular weight protein-like drug targets. Key methodologies including Potentiometry, Relaxometry, Polarimetry, and Refractometry (Kucherenko and Moiseev 1999, Valckenborg, et al. 2002, Šala, et al. 2011, Fedick, et al. 2017) continue to play important roles in drug research. Although NMR methodology does not directly yield data on chirality, conformational structure, and polarity, it provides more meaningful and sustainable insights when combined with the aforementioned techniques. Electrophoresis, for instance, is employed to elucidate target-

ligand interactions by focusing on electronegativity-based properties rather than solely on chemical properties, and is often used alongside biomolecular NMR techniques.

2.4. NMR combined with molecular biology approaches

NMR is integrated with various molecular biology methods to facilitate structural analysis of biological reactions triggered by drug activity. This approach employs metabolite PCR analysis, comprehensive protein NMR examinations, and blotting methods. The shared aspect of these combined techniques is their emphasis on metabolomics research. Furthermore, ^{31}P NMR is commonly employed to monitor changes in metabolism. Additionally, quantitative NMR results complement microscopic methods, allowing quantitative NMR assessment to reinforce qualitative findings. NMR chemical fingerprint studies help generate and validate genomics and proteomics data. The primary approach is based on NMR metabolomics to identify the presence of genome and proteome species. NMR techniques can be utilized to distinguish between Genetically Modified Organisms (GMO) and non-GMO plants, animals, and their resulting by-products (Kesanakurti, et al. 2020). The genomic modifications can be traced with 1D NMR titration spectra. Also, 3D folding features of protein structures can be illuminated with 1D and 2D NMR spectra. Thus, the protein conformations and the retrospective protein synthesis on the organism can be identified (Weininger, et al. 2025).

2.5. NMR combined with clinical approaches

Magnetic Resonance Imaging (MRI) is a fundamental tool in medical imaging, commonly used in both clinical settings and research, generating large datasets across diverse institutions worldwide. NMR techniques offer additional advantages that improve MRI techniques, as both methods are based on the same fundamental principles of magnetic resonance. In translational medicine research, many clinical samples are correlated with patients' medical images, leading to advancements in therapeutic and diagnostic strategies.

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Chapter 3

Recent Advancements in DNA Gel Electrophoresis on Pharmaceutical Sciences

Bekir Caglar Celikkaya [1]

Abstract

Gel electrophoresis is a fundamental technique for the separation of charged molecules in pharmaceutical sciences, and it is widely employed in biochemistry, molecular biology, and genetics. The primary methods of electrophoresis include gel electrophoresis, zone electrophoresis, free-flow electrophoresis, and capillary electrophoresis, with gel and capillary electrophoresis being the most prevalent in biological research. In DNA electrophoresis, DNA fragments are separated based on the number of base pairs. This technique utilizes agarose gels, which facilitate the movement of DNA—characterized by a slight negative charge—toward the positive electrode at the gel's end. While the use of electrophoresis has diminished due to advancements in DNA sequencing and polymerase chain reaction (PCR) technologies, gel electrophoresis remains a valuable tool, especially for investigating the interactions of DNA with enzymes. Recent research indicates that scientists are employing agarose gel electrophoresis (AGE) to study enzyme inhibition, structure-activity relationships (SAR), binding studies, pharmaceutical formulation properties, DNA genomics and transcriptomics. Despite the prevalence of newer methodologies, gel electrophoresis continues to provide significant insights in the realm of DNA research. In this section, the recent studies of AGE will be indicated, and the role of AGE in the recent area of pharmaceutical research will be discussed to enlighten the future perspective.

1. Electrophoresis in Pharmaceutical Research

Electrophoresis is a decisive analytical technique for separating charged molecules based on their movement through a baseline in response to an applied electric field. This method is widely utilized across different preparative

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https://doi.org/10.58830/ozgur.pub1094.c4354

and analytical applications, making it fundamental in biochemistry, molecular biology, and genetics (Sambrook et al. 1989).

Electrophoretic techniques are broadly used in various scientific field. The main principle of this technique is the movement of sample ingredients from one charge end to another. The main requirement of this technique is the sample should be charged electronically for the reason of migration. Various approaches are used that assembled in four main types of; gel electrophoresis, zone electrophoresis, free-flow electrophoresis and capillary electrophoresis (Rana et al. 2023). Thes techniques are used different migration baseline and distinct purposes for the relevant studies. In biological term, gel electrophoresis and capillary electrophoresis are used mostly, compared between all electrophoresis types.

During electrophoresis, molecules such as nucleic acids (DNA and RNA) and proteins are subjected to an electric current, prompting them to migrate through a gel or another matrix. The separation primarily results from differences in size, charge, and conformation; for example, smaller molecules generally travel faster than their larger counterparts. Common types of electrophoresis include agarose gel electrophoresis for DNA and RNA, which allows researchers to isolate specific fragments for cloning or sequencing, and polyacrylamide gel electrophoresis (PAGE) for proteins, which helps analyse protein purity, structure, and molecular weight. Techniques such as capillary electrophoresis enable scientists to separate different substances with remarkable precision and speed (Ausubel 1988).

1.1. DNA Gel Electrophoresis

DNA electrophoresis is the technique that determine the DNA fractions by their base pair number. This technique is widely used for DNA studies including DNA damage, replication, restriction and ligation. It is possible to see the DNA material can migrate from (-) end to (+) end as their charge is slightly negative (phosphate backbone). This negativity helps to moving forward in the same column. Thus, the DNA fragmentations are aligned by their molecular weight (parallel to base pair numbers) (Figure 1.1.). This method can be employed either horizontal or vertical gel electrophoresis.

Although various gelling agents can be employed for electrophoresis, agarose remains the most suitable choice. Agarose gels are widely used because traditional methods, such as sucrose density gradient centrifugation, only provide an approximate estimation of DNA fragment size (Lee et al. 2012). In contrast, agarose allows DNA fragments to migrate freely through

the gel matrix, maximizing the distance travelled and enabling more accurate and indicative separation of nucleic acids (Lee et al. 2012).

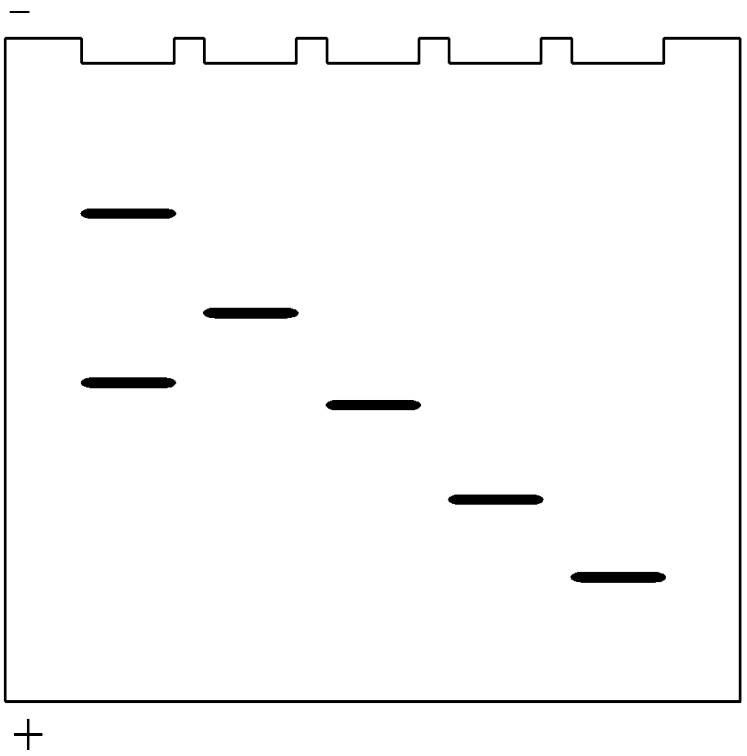

Figure 1.1. Illustration of gel electrophoresis. The DNA fragments can be shown on the gel as "bands" in separated columns.

1.2. DNA Gel Electrophoresis studies in the last decade

Although the utilization of agarose gel electrophoresis (AGE) has become increasingly limited, it remains a fundamental analytical technique in molecular biology and biochemistry. Its ability to resolve nucleic acid fragments and provide rapid, reliable results continues to make it valuable in many research settings. The electrophoretic migration of nucleic acids through agarose gels enables the separation of DNA fragments ranging from approximately 100 to 25,000 base pairs in length. Following electrophoresis, DNA bands can be visualized using UV-visible dyes that intercalate between nucleic acid bases. The widespread availability of agarose and the cost-effectiveness of its application protocol have contributed to the sustained relevance of this method, despite the advent of more sophisticated DNA-based analytical technologies (Semenov et al. 2023) .

Nevertheless, interest in electrophoretic techniques has gradually declined over the past decade, primarily due to major advances in DNA sequencing, PCR methodologies, and the integration of artificial intelligence into molecular modelling (Figure 1.2.). However, this decline reflects a methodological transition rather than a scientific retreat, as DNA-focused research-particularly within the pharmaceutical field continues to expand through the adoption of alternative analytical and computational strategies (Fonslow et al. 2009, Jaywant et al. 2024, Sowersby et al. 2024).

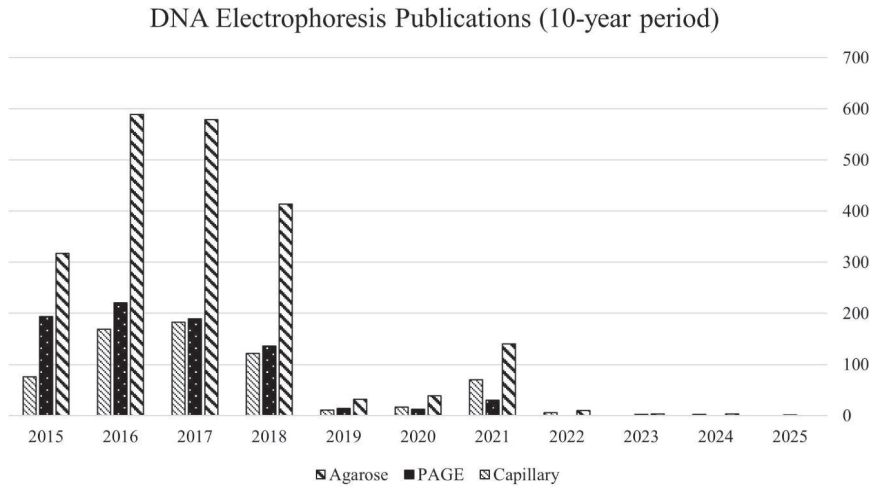

Figure 1.2. The bar chart of total publications counts of the fundamental electrophoresis techniques using with DNA-based materials (According to the PubMed Library.).

One factor contributing to the declining preference for electrophoresis is the increasing focus on short oligonucleotide studies in DNA research. Evaluating short DNA fragments with AGE presents challenges, as these sequences migrate rapidly through the gel, complicating their visualization and resolution. Nevertheless, despite these limitations, AGE continues to play a valuable role in molecular biology laboratories.

Its suitability for addressing straightforward research questions, together with its established reliability, reproducibility, and extensive troubleshooting flexibility, ensures that it remains widely used. Over the past decade, AGE has maintained relevance across several critical research areas, particularly in the analysis of nucleic acids and protein–nucleic acid interactions, where it continues to provide consistent and meaningful results (Semenov et al. 2023) (Figure 1.3.).

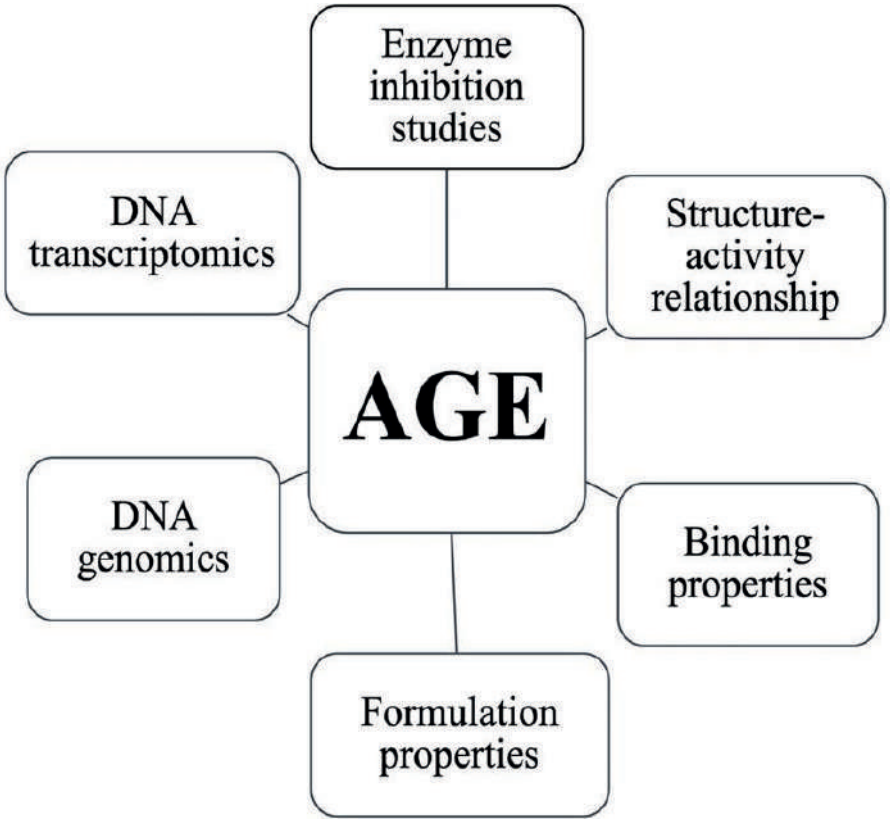

Figure 1.3. Research areas where active pharmaceutical studies have been conducted in the last 10 years.

2. Current Agarose Gel Electrophoresis methods on DNA fragment measurement

2.1. Enzyme inhibition studies

In the recent years, DNA material has been used as the model for enzyme inhibition that selected DNA-targeting enzymes using gel mobility and fraction assays. The DNA fragments clearly showed that the blockage of the functionality can be made by ligands, and it generates data about the mechanism of action for these compounds. Hence, the candidate compounds were investigated both DNA binding and functional interactions.

DNA topoisomerases are considered critical targets in the development of novel anticancer therapies. Compounds targeting these enzymes can act either by directly interacting with the DNA or by modulating the enzyme

itself. Protocols designed to evaluate the activities of topoisomerases and their inhibitors are collectively referred to as topoisomerase enzyme assays. These assays include a topoisomerase I activity test, which monitors the unwinding of supercoiled DNA; a topoisomerase II assay, which evaluates the decatenation of double-stranded DNA; and assays that measure DNA–protein covalent complexes, which represent essential intermediates in the reactions of both type I and type II topoisomerases with DNA (Osheroff et al. 1999, Nitiss et al. 2012).

As an example, tacrine–coumarin derivatives have been reported to exhibit activity against topoisomerase enzymes in lung carcinoma cells (Konkoľová et al. 2021). In that study, agarose gel electrophoresis was employed as a comprehensive assay to evaluate seven different compounds across a range of concentrations. The resulting data provided mechanistic insight into enzyme inhibition and were further supported by corresponding cell viability results. Similarly, agarose gel electrophoresis has been applied to investigate DNA–phytochemical interactions (Hsieh et al. 2020). In the present study, topoisomerase activity was assessed, and the DNA-unwinding capabilities of phytochemicals were evaluated using the different coiling forms of the pBR322 plasmid. Agarose gel electrophoresis enabled visualization of supercoiled DNA relaxation, demonstrating how ligand addition modulated topoisomerase-mediated DNA unwinding and highlighting the continued utility of AGE in functional nucleic acid research. In addition to investigating supercoiling mechanisms, other DNA-binding assays can be employed to evaluate compound–DNA interactions, such as those involving restriction endonucleases (Okumuş et al. 2022) and telomerases (Zhu et al. 2021). In these studies, electrophoretograms were used to visualize the effects of enzymatic reactions on DNA before and after treatment with the compounds. The DNA substrates varied depending on the enzyme under investigation, ranging from plasmid DNA to whole genomic DNA.

2.2. Structure-activity relationships and binding properties

Structure-activity relationships (SAR) studies have one of the greatest importance nowadays, due to identify the drug mechanism on various media. DNA-targeting treatment strategies need to be revealed the DNA binding mechanism of compounds and how resulted this binding on cellular metabolism. On the other hand, Electrophoretic Mobility Shift Assay (EMSA) studies is used to identify the binding effect of compounds on selected DNA targets. For example, the DNA stress studies can be assayed with EMSA, and revealing the immuno-modular response of DNA-binding compounds (Khan et al. 2022). Thus, compound binding induces

shifts in DNA fragments on the gel in a concentration-dependent manner. These observations are consistent with other DNA-related studies, further supporting the reliability of agarose gel electrophoresis. This demonstrates that AGE can continue to serve as a valuable tool for analyzing and explaining activity-related changes in DNA, both in current research and future applications.

There are new protocols using agarose gel electrophoresis and structural analysis were also published into a protocol book entitled "Bacterial Chromatin" (Dame 2024), which shows protocols including AGE and EMSA in current studies, focused on bacterial DNA. The application of AGE has become increasingly prominent in structure–activity relationship (SAR) studies of ligand molecules, while EMSA assays remain highly adaptable for evaluating new classes of compounds targeting diverse types of DNA. EMSA combined with AGE has also been employed for the in vitro assessment of temperature-dependent DNA binding (Hutin et al. 2024). Thus, the effect of temperature on DNA binding was revealed, and the binding kinetics were quantified using AGE. Both complexed and free DNA were visualized on the same gel under varying temperature conditions. These data provide insights not only into binding kinetics but also into the physicochemical properties of the DNA fragments, yielding results that are comparable to those obtained using other DNA-related techniques, such as PCR.

DNA binding dyes are used in multiple purposes in pharmaceutical research. In order to show the DNA fragments under the UV light, dyes are playing crucial roles. For different purposes, various DNA dyes were commercially available. To indicate the mechanism of binding of these dyes, a comprehensive EMSA assay was applied to DNA (Bawane et al. 2024). According to this assay, various dyes have been identified their identical retention profiles according to the DNA-binding capacity. The relative binding levels of dyes are differed from each other, and this will result a signature pattern in the AGE-EMSA.

2.3. Formulation properties

Drug formulation systems are used for better drug activity and lower cytotoxicity. DNA-based AGE strategies were used to test the plasmid DNA interactions of new liposomal formulations (Manturthi et al. 2022). The application involved both the free and complexed forms of the therapeutic carrier interacting with DNA, resulting in a characteristic shift on AGE that reflected complex formation. Not only drug formulation, but also vaccine

formulations have been standardized with AGE interaction studies. For deciding the best composition of viral vaccine formulation, viral capsid and nucleic acid EMSA assay were done simultaneously (Sacherl et al. 2023).

New formulation strategies are used for different purposes that targeting different biological mechanisms. Photosensitization is one of them, and this strategy promotes to reduce the cellular integrity by the light exposure. AGE study was applied in a study that developing a new formulation strategy for vitamin E photooxidation and potential DNA damage (Teychené et al. 2020). The DNA damage was profiled with AGE-EMSA in this study; hence the radical oxygen species were identified. T4 Endonuclease enzyme was treated before and after irradiation step, and the fragment response was distinct. After quantifying the fragments, the capacity of photosensitization of vitamin E was determined.

Recombinant plasmid DNAs are novel strategies for vaccines and gene therapy. Their formulations are usually in colder temperatures to keep their stability maximize. Defining the stability characteristics and formulation integrity of components, AGE used to show the potential plasmid damage (Kieu Doan et al. 2023). Consequently, optimized conditions were established to enhance the stability of these plasmid DNAs. EMSA was successfully applied, with results depending on both incubation time and the concentration of the protective polymer.

2.4. DNA Genomics and transcriptomics

Gel electrophoresis continues to contribute to genomics and metabolomics studies, such as DNA footprinting assays in diverse contexts. Although PCR has largely supplanted this method, several confirmatory gel-based assays have still been reported in recent studies. The Electrophoretic Mobility Shift Assay (EMSA) on agarose gel provides the data about the modifications on promoter regions and gene expression profiling. Anti-sense oligonucleotides were used to inhibit these transcriptional regulator and resulted the promoter region inactivity of gene expression (Numata et al. 2024). Additionally, gene mutations can be monitored using EMSA with various DNA fragments together. The mutant genes were found identical shift distance according to their genomic variations, especially on promoter regions which responsible with the transcriptomic functionality (Ausubel 1988, Gurevich et al. 2010).

Overall, these findings demonstrate that EMSA and gel electrophoresis remain versatile and reliable tools for investigating DNA-ligand interactions, assessing enzymatic activity, and monitoring genetic variations, providing valuable mechanistic and functional insights that complement modern molecular techniques.

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Chapter 4

Current Developments and Innovative Approaches in Nanoparticle-Mediated Cancer Therapies

Sumeyye Idil Celikkaya[1,2,3]

Abstract

Recently, cancer is one of the significant reasons for deaths including all diseases. Although there are many different treatment options, the deaths majorly occurred due to high toxicity or drug resistance related to the cancer chemotherapeutics. There are many studies that can reduce toxicity and drug resistance using with nanotechnology approaches in drug delivery of cancer treatments. Targetable nanotechnological molecules are designed to support protect healthy tissues from high-level cytotoxic of chemotherapeutic agents by allowing the drug to penetrate inside to the tumor cell efficiently. In addition, due to these features, they prevent drug resistance by binding to specific targets in the tumor. This will help to select cancer cells more than the healthy cells preferably. In this section, a review is made about the innovative usage of nanotechnology in cancer and future perspectives. Various material types and nanoparticulation approaches will be discussed.

1. Nanotechnology in Cancer Treatment

Cancer is defined by the uncontrolled and excessive proliferation of cells, which is followed by their dissemination throughout the body, ultimately leading to mortality (Jin et al., 2020). Cancer ranks as the second deadliest disease globally, responsible for over 10 million deaths annually. Traditional cancer therapies, including radiotherapy and chemotherapy damage to

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https://doi.org/10.58830/ozgur.pub1094.c4355

healthy tissues and the possibility of insufficient tumour reduction during surgery. In addition, the emergence of multidrug resistance in patients during treatment presents another burden. The complex character of the tumour microenvironment underlines the need to develop renewed treatment options (Bray *et al.*, 2018; Baghban *et al.*, 2020; Sun *et al.*, 2023).

Nanotechnology has occurred as a significant tool in the field of oncology, particularly for its potential in early cancer diagnosis, treatment, and overcoming therapeutic resistance. By exploiting materials at the nanoscale, researchers have designed various diagnostic instruments that allow for the detection of tumours at much earlier stages than traditional methods (Hu *et al.*, 2016). Nanotechnology encompasses subfields including drug delivery, gene therapy, diagnostics, biomarker mapping, targeted therapy, and molecular imaging (Jin *et al.*, 2020). One of the most important advantages of nanotechnology is the increase in drug side effect profiles. Many cancer drugs can also affect the healthy tissues so lead to toxicity and numerous side effects. However, nanotechnological drugs present the possible to target medication more precisely, provided that their effects are limited to tumour tissues (Hu *et al.*, 2018; Ye *et al.*, 2018).

Nano-oncology is a multidisciplinary field that integrates various disciplines, including biology, chemistry, engineering, and medicine. The goal of cancer treatment is to deliver drugs to target cells in a controlled manner. However, the process of redesigning and introducing a new active substance can be quite lengthy. Nanotechnology accelerates this process, making it faster and easier to modify existing drugs that are already effective in treatment. Additionally, nanotechnology enhances the efficacy of these drugs at lower concentrations, resulting in reduced toxicity (Alrushaid *et al.*, 2023). The most important advantage of cancer treatment using nanotechnological drugs is the ability to selectively target malignant cells. Identifying markers that are present in cancer cells but are uncommon or absent in healthy tissues simplifies the application of nanotechnology. Specific targets, such as folate receptors, transferrin, antibodies are commonly found in a variety of cancers, including breast, ovarian, brain, and lung cancers (Figure 1) (Al-Thani *et al.*, 2024).

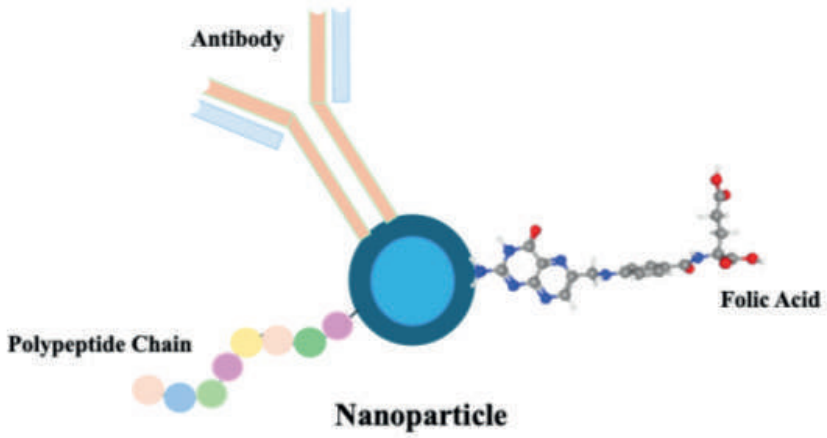

Figure 1. Demonstration of Surface Functionalization of Nanoparticles.

1.1. Nanoparticles

Nanoparticles are nano-objects that can be spherical, cylindrical, hollow, and have dimensions between 1-100 nm. They can generally consist of a single layer or several different layers. These layers can be metal ions such as gold or silver, or polymers (Machado *et al.*, 2015). Nanoparticles are basically divided into 3 classes: organic, inorganic and carbon-based. Liposomes, micelles and dendrimers are organic nanoparticles. The most prominent features of these nanoparticles are that they contain organic substances such as lipids, carbohydrates, polymers or proteins in their structures. For this reason, this group of nanoparticles is non-toxic and can be eliminated by breaking down from the body. Today, this group of nanoparticles is used for biomedical purposes such as cancer treatment (Gujrati *et al.*, 2014; Ng and Zheng, 2015; Pan and Zhong, 2016). Inorganic nanoparticles are composed of metal or ceramic. Metal nanoparticles are examined under three subheadings as monometallic, bimetallic and polymetallic. Metal nanoparticles have optical and electrical properties due to their surface plasmon properties. These properties increase their surface functionalizing. They also increase their potential for use in biomedical applications (Toshima and Yonezawa, 1998; Nascimento *et al.*, 2018). Carbon-based nanoparticles consist solely of carbon atoms and are highly biocompatible, making them used in drug delivery and tissue engineering applications (Long, Nascarella and Valberg, 2013; Ahlawat *et al.*, 2021).

1.2. Nanodiamonds

These carbon-based agents are distinguished from other nanoparticles by their very high biocompatibility (Figure 2). The nanodiamonds developed by (Chan *et al.*, 2017) have loaded with doxorubicin. They used this agent not only to target the cancer cell but also to ensure that it can enter the mitochondria of the cell directly. These nanodiamonds, which can be targeted not only to the cell but also to the mitochondria of the cell, were able to overcome resistance in resistant breast cancer cells.

Figure 2. Demonstration of nanodiamonds with different structures.

1.3. Liposomes

Liposomes, which have amphipathic properties, are vesicles consisting of a double layer and can be of different sizes. The sizes of those used for cancer usually vary between 10-200 nm. In addition to encapsulating and transporting drug molecules, their surfaces can also be coated with antibodies, aptamers or receptors (Figure 3). Recent studies on liposomes currently in use are exciting. Liposomal doxorubicin, which was developed to prevent metastasis, is promising for breast cancer patients.

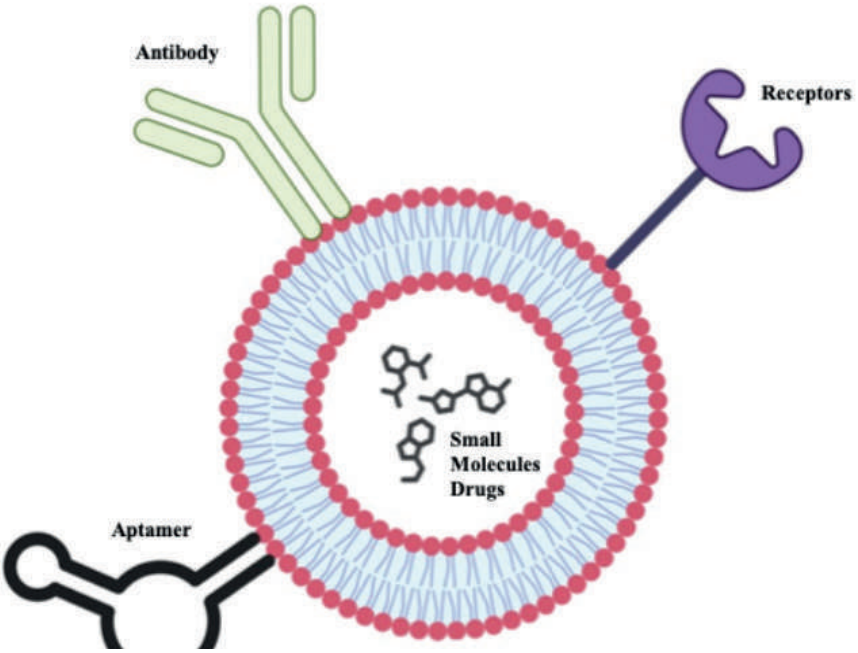

Figure 3. Functionalizing of liposomes to form drug-loaded complex.

2. Targeted Delivery

Drug targeting with nanoparticles can be divided into active and passive mechanisms. Active targeting involves using nanoparticles whose surfaces are selectively functionalized with specific agents. In contrast, passive targeting relies on the Enhanced Permeability and Retention (EPR) effect, which facilitates the accumulation of nanoparticles within tumour tissues due to leaky vasculature and impaired lymphatic drainage. EPR allows preferential localization of nanoparticles in tumour microenvironments, thereby increasing the effectiveness of drug delivery systems in oncology (Sun *et al.*, 2023).

In active targeting, drugs bind to receptors on the cancer cell surface and enter the cell by endocytosis. In passive targeting, drugs accumulate in the tumour tissue with the EPR effect. In fact, the most preferable targeting is active targeting. However, the fact that specific targets have not been discovered in some tumour tissues is the biggest obstacle to this. However, in such cases, it would be useful to take advantage of the EPR effect. For example, agents such as doxorubicin progress to brain tumours with the

EPR effect. Ultimately, both targeting aims to increase the accuracy of drug delivery and reduce toxicity (Zhang *et al.*, 2019; Rao *et al.*, 2022).

3. Nanoparticle-Mediated Synergistic Therapy

Combination therapy is the use of more than one drug together. The advantages of this treatment are to achieve the same or greater effect with a lower drug dose and to reduce side effects. Using two or more drugs affects different signalling pathways, thus preventing drug resistance (He *et al.*, 2016).

One of the advantages of nanoparticle drug delivery systems in combination therapies is that they allow multiple drug molecules to be loaded onto the same agent. In addition, they improve drug solubility, transport drugs to the target area, and protect the drug from immediate metabolism. Nanoparticles increase synergistic effects by providing more consistent pharmacokinetics of drugs. These nanoparticles can be administered orally, transdermal, or by injection. This increases patient compliance with treatment (Li *et al.*, 2024). Approved in 2017, Vyxeos is a liposomal formulation of cytarabine and daunorubicin loaded together. It has been approved for use in acute myeloid leukaemia and myelodysplasia (Krauss *et al.*, 2019).

4. Nanoparticle-Mediated Thermal Therapy

Nanoparticles designed to transmit heat to tumor cells aim to minimize damage to healthy tissues. The aim of the treatment is for the heat produced by the nanoparticles to kill cancer cells. Targetable nanoparticles heat the tissue locally. Nanoparticles that absorb light energy and convert this energy into heat at a specified wavelength are usually prepared using metals such as gold. Magnetic nanoparticles, on the other hand, bring tumor cells to a temperature where they can die when exposed to a magnetic field. Iron oxide nanoparticles are usually used in these cases. Although it may seem advantageous, it is very important for the tumor to reach the correct temperature and it is very difficult to monitor the situation (Bravo *et al.*, 2024).

Figure 4. Nanoparticle-mediated thermal therapy.

5. Personalized Nanomedicine

Personalized medicine aims to develop treatments tailored to each individual by addressing genetic and environmental factors that can affect the effectiveness and safety of treatment (Fornaguera and García-Celma, 2017).

The main goal of personalized medicine is to deliver the right drug to the right patient in the right dose. The targetability of nanoparticles makes them advantageous for personalized treatment because the dose can be easily adapted to the patient. In addition to all these, nanotechnology is also used to increase the bioavailability and binding affinity of the drug (Alghamdi *et al.*, 2022).

6. Future Perspective

As shown in Figure 5, there are many drugs approved for use in cancer treatment. Although the amount of these drugs is not large, many nanotechnological drug studies currently being studied are promising for the diagnosis and treatment of cancer. It is quite clear that nanotechnology, which offers more effective treatments with less toxicity and side effects as well as lower doses, will replace conventional treatment in the near future. In addition, drug resistance is a very big problem in cancer. Targetable treatments have been developed thanks to nanotechnology, and it is seen that resistance can be prevented thanks to these treatments (Puttasiddaiah *et al.*, 2025).

According to recent studies, new nanopharmaceuticals that elicit proptosis in cancer cells have been developed. These drugs increase selectivity

while minimizing unwanted effects and inflammation. In addition, these latest studies aim to examine and reveal the complex connections between proptosis, apoptosis and necrosis. The elucidation of these complex mechanisms with nanotechnology will bring significant success in cancer treatment in the future (Deivayanai *et al.*, 2024).

Approval (year)	Product	Nanoparticle material	Drug/Mechanism	Indication
EMA (2019)	Hensify (NBTXR3)	Hafnium oxide nanoparticle	Radiotherapy	Locally advanced soft tissue sarcoma (STS)
EMA (2019)	Pazenir	Nanoparticle-bound albumin	Paclitaxel	Metastatic breast cancer, metastatic adenocarcinoma of the pancreas, non-small cell lung cancer
FDA (2017) EMA (2018)	Vyxeos	Liposome	Cytarabine/Daunorubicin	Acute myeloid leukemia
FDA (2015)	Onivyde	Liposome	Irinotecan	Pancreatic cancer, colorectal cancer
EMA (2010, 2013)	NanoTherm	Iron oxide nanoparticles	Thermal ablation with magnetic field	Glioblastoma, prostate, and pancreatic cancer
FDA (2012)	Marqibo	Liposome	Vincristine	Acute lymphoblastic leukemia
EMA (2009)	Mepact	Liposome	Mifamurtide MTP-PE	Osteosarcoma
South Korea (2007)	Genexol-PM	PEG-PLA polymeric micelle	Paclitaxel	Breast, lung, ovarian cancer
FDA (1994, 2006)	Oncaspar	Polymer protein conjugate	Pegaspargase/L-asparaginase	Acute lymphoblastic leukemia
FDA (2005)	Abraxane	Nanoparticle-bound albumin	Paclitaxel	Breast and pancreatic cancer, non-small-cell lung cancer
FDA (1999)	DepoCyt	Liposome	Cytarabine	Neoplastic meningitis
FDA (1996)	DaunoXome	Liposome	Daunorubicin	Kaposi's sarcoma
FDA (1995, 1999, 2007), EMA (1996, 2000), Taiwan (1998)	Doxil, Caelyx, Myocet, and Lipo-Dox	Liposome	Doxorubicin	Metastatic breast cancer, ovarian cancer, Kaposi's sarcoma, multiple myeloma

Figure 5. Approved nanotechnological drugs used in cancer treatment.

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Chapter 5

Biotechnological Therapeutics: Advances and Future Outlook

Pakize Canturk[1]

Abstract

In today's world where current medical treatments have reached their peak, many highly effective drugs have been developed with various technologies and unfortunately, despite the development of sophisticated drugs, there are still incurable diseases in the world. However, due to the lack of sufficient funds on a global scale and the inability to access treatments due to the inadequacy of various health policies, many patients cannot be provided with the magnificent fountain of life called "treatment". To some extent, even the limited focus area on developing new scientific perspectives can be compatible with this sad result. The development of biotechnological drugs and their prominent applications in current treatments are increasingly receiving investment and are considered worthy of attention. In addition to the traditional production of many drugs used in treatment, technological developments inevitably change the fate of drug development. In this section, by giving a little favor, examples were presented where biotechnological drugs seem to have won the war against conventional drugs.

1. Introduction

Many conventional drugs have been developed and used in the treatment of various diseases, but some of these diseases are so challenging that since no effective drug has yet been found for their treatment, we have long since chosen to resort to various alternative drug development methods. In fact, as the treatment of incurable diseases becomes more complex, we expect more efficiency from biotechnological drug development methods.

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https://doi.org/10.58830/ozgur.pub1094.c4356

Biotechnological therapeutics can be broadly classified into recombinant protein–based therapeutics, including growth factors, hormones, enzymes, and cytokines; monoclonal antibodies (mAbs) and antibody derivatives such as humanized, fully human, and bispecific antibodies as well as antibody-drug conjugates; nucleic acid-based therapies encompassing mRNA therapeutics, siRNA and antisense oligonucleotides, and CRISPR-based platforms; cell and gene therapies including CAR-T cell therapies and gene replacement or gene-editing approaches; and vaccines developed using biotechnological methods, such as mRNA vaccines (Crommelin et al., 2013; Dey et al., 2024). Many of these biotechnological therapeutics can be considered groundbreaking applications in their own right. For example, monoclonal antibody–based therapeutics and their derivatives demonstrate how recombinant and engineered proteins can be translated into effective, rapidly deployable treatment options, particularly in disease areas where conventional pharmacological approaches are insufficient. Likewise, CRISPR-based platforms-within the broader category of nucleic acid–based and gene-editing therapies-highlight the potential of highly precise, target-specific interventions that extend the therapeutic landscape beyond traditional modalities (Huber et al., 2026; Rajewsky, 2019).

2. Current Advances in Biotechnological Therapeutics

2.1. Monoclonal Antibodies

Monoclonal antibodies (mAbs) have emerged as highly effective therapeutic agents for the treatment and management of a wide range of chronic diseases, including cancer, cardiovascular disorders, immune-mediated conditions, and neurological diseases (Colwill et al., 2025; Lee et al., 2025; Saha et al., 2025). MAbs can also target cytotoxic small molecules (e.g., ADCs) and can affect the immune system by either strengthening or suppressing it (Mould & Meibohm, 2016). Monoclonal antibodies represent one of the leading modalities in biotechnological drug development, with multiple factors influencing the design and optimization of their production processes. The development of mAb manufacturing processes must address several critical control parameters, including product purity, stability, scalability, compatibility with large-scale manufacturing protocols, and reliable raw material supply, all of which contribute substantially to overall production costs. Aggregation represents a critical degradation pathway for therapeutic proteins such as antibodies and remains a central challenge in antibody developability and formulation. Advanced molecular simulation approaches, when accurately interpreted, enable the identification of

aggregation-prone regions and physicochemical liabilities that can compromise stability. Furthermore, the increasing resolution of structure-based simulation and interaction analyses supports rational antibody engineering, facilitating improved developability profiles while enhancing target binding and functional performance. Moreover, practical limitations-such as expression system performance and cost constraints-necessitate continuous refinement of laboratory and industrial strategies to achieve efficient and optimal mAb production (Chennamsetty et al., 2009; Shukla et al., 2017).

The number of monoclonal antibodies (mAbs) has been steadily increasing in recent years; as of 2021, 118 therapeutic monoclonal antibodies (mAbs) have been approved for the European market, with over 294 mAbs reported to have been approved in total (Chiu & Gilliland, 2016; Gogesch et al., 2021). The use of monoclonal antibodies (mAbs) and other biological drugs in the treatment of many diseases is constrained by several factors, one of which is the limited incorporation of patients' genetic profiles into treatment decision-making. In this context, prioritizing therapies tailored to individual genetic characteristics has been proposed as a more effective strategy. Accordingly, pharmacogenomics holds significant potential to inform mAb dose optimization, reduce interpatient variability, and substantially influence therapeutic efficacy and drug response (Lee et al., 2025; Okusanya et al., 2025).

Monoclonal antibodies (mAbs) constitute one of the largest and most extensively utilized classes of biological therapeutics across a broad range of clinical indications. The antigen-binding sites of mAbs are formed by the variable domains of the heavy (HC) and light (LC) chains, which collectively determine antibody specificity and binding affinity. The Y-shaped constant region of the antibody, referred to as the Fc (fragment crystallizable) domain, is composed of two glycosylated heavy-chain constant domains and mediates effector functions through interactions with immune receptors and complement components. The variable (V) regions of the Fab fragment constitute a critical determinant of antigen or target recognition. The antigen-binding site is formed by the spatial convergence of six hypervariable complementarity-determining regions (CDRs), with three contributed by the heavy chain and three by the light chain, collectively defining binding specificity and affinity (Chen & Zhang, 2021; Chiu & Gilliland, 2016).

With technological advancements enabling the development of higher-level MAbs, innovations in MAbs such as antibody-drug conjugates (ADCs), fusion proteins, and other derivatives with high affinity and efficiency are

expected for the development of therapeutic monoclonal antibodies (Mould & Meibohm, 2016). In recent years, numerous studies have been conducted on the modification of mAb structures, revealing the numerous advantages that can be gained through changes in the mAb structure. mAbs used in cancer treatment are responsible for recognizing cell surface proteins in target cells. They then aim to kill target cells through multiple mechanisms involving interaction with effector Fc gamma receptors. Therefore, researchers have developed extensive and elegant modification capabilities to fine-tune Fc functions by strengthening or weakening the effector functions of mAbs (He et al., 2025; Kang & Jung, 2019).

Expanding the number of molecular targets can substantially influence both the efficacy and functional limitations of antibody constructs. In this context, the development of bispecific antibodies (BsAbs) has emerged as a successful strategy to enhance therapeutic performance. BsAbs are engineered antibodies that contain two distinct antigen-binding domains capable of recognizing either two different antigens or two separate epitopes on the same antigen, thereby enabling simultaneous modulation of multiple disease-driving pathways. Owing to their capacity for concurrent target engagement, BsAbs offer unique therapeutic advantages and have gained increasing prominence across a wide range of clinical applications (Chiu & Gilliland, 2016; Ma et al., 2021). Bispecific antibodies appear to be a highly preferable option compared to monoclonal antibodies due to their lower resistance rates under cytotoxic effects, tumor formation, and infection conditions, as they target two different antigens (Ma et al., 2021). Figure 1 illustrates the mechanism of action of a bispecific antibody in a simplified manner.

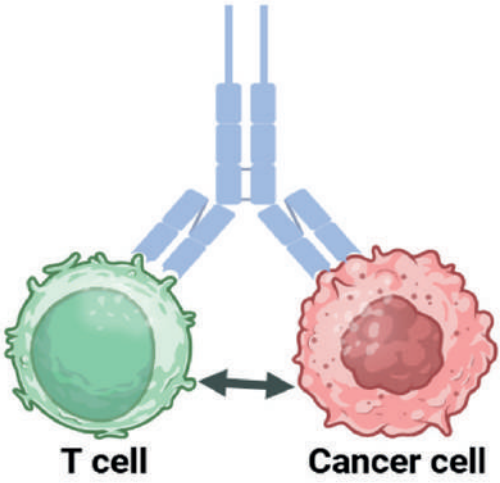

Figure 1. An example illustrating the mechanism of bispecific antibodies, specifically Catumaxomab, was adapted from Ma et al. with modifications (Ma et al., 2021). Figure created with BioRender.com

2.2. RNA-based Therapies

RNA molecules offer compelling alternatives and new treatment possibilities in the field of biotechnological drug development. The therapeutic versatility of RNA interference (RNAi) has catalyzed its application across a broad spectrum of human pathologies. Significant clinical progress is currently being made in leveraging RNAi-based platforms to target the molecular drivers of neurodegenerative disorders, infectious diseases, various malignancies, and cardiovascular diseases (Parsamanesh et al., 2024). Modern transcriptomic intervention strategies encompass a broad range of oligonucleotide-based modalities. These include RNA interference (RNAi) platforms, such as small interfering RNA (siRNA), microRNA (miRNA), and short hairpin RNA (shRNA), alongside other sophisticated approaches like antisense oligonucleotides (ASOs). While these tools differ in their intracellular processing and molecular structures, they collectively offer unprecedented precision in modulating gene expression at the post-transcriptional level (Germain et al., 2023; Liu, 2024). With these

characteristics, ribonucleic acid (RNA) therapeutics provide an effective and targeted treatment option when existing therapies suffer from target selectivity or low efficacy (Torrisi et al., 2026).

RNA interference (RNAi) is fundamental to a variety of biological processes, ranging from post-transcriptional gene regulation to the defense against RNA virus infections. A critical function of the RNAi pathway is the suppression of transposable elements (TEs)-genomic sequences capable of causing deleterious mutations if left unregulated. Dicer proteins facilitate this by identifying and cleaving TE-derived transcripts, thereby initiating gene silencing. Beyond its role in genomic stability, Dicer acts as a cellular sentinel, recognizing endogenous double-stranded RNA (dsRNA) to trigger a precise inhibitory response that maintains cellular homeostasis (Cornec & Poirier, 2023; Jadhav et al., 2024; Wang & Li, 2024). Following the early demonstration of antisense oligonucleotide–mediated viral inhibition by Stephenson and Zamecnik in 1978, RNA-based therapies gradually advanced toward clinical application. In 2018, patisiran became the first RNA interference (RNAi)-based drug to receive approval from both the FDA and the European Commission for the treatment of hereditary amyloidogenic transthyretin (hATTR) amyloidosis with polyneuropathy. This milestone was followed by the FDA approval of givosiran for adult patients with acute hepatic porphyria (AHP) (Hu et al., 2020; Torrisi et al., 2026). During the COVID-19 pandemic, RNA-based drugs and vaccines gained global prominence as effective therapeutic and preventive tools. The urgent need for rapid development and deployment underscored the potential of RNA technologies. In parallel, their versatility across multiple indications became increasingly clear, contributing to a growing pipeline of RNA-based therapeutics entering clinical development (Hu et al., 2020; Sparmann & Vogel, 2023).

Chemically synthesized siRNAs represent a highly promising therapeutic modality, characterized by their streamlined and rapid production. Beyond their ease of manufacture, these therapeutics offer a distinct mechanism of action by selectively silencing disease-associated genes at the post-transcriptional level (Ebenezer et al., 2025). The versatility of RNA-based drugs has fueled a broad expansion of their therapeutic reach. Notably, these agents can be programmed to target specific oncogenes or disrupted signaling pathways, allowing for highly individualized treatment strategies. This capability underpins the development of personalized mRNA vaccines, which are meticulously optimized based on the genetic characteristics of a patient's tumor (Hu et al., 2020; Rossi & Rossi, 2021).

Recent investigative studies highlight the clinical evaluation of Alnylam's cemdisiran and Regeneron's pozelimab as a dual-action therapy for paroxysmal nocturnal hemoglobinuria (PNH). By combining pozelimab-a monoclonal antibody-with the siRNA cemdisiran, researchers aim to target distinct components of the complement cascade. This combinatorial approach suggests a synergistic potential to modulate inflammatory responses and complement activation more robustly than monotherapy. By extension, this therapeutic synergy may prove effective in other pathologies driven by complement dysregulation, including atypical hemolytic uremic syndrome (aHUS), age-related macular degeneration (AMD), and various autoimmune disorders (Ebenezer et al., 2025).

Next-generation platforms, such as CRISPR-Cas13, are poised to revolutionize the landscape of RNA-targeted interventions by providing unprecedented precision in transcriptomic modulation. A particularly compelling frontier in this field lies in the development of combinatorial therapies. By integrating RNA-interference (RNAi) with biomaterial engineering or regenerative cell-based therapies, researchers can create synergistic platforms that address complex pathologies-such as the inhibitory environment of the injured spinal cord-through multiple therapeutic axes (Chudakova et al., 2025). Despite its immense potential, the clinical translation of RNA-based therapeutics is hindered by significant pharmacological barriers. These challenges primarily include suboptimal pharmacokinetic profiles, the inherent difficulty of achieving efficient intracellular delivery across biological membranes, and the risk of triggering adverse immune-related toxicities. Overcoming these hurdles is essential for ensuring that RNA drugs can reach their intended targets without inducing systemic inflammatory responses (Sparmann & Vogel, 2023).

2.3. Innovations in Gene Therapy; CRISPR

CRISPR-based gene therapies, leveraging the programmable nature of CRISPR-Cas (clustered regularly interspaced short palindromic repeats (CRISPR)-associated proteins) nucleases, represent a transformative approach to modern medicine. These systems facilitate precise genetic modifications-including gene disruption, the correction of pathogenic defects, or the introduction of novel cellular functions-to address a wide array of severe pathologies. However, the successful clinical adoption of these tools is contingent upon rigorous evaluations of their long-term safety profiles, immunogenic potential, and the development of high-resolution regulatory frameworks to ensure precise and predictable genetic outcomes (Banerjee et al., 2021; Huber et al., 2026; Ji et al., 2025). Originally discovered as

adaptive immune mechanisms in prokaryotes, CRISPR-Cas9 established the foundation for programmable genome editing by enabling efficient, site-specific DNA cleavage. Since its inception, the field has expanded to include over 300 characterized CRISPR-Cas systems, including the structurally and functionally distinct Cas12 and Cas13 families. The development of engineered Cas variants has further refined editing specificity, significantly reducing off-target effects. While CRISPR-based gene therapies have transitioned into clinical trials for a variety of pathologies, critical challenges-specifically regarding delivery efficiency, molecular precision, and long-term safety-must be fully addressed to ensure clinical success (Gasiunas et al., 2012; Ji et al., 2025).

The clinical success of CRISPR therapies is heavily dependent on overcoming delivery-related hurdles. While viral systems like AAV and lentivirus are widely utilized, they present a complex risk profile characterized by limited cargo size and host immune activation. A major concern remains the lack of control over the duration of Cas expression; prolonged presence of the editor can lead to cumulative off-target activity. Moreover, the threat of insertional mutagenesis-where the vector integrates into the host genome in an unintended manner-highlights the urgent need for more precise, transient delivery mechanisms (Ji et al., 2025; Saha et al., 2019). The limitations of the CRISPR-Cas9 system-namely its susceptibility to off-target effects and the challenges associated with non-specific delivery-have necessitated the diversification of the genome-editing toolkit. While Cas9 remains the dominant platform, non-nuclease technologies are gaining attraction as safer or more controlled alternatives.

Triplex-forming peptide nucleic acids (PNAs) represent a notable advancement in this category; these synthetic analogs can bind with high affinity to double-stranded DNA, creating triplex structures that stimulate site-specific gene correction without the double-stranded breaks typically required by CRISPR-based systems (Saha et al., 2019). While many current gene-editing techniques utilize donor templates to facilitate precise sequence correction, recent evidence suggests that peptide nucleic acids (PNAs) possess an intrinsic propensity to aggregate with single-stranded DNA (ssDNA) donor templates. This clustering phenomenon can manifest as false-positive signals in PCR-based readouts, potentially leading to the overestimation of editing efficiencies. Interrogating and mitigating such artifacts is critical for the rigorous development of optimized gene-editing agents (Ho et al., 2021). The advent of CRISPR-Cas9 technology has significantly transformed the landscape of epigenetics, offering a sophisticated toolkit for both genomic and epigenomic manipulation. Leveraging its inherently modular design,

Cas9 can be adapted-typically through the use of catalytically inactive 'dead' Cas9 (dCas9)-to serve as a scaffold for various epigenetic modifiers. This multi-processing capability facilitates the precise and dynamic modulation of epigenetic states within living cells. By enabling site-specific alterations to DNA methylation or histone acetylation, these tools allow researchers to interrogate the functional consequences of epigenetic regulation in real time, providing unprecedented insights into gene-regulatory networks (Pulecio et al., 2017).

Among the diverse strategies within biotechnological drug development, Chimeric Antigen Receptor (CAR) T-cell therapy has emerged as a transformative modality. While its clinical application is currently focused on lymphomas in select regions and is occasionally complicated by adverse events-specifically cytokine release syndrome (CRS) and neurotoxicity-it represents a vital therapeutic alternative for patients refractory to conventional treatments**.** The study by Stadtmauer et al. is particularly noteworthy, as it lays the groundwork for future research into CRISPR-engineered cancer immunotherapies. In this phase I clinical trial, the authors evaluated the safety and feasibility of CRISPR-Cas9-mediated gene editing in three patients with advanced malignancies. They extracted T lymphocyte cells from the patients and used the CRISPR-Cas9 system to modify three genes (TRAC, TRBC, and PDCD1) to enhance antitumor immune responses. In addition, a cancer-specific transgene, NY-ESO-1, was introduced to enable tumor recognition. The edited T cells were subsequently infused back into the patients, were well tolerated, and demonstrated sustained persistence for an approximately nine-month period (Stadtmauer et al., 2020). One of the notable factors in this study is the absence of any clinical toxicity associated with genetically engineered T cells. Bone marrow and tumor biopsies showed that T cells migrated to tumor sites in all three patients, but residual tumor tissue was detected in tumor biopsies in both myeloma patients. Nevertheless, the genetically engineered T cells were shown to be effective on their target. As summarized in Figure 2, cancer cell elimination mediated by CRISPR-edited T cells is driven by enhanced anti-tumor effector function, resulting in increased cytotoxic activity against tumor cells.

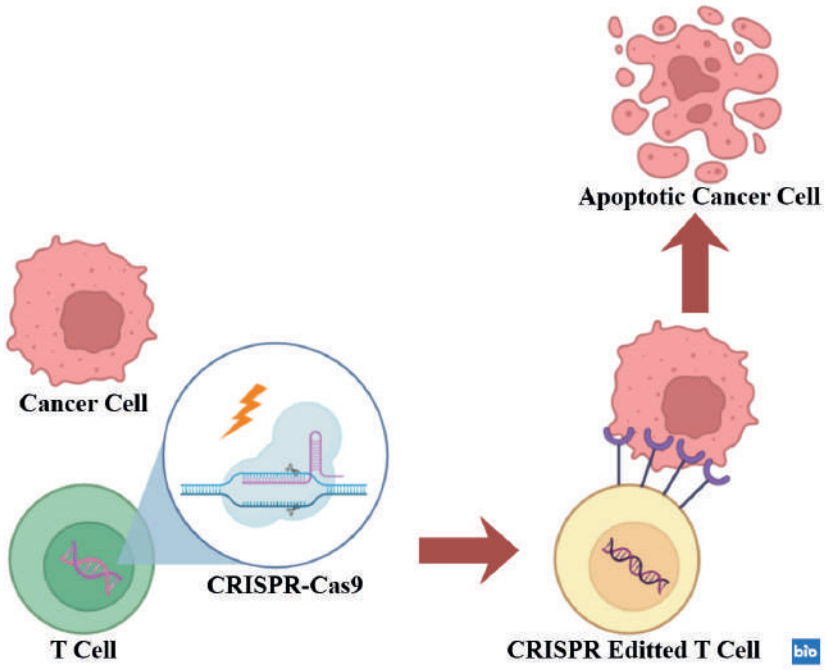

Figure 2. Schematic illustration of cancer cell elimination mediated by CRISPR-edited T cells. This approach enhances T-cell cytotoxic activity by genetically reprogramming T cells to mount an improved anti-tumor immune response. The figure was adapted and simplified from Stadtmauer et al. (2020). Figure created with BioRender.com

3. Discussion

Biotechnology-based therapeutic products have a wide range of clinical applications, including gene therapies, CAR-T cell therapies, RNA-based therapeutics, nucleic acid vaccines, recombinant proteins, and monoclonal antibodies. These modalities have achieved significant clinical success across oncology, immunological and infectious diseases, neurological disorders, and autoimmune conditions. Among biotechnological drugs, monoclonal antibodies are the first that come to mind due to their prominent applications.

Monoclonal antibodies (mAbs) are a key class of biotechnological therapeutics, with production process design shaped by multiple technical and economic factors. Limitations such as expression system efficiency and production costs require continual optimization of mAb manufacturing strategies. Detailed investigation of adverse immune-mediated drug reactions to monoclonal antibodies (MAbs) in humans is necessary; when

examining the formation of anti-drug antibodies (ADAs) in drug-treated animals, pharmacological studies designed with short timeframes yield more favorable results than toxicological analyses conducted over longer periods. Furthermore, while preclinical pharmacology studies often require animal models of a specific disease or condition, toxicology studies may not fully conform to these models, encouraging interdisciplinary collaboration (Mould & Meibohm, 2016). By targeting immune mediators including cytokines, integrins, and lymphocyte trafficking pathways, monoclonal antibodies (mAbs) have emerged as effective therapeutic agents for inflammatory bowel disease, a disorder associated with a substantial reduction in quality of life (Colwill et al., 2025). Closely related to monoclonal antibody production, biosimilar drugs have attracted considerable attention due to their potential to reduce manufacturing costs, and numerous biosimilar candidates are currently undergoing regulatory approval processes. Furthermore, the globalization of biological manufacturing enables countries to establish local production capabilities, thereby improving accessibility and supporting more sustainable healthcare systems (Chennamsetty et al., 2009; Dey et al., 2024).

RNA therapeutics are designed to modulate gene expression and protein synthesis for disease prevention and treatment. While the development of RNA-based therapeutics necessitates precise computational and molecular interaction analyses, these agents provide significant advantages in safety, efficacy, and manufacturing efficiency. Their cost-effectiveness, along with favorable storage and distribution characteristics, underscores their growing importance. Notably, RNA-based drug development occupies a unique therapeutic niche, offering a viable pathway toward personalized treatments for a myriad of diseases that have historically remained refractory to conventional interventions (Torrisi et al., 2026). Complementing these advancements, advanced drug delivery systems serve as a cornerstone for maximizing therapeutic efficacy and ensuring precise target engagement. A critical imperative in this field is the optimization of intracellular transport and the stability of biotechnological agents. This is increasingly achieved through strategic chemical modifications or the integration of sophisticated structural frameworks.

The evolution of RNA delivery is perhaps most evident in the dramatic enhancements to both potency and metabolic longevity. Recent breakthroughs have successfully reduced therapeutic requirements to the microgram level while extending drug half-lives from minutes to several months. A hallmark of this progress is the development of GalNAc-siRNA conjugates, which permit infrequent subcutaneous administration-occurring

as rarely as twice annually-and offer expanding potential for targeted delivery to the renal, central nervous, and ocular systems (Ebenezer et al., 2025). Similarly, exosomes have emerged as robust vehicles for miRNAs, acting as pivotal mediators of gene expression within the oncogenic landscape. By exporting tumor-suppressor miRNAs, these vesicles can actively facilitate tumor progression; however, current strategies to modulate this biogenesis often disrupt essential physiological functions. Through the standardization of molecular profiling, exosomal miRNAs are poised to become highly precise, non-invasive biomarkers for cancer diagnosis (Thind & Wilson, 2016).

CRISPR technologies provide unique opportunities for both preventing and treating disease by directly correcting harmful genetic mutations. By utilizing targeted gene disruption or precise correction, CRISPR can effectively stop a disease from advancing or restore a gene's natural function. Despite this potential, researchers must still overcome critical hurdles, such as off-target effects and accidental DNA changes that could lead to genomic instability. Similarly, CAR T-cell therapy faces barriers beyond its high price point. A comprehensive assessment of its long-term success and safety profile is still required. To make this treatment more accessible to the general population, it is essential to develop the technical expertise and infrastructure-the 'know-how'-necessary to scale production and reduce costs.

Addressing the remaining challenges in biotechnological therapeutics will require the accelerated development of next-generation biotherapeutics, the integration of personalized and precision biomedicine, the adoption of AI-driven drug design and protein engineering strategies, and continued advances in delivery technologies. Together, these efforts are expected to drive more effective, accessible, and durable therapeutic solutions in the coming years. Perhaps it's time to focus on some "elegant biological details" to make monoclonal antibodies (mAbs) and other biological drugs more useful in treating many diseases. Especially when considering the development of personalized medicine, pharmacogenomics seems to have the potential to change mAb dose optimization. From this perspective, improving biological therapies based on individual genetic profiles that can significantly influence drug response has become one of the priority issues to be addressed.

In conclusion, despite substantial progress in addressing many of the challenges associated with biotechnological drug development, important limitations persist, including high manufacturing costs, logistical constraints related to storage and distribution, and the need to mitigate unwanted

immune responses. Nevertheless, the consistently promising clinical and translational outcomes achieved to date underscore the transformative potential of these therapies. Continued innovation in bioprocessing, formulation, and molecular design is therefore expected to further expand their accessibility, effectiveness, and long-term clinical impact.

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Editor: Pakize Cantürk

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