EGAS MONIZ SCHOOL of HEALTH & SCIENCE | INSTITUTO UNIVERSITÁRIO EGAS MONIZ

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MESTRADO EM ANÁLISES CLÍNICAS

NANOTECHNOLOGY APPLICATIONS IN DRUG DELIVERY AND CLINICAL DIAGNOSTICS

Trabalho submetido por
Yosra Sdiri
para a obtenção do grau de Mestre em Análises Clínicas

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Prof. Doutora Ana Isabel Fernandes

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List of abbreviations

- **AgNCs:** Silver nanoclusters
- **AuNPs:** Gold nanoparticles
- **BBB:** Blood-brain barrier
- **CGM:** Continuous glucose monitoring
- **CNTs:** Carbon nanotubes
- **CPT:** Camptothecin
- **CuSNPs:** Copper sulphide nanoparticles
- **CUR:** Curcumin
- **CT:** Computed tomography
- **DGL:** Dendritic glycopeptide-lipid
- **DIC:** Disseminated intravascular coagulation
- **DOX:** Doxorubicin
- **EPR:** Enhanced permeability and retention
- **FA-BSA-AuNCs:** Folic acid-bovine serum albumin-gold nanoclusters
- **FI:** Fluorescence imaging
- **FLIM:** Confocal fluorescence lifetime imaging microscopy
- **FSNPs:** Fluorescent silica nanoparticles
- **GQD-FA:** Graphene quantum dots conjugated with folic acid
- **GQDs:** Graphene quantum dots
- **GRAS:** Generally recognized as safe
- **HES:** Hydroxyethyl starch
- **HSV-TK:** Herpes simplex virus thymidine kinase
- **IONPs:** Iron oxide nanoparticles
- **iNPs:** Immuno-nanoparticles
- **LNPs:** Lipid nanoparticles

- **LPHNPs:** Lipid-polymer hybrid nanoparticles
- **LPNs:** Lipid polymer hybrid nanoparticles
- **MNPs:** Magnetic nanoparticles
- **MOF:** Metal-organic framework
- **MRI:** Magnetic resonance imaging
- **MSNs:** Mesoporous silica nanoparticles
- **MSNPs:** Mesoporous silica nanoparticles
- **NLCs:** Solid lipid nanocarriers
- **NPs:** Nanoparticles
- **PA:** Photoacoustic imaging
- **PET:** Positron emission tomography
- **PK:** Pharmacokinetics
- **PLGA:** Polylactic acid glycolic acid
- **POx:** Polyoxazoline
- **PT:** Photoacoustic temperature
- **PTX:** Paclitaxel
- **PVP:** Poly(vinylpyrrolidone)
- **QDs:** Quantum dots
- **RONSS:** Reactive oxygen, sulphur, and nitrogen species
- **ROS:** Reactive oxygen species
- **SiNPs:** Silica nanoparticles
- **SLNs:** Solid lipid nanoparticles
- **SPIONs:** Superparamagnetic iron oxide nanoparticles
- **TME:** Tumor microenvironment
- **USINPs:** Ultrasmall iron nanoparticles

Abstract

The following work focuses on the potential of nanoparticle-based strategies to transform healthcare, namely in drug delivery and clinical diagnosis. Additionally, it investigates the design and use of nanomaterials in such applications. A variety of nanocarriers, such as inorganic nanoparticles like silica, iron oxide, and gold, and polymeric nanoparticles including polymersomes, dendrimers, and nanogels, which contribute to the enhancement of to the drug delivery techniques, are described. The potential of lipid-based nanomaterials, such as liposomes, niosomes, and solid lipid nanoparticles, to improve drug bioavailability and targeting effectiveness is also discussed. Nano-based biomedical technologies, such as monitoring systems and nanobiosensors, demonstrate improvements in precision diagnostics and real-time patient monitoring in the diagnostic field. Focus is placed on the use of nanoparticles in cancer treatment, emphasizing their efficacy in targeted therapy and in theragnostic applications, which combine therapeutic and diagnostic properties. The monography also emphasizes on the optimization techniques for the safety and effectiveness of nanoparticles, namely increased drug loading capacity, targeted delivery, and biocompatibility. Lastly, the challenges and potential prospects of nanotechnology in healthcare are examined, considering both the revolutionary possibilities and the obstacles that must be removed for wider clinical application.

Keywords: nanotechnology, nanoparticles, drug delivery, diagnostics, theragnostics

Resumo

O presente trabalho foca-se no potencial das estratégias baseadas em nanopartículas para transformar a área da saúde, nomeadamente na administração de fármacos e no diagnóstico clínico. Adicionalmente, investiga a conceção e utilização de nanomateriais para tais aplicações. Uma variedade de nanotransportadores, incluindo nanopartículas inorgânicas, como sílica, óxido de ferro e ouro, e nanopartículas poliméricas, tais como polimersomas, dendrímeros e nanogeles, que contribuem para o aperfeiçoamento das técnicas de veiculação de fármacos, é descrita. Também é discutido o potencial dos nanomateriais à base de lipídios, como lipossomas, niossomas e nanopartículas de lípidos sólidos, para melhorar a biodisponibilidade e a eficácia da vetorização de fármacos para os alvos específicos. As tecnologias biomédicas baseadas em nanotecnologia, como os sistemas de monitorização e os nanobiossensores, demonstram avanços na precisão diagnóstica e na monitorização em tempo real dos pacientes na área do diagnóstico. Dá-se uma atenção especial à utilização de nanopartículas no tratamento do cancro, enfatizando a sua eficácia em terapias direcionadas e em aplicações teranósticas, que combinam propriedades terapêuticas e diagnósticas. A monografia também aborda as técnicas de otimização para a segurança e eficácia das nanopartículas, nomeadamente o aumento da capacidade de transporte de fármacos, o direcionamento e a biocompatibilidade. Finalmente, são analisados os desafios e as perspetivas futuras da aplicação da nanotecnologia na saúde, considerando tanto as possibilidades revolucionárias quanto os obstáculos que precisam ser superados para uma aplicação clínica mais ampla.

Palavras-chave: nanotecnologia, nanopartículas, veiculação de fármacos, diagnóstico, *theragnostics*.

A. Nanomaterials and carriers utilized in drug delivery systems and clinical diagnostics

Several life-threatening diseases remain without any effective treatment or cure and only symptomatic and pain management are possible. These diseases have caused a great deal of suffering and financial loss to the patients and to the overall health care systems. In addition, the increasing mortality rates caused by antibiotic resistance and super bugs have urged the scientific community to seek alternatives to the conventional medicines, thus the high emergence of developments in nanotechnology and the nanomedicine fields.

Nanomedicine is based on the use of nanoparticles (NPs) ranging from 1 to 1000 nm in size (mishra et al. 2023), categorized into 2 groups based on the nature of their constituents and physiochemical properties, namely inorganic NPs like carbon nanotubes, silicon, copper, and gold, as well as organic carriers such as liposomes and polymer-based nanomaterials." (Marin-Silva et al., 2023; Pérez-Herrero & Fernández-Medarde, 2015).

Drug delivery remains one of the most important domains where nanoparticles are employed to solve the numerous shortcomings of conventional medicines. Many drugs are characterized by a poor bioavailability, insolubility and high toxicity causing a great deal of side effects to patients and reducing the efficacy of the treatment. Antineoplastic drugs employed in cancer treatment are an example of such drugs, lacking selectivity and precise drug delivery to the tumoral cells; the high doses thus required cause distress to the healthy cells and in time multidrug resistance may be observed (Mishra et al., 2023).

Another disadvantage of standard pharmaceutics is the risk of being degraded in the case of cytosolic drugs via the endocytic pathways or metabolized before it even reaches its target through first pass metabolism (Mishra et al., 2023). On the other hand, nano- based drug delivery systems have numerous advantages namely an increased bioavailability, biocompatibility and efficacy, enhanced drug circulation time and a reduced recognition by the immune system in breast cancer, for instances (Mirza & Karim, 2021).

Thanks to their small size, nanoparticles of 20 nm escape endocytic degradation or other types of degradation such as hydrolytic, enzymatic and oxidative degradation, pH-sensitive degradation, reductive degradation, thermal and mechanical degradation (Mohammapdour & Ghandehari, 2021) which guarantees an uninterrupted release of drugs. Certain nanoparticles

can even reach the central nervous system (Baskin et al., 2021). In cancer, the precise cell targeting and cellular pathways of NPs allows the avoidance of drug entering non tumoral cells and delivery of an increased drug payload thus reducing the administered doses and costs (Gyanani et al., 2021). More recently, some nanocarriers have been developed to include a therapeutic, as well as a diagnostic approach, referred to as theragnostics (Nagaraju et al., 2021).

1. Nanoparticle-based drug delivery systems

1.1. Polymeric nanoparticles

1.1.1. Polymersomes

Polymersomes are artificial nanosized vesicles made from amphiphilic block copolymers structured in a single layer membrane (unilamellar) allowing a better control of the internal and external flux into and out of the vesicles. Polymersomes based drug delivery systems are also characterized by a colloidal state maintaining their structure and do not break down easily. Due to their thick bilayer, polymersomes have a limited penetration keeping the encapsulated drug protected from interactions with the external environment until reaching the target site. In addition, the bilayer thickness allows them to carry a larger or more complex drug molecule, guarantees a higher encapsulation and a better adaptability (Aibani et al., 2020). Polymersomes can be engineered to carry hydrophobic and hydrophilic drugs due to their amphiphilic polymeric structure (Anajafi & Mallik, 2015) for co-delivery in combination therapy. An unconventional example is the concurrent delivery of paclitaxel and doxorubicin – a hydrophobic-hydrophilic drug pair – encapsulated within specialized co-delivery polymersomes that are targeted using folate. This targeted combination therapy yielded significantly better outcomes compared to non-targeted co-delivery polymersomes (Zhu et al., 2017).

Techniques such as nanoprecipitation, film rehydration, electroformation and emulsification, amongst many others, can be used to create polymersomes that respond to changes in pH, temperature, magnetic fields, enzymes, light, changes in redox potential, etc. (A. K. Sharma et al., 2020).

Several diseases and pathological states implicate the production of higher levels of reactive oxygen, sulphur and nitrogen species (RONSS). RONSS can be utilized in the creation of activatable theragnostic nanomaterials, serving as triggers or stimuli. By carefully designing spacer chemistry, ROS-responsive polymersomes can be developed with minimal side effects, such as reduced heart toxicity and weight loss, compared to the administration of free Doxorubicin (DOX). It has been suggested that selective nanomedicines responsive to the tumour microenvironment (TME) can be used to create ROS-responsive polymersomes, which have significant potential for treating diseases associated with high ROS levels, including

aging, diabetes, cardiovascular diseases, obesity, and chronic inflammation (Deng et al., 2017; Jäger et al., 2020).

1.1.2. Dendrimers

Dendrimers are polymeric drug delivery systems characterized by precisely defined size, shape, molecular weight, and uniformity (monodispersity). They were first introduced by Vogtle in 1978 (Mishra et al., 2023). Thanks to their unique physicochemical properties and structural design, dendrimers offer prolonged drug effects and enhanced adaptability, making them highly applicable in cancer treatment. Their potential for targeted drug delivery is significant, owing to the high density of chemical groups, available on their surface for derivatization, relative to their molecular volume. Their spherical shape, nanoscale size, uniform distribution, lipophilic nature, extensive branching points, and ability to penetrate cell walls make them ideal candidates for drug delivery systems. While their toxicity poses challenges in biological applications, ligand coating can expand their use across various applications. Additionally, their capacity to encapsulate or conjugate with both hydrophobic and hydrophilic molecules of high molecular weight support their role as effective drug delivery agents (Kannan et al., 2014; Wei et al., 2021). The use of dendrimers for therapeutic delivery is limited by regulatory concerns, but one suggested solution is that the toxicity associated with cationic dendrimers can be mitigated through PEGylation (Tripathy & Das, 2013).

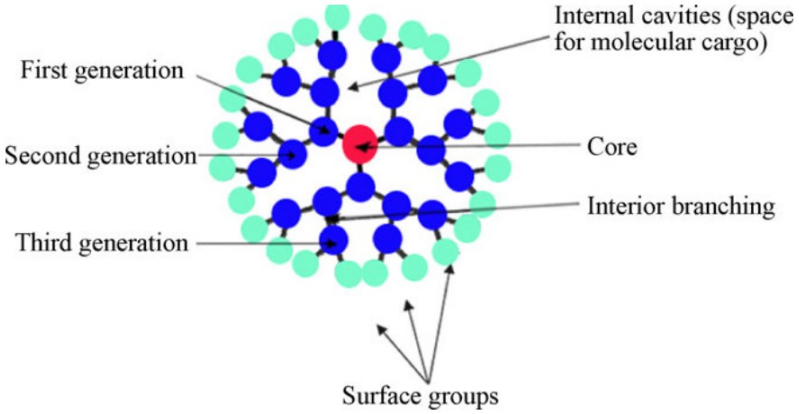

Figure 1. General structure of dendrimers (Jain et al., 2010)

1.1.3. Polymer micelles

Polymeric micelles are of small size (under 100 nm), which allows a slow clearance from the body via the kidneys and their accumulation in tumour tissues because of the Enhanced Permeability and Retention (EPR) effect (Figure 2). They are made from amphiphilic block copolymers, which not only form micelles but are also useful in creating other nanostructures for drug delivery. These copolymers self-assemble into micelles in an aqueous environment, with the hydrophobic parts forming the core and the hydrophilic parts forming the outer shell. These properties make polymeric micelles effective for delivering drugs to tumour sites, enhancing treatment efficacy while minimizing side effects (Mishra et al., 2023).

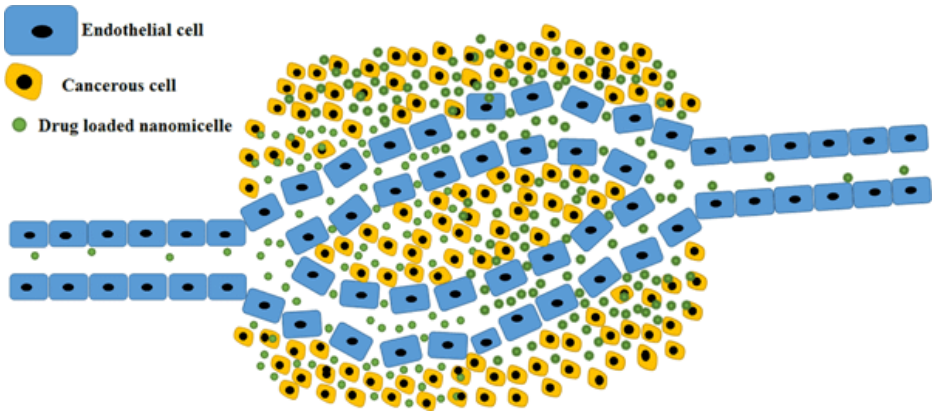

Figure 2. Illustration of the Enhanced Permeability and Retention (EPR) effect. (Patel & Thakore, 2018).

Polymeric micelles, though costly due to the need for high-purity monomers, complex synthesis, and challenges in drug loading, stability, biosafety, and functionalization, exhibit remarkable anticancer effectiveness, particularly when delivering gemcitabine. A novel micelle formulation using PEG-PCL-Ptyr polymers shows significant promise in delivering 10-hydroxycamptothecin, a drug that can halt cancer cell growth in the S phase and trigger apoptosis. Additionally, while traditional oral and intravenous treatments for respiratory diseases have been hampered by side effects and poor drug delivery to the lungs, polymeric micelles offer a more effective alternative, reducing side effects, improving drug solubility, and enabling administration via inhalation (Y. Guo et al., 2021; Pham et al., 2021)

1.1.4. Nanogels

Nanogels, often termed the next-generation drug delivery systems, are a type of three-dimensional hydrogels with sizes ranging from approximately 1 to 1000 nanometres (Mishra et al., 2023). Nanogels are widely utilized in drug delivery due to their stability, flexibility, and responsiveness to changes in the external environment. They consist of swellable hydrophilic polymer networks interconnected by various bonds, including covalent, hydrogen bonds, and van der Waals interactions. Thanks to their biocompatibility, nanogels are suitable for theragnostic applications combining both therapy and imaging. Additionally, their sensitivity to stimuli can be tailored by selecting specific polymers and crosslinkers (Neamtu et al., 2017; Pham et al., 2021).

Nanogels have the capacity to carry substantial drug payloads and retain significant water content, allowing targeted delivery of therapeutic agents to cancer tissues. By utilizing the pH disparity between tumours and normal tissues where normal tissues have a higher pH – pH-sensitive nanogels have been engineered to target and release anticancer drugs specifically within tumours. Additionally, nanogels created with emulsions such as hyaluronic acid and polyethyleneimine have proven highly effective in delivering DOX to ovarian cancer cells (Z. Li et al., 2021; Limiti et al., 2022).

1.1.5. Nanospheres

Nanospheres are spherical structures with a solid core (Figure 3). In these matrix-type nanodevices, drugs can be uniformly distributed within the core or applied to the surface, allowing the drug to diffuse into the target tissue. The advantages of nanospheres include their capacity to carry a large amount of poorly soluble drugs, as well as their ability to provide sustained and targeted release of therapeutic agents. However, nanospheres tend to show lower drug uptake in intracellular environments but release drugs more quickly than nanorods, highlighting the impact of shape on drug delivery and release within cells (Pada et al., 2019). A novel drug delivery system was developed using doxorubicin as a model drug, featuring hollow nanospheres encapsulated with polyacrylic acid that responds to both pH and temperature. This system demonstrated enhanced drug loading capacity and no cytotoxicity. Such drug delivery systems hold significant promise for cancer treatment therapies (K. Zhang et al., 2021). Nanomaterials have the potential to address challenges such as bacterial

resistance, the need for high drug doses, and low bioavailability in the eye, making them also suitable for ocular drug delivery. For instance, chitosan-cyclodextrin nanospheres were developed to effectively deliver the drug levofloxacin in collyrium (De Gaetano et al., 2021).

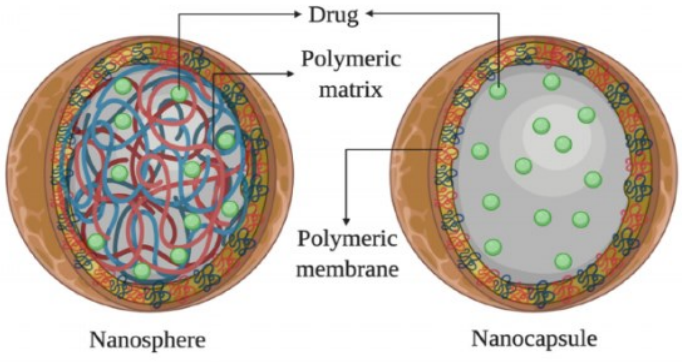

Figure 3. Schematic representation of the distinct morphology of a nanosphere and a nanocapsule (Baldim et al., 2020)

1.1.6. Nanocapsules

Nanocapsules are characterized by a core region, which may either be void or filled, enveloped by a shell-forming material that encases the internal structure (Bazylińska & Wilk, 2017). The core–shell architecture enhances the versatility of nanocapsules, thereby rendering them particularly advantageous; these nanoparticles facilitate the encapsulation of molecules possessing diverse physicochemical characteristics and existing in various physical states (solid, liquid, or semi-solid). The polymeric shell serves to prolong the lifespan of the encapsulated entities by safeguarding them from detrimental environmental conditions such as fluctuations in temperature and pH, exposure to light radiation, and enzymatic activity (Lima, Gratieri, Cunha-Filho, & Gelfuso, 2022).

1.2. Inorganic nanoparticles

1.2.1. Silica nanoparticles

Silica nanoparticles (SiNPs) are valued in drug delivery for their biocompatibility, extensive surface area, and ability to be easily functionalized. These nanoparticles are available in several forms: non-porous, mesoporous, hollow mesoporous, and core-shell silica, with or without surface modifications. Mesoporous SiNPs (MSNs) are favoured for their adjustable internal structures, biocompatibility, and ease of functionalization, and have a high capacity for drug loading. Additionally, they are effective in delivering antimicrobial drugs, helping to minimize side effects (Selvarajan et al., 2020a).

According to Wang et al.2014 SNs are used in both controlled and directed drug delivery, where they can facilitate either rapid or sustained drug release. MSNs are also employed in bioimaging and as bioactive materials for tissue regeneration. In cancer therapy, MSNs are particularly effective as drug delivery systems due to the excellent stability of their colloids, allowing them to carry multifunctional drugs. Additionally, therapeutic molecules can be attached to the surface of MSNs through surface functionalization using various additives. Depending on the need, the drug release can be triggered by external or internal stimuli (Bharti et al., 2015; Selvarajan et al., 2020b) MSNs carrying pH-sensitive lipids and coated with polyacrylic acid were employed to deliver two anticancer drugs – arsenic trioxide and paclitaxel – simultaneously. This approach enhanced the overall therapeutic effectiveness by extending circulation time and improving targeting to tumour sites (Vallet-Regí et al., 2017). The production of SiNPs can be costly when using synthetic organic precursors, which are not only expensive but also non-biocompatible and potentially polluting. However, aligning with the principles of a circular economy to promote sustainability, SiNPs can also be produced using agricultural residues such as corn cobs, coffee and rice husks, wheat husks, and sugarcane bagasse. Despite this, synthetic precursors remain more commonly used in SiNP fabrication. Notably, the inclusion of SiNPs in face masks has been shown to enhance their effectiveness in preventing SARS-CoV-2 infection (Prabha et al., 2020).

1.2.2. Quantum dots

Quantum dots (QDs), which range in size from 1 to 20 nanometres, are zero-dimensional nanomaterials with a radius comparable to that of a Bohr exciton. They exhibit a broad absorption spectrum along with exceptional brightness and durability, making them suitable for various applications due to the quantum confinement effect, particularly in disease detection and monitoring drug metabolism in the body. Graphene Quantum Dots (GQDs), a significant class of carbon-based nanomaterials, are gaining importance in fields like tissue engineering, stem cell research, and drug delivery. GQDs offer lower toxicity and improved biocompatibility compared to quantum dots made from selenium, tellurium, or metal sulphides. Additionally, both doped and pure GQDs can be produced using either constructive or reductive approaches through environmentally friendly synthesis methods (Biswas et al., 2021; Ghahramani & Javanmardi, 2021).

GQDs can target drugs across the blood-brain barrier and into tumours, offering potential solutions to the therapeutic challenges associated with treating conditions like Parkinson's and Alzheimer's diseases (Henna & Pramod, 2020). Wang et al. (2014) developed a nanoassembly composed of GQDs conjugated with folic acid (GQD-FA) for the delivery of the anticancer drug DOX. Their research demonstrated that this nano assembly effectively delivered the drug specifically to cancer cells, avoiding non-cancerous cells, and ensuring efficient drug delivery. The DOX-GQD-FA system successfully targeted HeLa cells, and the stable fluorescence emitted by the GQDs allowed for real-time monitoring of doxorubicin's release and targeting (X. Wang et al., 2014). Recently, biocompatible MXene-based QDs have become increasingly popular in cancer theragnostics. These materials, with the general formula $M_{n+1}X_nT_x$, are composed of transition metal carbides, carbonitrides, and nitrides, featuring two-dimensional planar structures. MXene QDs have distinct surface terminations and a wealth of functional groups, making them suitable for use as nanodrugs and in biomedical applications, including as biological sensors, in bioimaging, theragnostics, and photothermal drug delivery (H. Huang et al., 2022; Tareen et al., 2022).

1.2.3. Iron oxide nanoparticles

Drug delivery using iron oxide nanoparticles (IONPs) is facilitated by magnets, which help enhance the drug's effectiveness under physiological conditions. The appeal of magnetic

nanoparticles lies in their ability to be directed by a magnetic field, coupled with their biocompatibility and the easy modification of their surfaces to attach drug molecules. These nanoparticles control drug delivery by adsorbing and releasing the drug in a regulated manner (Mishra et al., 2023). IONPs linked with anticancer drugs can be precisely directed to gliosarcoma tumours using magnetic fields, ensuring that the drugs accumulate at the tumour site. This accumulation has been successfully measured using MRI. However, a common issue with bare IONPs is their tendency to aggregate, which can interfere with effective drug delivery. To overcome this challenge, IONPs are often coated with stabilizing agents (Mishra.,2023). According to Nedyalkova et al. (2021), superconducting magnets, localized magnet implants, and magnetic stents can produce the necessary magnetic field gradients for effective in vivo drug delivery using superparamagnetic iron oxide nanoparticles (IONPs). Therefore, it can be asserted that superparamagnetic IONPs are at the forefront of cancer treatment technologies. They offer exceptional theragnostic potential, allowing for both targeted drug delivery and real-time imaging (Nedyalkova et al., 2021)

The delivery of the anticancer drug 5-aminolevulinic acid was investigated using carbon nanotubes (CNTs), carboxyl-functionalized CNTs, and IONPs. The study found that drug delivery using functionalized CNTs and IONPs was effective, with functionalization occurring through hydrogen bonds and van der Waals interactions. Additionally, the in-situ synthesis of active cisplatin II resulted in prolonged death of ovarian cancer cells when IONPs loaded with cis-diamminetetrachloroplatinum IV were used for targeting (Gutiérrez-Romero et al., 2021).

1.2.4. Gold nanoparticles

The surface of gold nanoparticles (AuNPs) can be functionalized with various therapeutic ligands. They are also useful in photothermal therapy, anti-angiogenic therapy, and radiotherapy. Their biocompatibility, low cytotoxicity, and unique optical properties make them particularly well-suited for applications in cancer theragnostics (Nejati et al., 2021; Yafout et al., 2021). AuNPs are quickly absorbed due to their small size and stability. Additionally, their fabrication process is straightforward and reproducible (Lacroce et al., 2021). AuNPs can be integrated with thermoresponsive hydrogels, which release the drug when exposed to an appropriate temperature stimulus. Having an average size of around 5 nm AuNPs can create temporary and reversible pores in the stratum corneum. This property allows protein drug-AuNP combinations to be used for needle-free, self-administered transcutaneous

vaccination. In malignant tumours, AuNPs can facilitate drug delivery due to their distinctive physicochemical and photonic properties. For instance, the anticancer drug methotrexate was successfully loaded onto gelatine-coated AuNPs to specifically target MCF-7 breast cancer cell lines (Khodashenas et al., 2021; Lacroce et al., 2021). In addition to their drug delivery capabilities, AuNPs can also exhibit inherent cytotoxicity against cancer cells (Jha et al., 2018).

1.3. Lipid based nanomaterials for drug delivery

Lipid-based platforms for drug delivery are among the most prevalent types of FDA-approved nanomedicines. lipid nanoparticles LNPs are mainly divided into several subgroups namely liposomes, niosomes, solid lipid nanoparticles, nanostructured lipid carriers, lipid polymer hybrid and nanoemulsions (Mehta et al.,2023).

LNPs are unique due to their ability to form structures like micelles within their core, and their morphology can be adjusted based on synthesis and formulation conditions. Structurally, they are spherical, uniformly sized, and stable, with enhanced cellular penetration efficiency and positive zeta potentials. However, their use as drug carriers is somewhat limited because they have a reduced drug payload, often being taken up by the liver and spleen. Despite this, LNPs have significant potential in gene therapy, effectively delivering nucleic acids. Notably, LNPs are neutral at pH 7 but become acidic in endosomes, facilitating cellular delivery by promoting endosomal escape (Basha et al., 2021; Patel et al., 2019; Vhora et al., 2019).

LNPs hold significant promise as drug delivery systems due to their ability to carry drugs across difficult barriers, such as the BBB and plasma membrane. They offer extended pharmacokinetics and reduced side effects, making them suitable for delivering siRNA, CRISPR complexes, and even vaccines in liposomal forms (Fan et al., 2021).

Additionally, they offer excellent biocompatibility, straightforward fabrication, easy scalability, and targeted delivery with minimal toxicity. They are suitable for both oral and parenteral delivery routes and are FDA-approved with GRAS status. Pharmacokinetic fluctuations, particularly those affecting the absorption of Erlotinib HCl for treating metastatic lung cancer, have been a challenge due to its poor oral bioavailability. This issue was addressed and improved by utilizing solid lipid nanoparticles for its delivery (Rampaka et al., 2021).

1.3.1. Liposomes

Liposomes and micellar structures, formed from amphiphilic molecules like surfactants, phospholipids, and block co-polymers, are highly effective drug delivery agents due to their properties such as biodegradability, bioavailability, and biological compatibility, as well as their ability to enhance patient compliance. Liposomes (Fig. 4a) are structurally classified as either unilamellar or multilamellar, and their stability can be tailored both in laboratory settings and within living organisms. This adjustment is achieved by managing aspects such as nanoparticle surface charge, size, lipid composition, the quantity of lamellae, and the addition of ligand-based surface modifications. Stability can be further improved through PEGylation, with PEGylated liposomes showing increased anti-cancer effects, particularly in targeting drug-resistant mammary gland and ovarian cancers with DOX. Liposomes are versatile, capable of simultaneously entrapping both hydrophilic and lipophilic molecules, which makes them highly adaptable for various drug delivery needs. Their ability to degrade easily within the body at physiological pH, while encapsulating hydrophilic agents in their aqueous core and lipophilic drugs in their lipid layers, further enhances their effectiveness as drug delivery systems (Edis et al., 2021; Hare et al., 2017; Osorno et al., 2021; Sarfraz et al., 2018) .The antigen-antibody-dependent cytotoxicity to cells was significantly increased when DOX was encapsulated in liposomes conjugated with antihuman-CD71 monoclonal antibodies. For the treatment of B chronic lymphocytic leukaemia, anti-CD37 monoclonal antibodies conjugated to liposomes were used, offering a potentially more effective approach for personalized nanomedicines in treating B cell malignancies (Mishra et al., 2023).

1.3.2. Niosomes

Niosomes are another type of vesicle-based drug delivery system, characterized by their spherical shape and bilayer structure. They are created through the organization of non-ionic surfactants in a water-based environment, with cholesterol being a key component of their membrane. The size of niosomes is affected by the amount of cholesterol present, the hydrophilic-lipophilic balance (HLB) of the surfactant, and the techniques used for size reduction (Nowroozi et al., 2018; Yasamineh et al., 2022).

Niosomes are favoured for drug delivery due to their stability and cost-effective preparation process. They enhance the pharmacological potential of drugs by preventing their clearance

from circulation, shielding them from the biological environment, and ensuring that their effects are limited to targeted cells only (R. Sharma et al., 2022)

Niosomes can encapsulate both hydrophilic and hydrophobic substances, enabling their use in topical and targeted drug delivery applications. By attaching specific ligands to the surface of niosomes, drugs can be directed to the appropriate receptors. Niosomes can be administered through ocular and transdermal routes and are utilized in cancer treatment (Aparajay & Dev, 2022; Witika et al., 2022).

1.3.3. Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are typically disc-shaped or flat ellipsoidal particles, ranging from 50 to 1000 nm in size. Drugs within SLNs can be in the lipid core, lipid shell, or uniformly dispersed throughout the lipid matrix. SLNs offer several technical advantages, such as protecting therapeutic agents from chemical and enzymatic breakdown, providing robust physical stability, supporting scalable production, avoiding the need for organic solvents, simplifying sterilization, and enabling the co-delivery of multiple active ingredients. Additionally, they show considerable versatility across various administration routes and are valued for their biodegradability and biocompatibility. However, SLNs also face notable technological hurdles, including limited drug-loading capacity, shorter shelf life requiring cold storage, inadequate long-term drug retention, lower drug-carrying efficiency, particle size variability, and high processing temperatures (Mehta et al.,2023).

1.3.4. Nanostructured lipid carriers

To overcome the limited drug loading capacity of other types of solid lipid nanocarriers (NLCs). Nanostructured lipid carriers were developed to integrate both solid and liquid lipids within their cores. This disordered structure guarantees a higher drug payload capacity and a more efficient drug liberation rate. The addition of ligands or polymers to NLCs enhances hydrophilic and hydrophobic drug delivery (Khan, Sharma, & Jain, 2022) RNA and pDNA loaded NLCs are an example of efficient carriers for gene therapy. NLCs have demonstrated significant potential in enhancing the bioavailability of orally administered pharmaceuticals and have been the subject of clinical investigations concerning a diverse array of therapeutic

agents, encompassing COVID-19 mRNA vaccines, anticancer and antiviral compounds, and antioxidants (Mehta et al.,2023).

1.3.5. Lipid polymer hybrid nanoparticles

Lipid polymer hybrid nanoparticles (LPNs) represent a significant category of lipid nanoparticles that amalgamate the beneficial properties of lipids and polymers for a diverse array of biomedical applications (Hadinoto, Sundaresan, & Cheow, 2013). LPNs possess polymeric cores that encapsulate therapeutic agents, complemented by lipid/lipid-PEG outer layers, which serve as a "stealth" coating to enhance *in vivo* circulation. Lipid-polymer nanoparticles (LPNs) have proven effective in encapsulating a range of pharmaceuticals, including nucleic acids, enabling prolonged drug release and improved stability. Additionally, modifying the polymer surface with functional groups increases the accuracy of drug targeting to specific cells or tissues. Due to their numerous advantages, LPNs are gaining recognition as an advanced alternative to traditional liposomal and polymer-based drug delivery systems. Their applications span areas such as targeted combination therapies, cancer gene therapy, vaccine formulation, and advanced diagnostic imaging methods. (Hadinoto et al.,2013). Notwithstanding, LPNs face certain obstacles, such as the potential toxicity associated with polymer components and challenges related to achieving uniform particle size and morphology (Mehta et al., 2023).

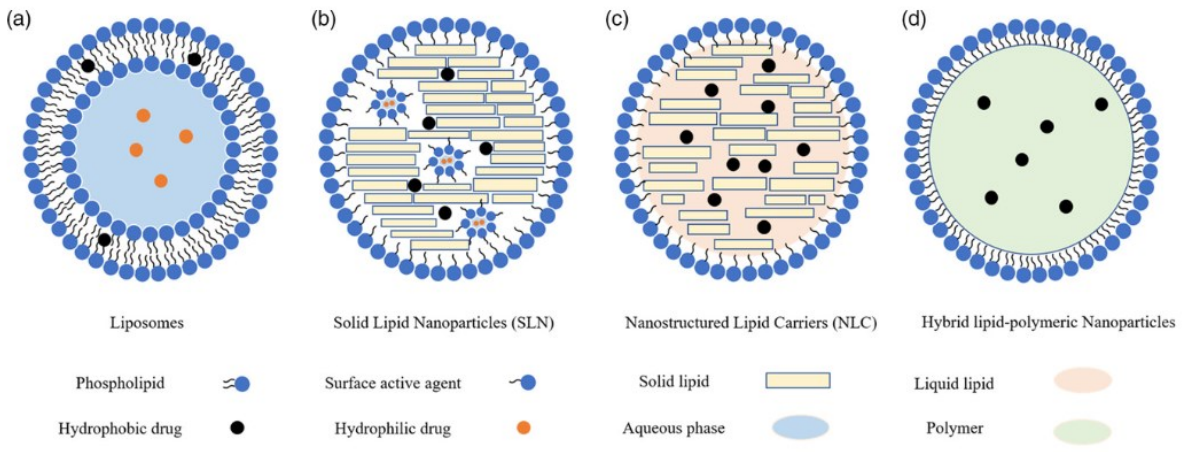

Figure 4. A schematic diagram of four different classes of lipid-based nanoparticles (Xu et al., 2022)

1.3.6 Nanoemulsions

With sizes typically ranging from 10 to 100 nm, nanoemulsions are highly effective in drug delivery. They are created by dispersing one liquid phase as droplets within another, resulting in what are known as liquid emulsions. These emulsions are stabilized by surfactants, including peptide and protein surfactants, as well as phospholipids. Nanoemulsions offer significant advantages as drug delivery systems by enabling controlled drug release, protecting drugs from degradation, and ensuring excellent bioavailability while delivering them to the intended targets (Wilson et al., 2022). Oil-in-water or water-in-oil dispersions can facilitate ocular drug delivery when these heterogeneous nano emulsions are used in combination with surfactants and co-surfactants (Choradiya & Patil, 2021). Bio-based nanoemulsions can be synthesized for the treatment of dementia and Alzheimer's, facilitating the delivery of stilbenoids and repurposing FDA-approved drugs. Additionally, a cholesterol-rich nanoemulsion was developed for delivering aluminium phthalocyanine chloride to glioblastoma in humans (D'Arrigo, 2023; Tedesco et al., 2021). Below is the scheme of a nanoemulsion general structure.

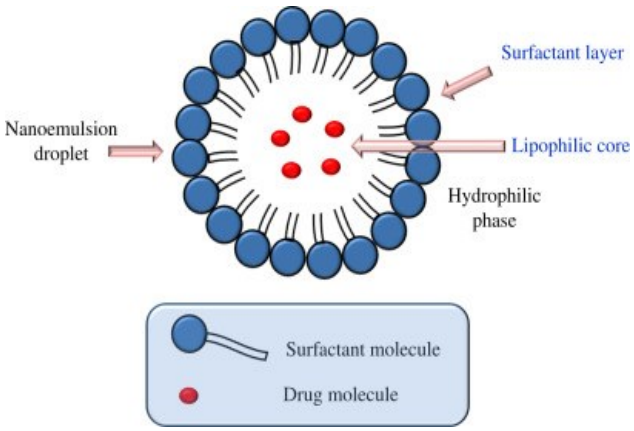

Figure 5. Nanoemulsion general structure (Patel et al., 2018)

2. Nanoparticle-based diagnosis systems

In imaging, tumour-targeting contrast agents designed to bind NP surface ligands to cancer biomarkers can enhance diagnostic precision and therapeutic monitoring. However, identifying ligands with high affinity for these biomarkers remains a challenge. NPs outperform traditional contrast agents by enabling precise molecular targeting, extending blood circulation time, and allowing controlled biological clearance, which supports prolonged imaging sessions (Bayford et al., 2017).

Superparamagnetic nanoparticles (MNPs), typically composed of an iron oxide core with a polymer or carbohydrate coating, are particularly useful in MRI applications, such as cardiovascular imaging. Their size and pharmacokinetics make them ideal for visualizing cellular and molecular processes, including arterial plaque and heart muscle damage (Tedesco et al., 2021). Similarly, metal oxide nanoparticles, like ^{18}O-enriched aluminium oxide (Al_2O_3), are effective in PET imaging, where they generate ^{18}F-labeled nanoparticles via proton irradiation reactions (Xing et al., 2014). Solid NPs have also demonstrated potential in improving ultrasound imaging in tissue phantoms and *in vivo* studies (Bayford et al., 2017).

Platinum nanoparticles (Pt-NPs) exhibit unique interactions with cellular processes, such as disrupting apoptosis and inhibiting ultrasound-induced autophagy. This inhibition enhances apoptosis by counteracting autophagy's pro-survival role (Jawaid et al., 2016). Additionally, gold nanoparticles (AuNPs) targeted with anti-Her2 antibodies significantly improve tumour imaging in breast cancer models, with active targeting proving more effective than passive approaches (Charmi et al., 2022).

Beyond imaging, nanoparticles play a critical role in infectious disease diagnosis. Fluorescent silica nanoparticles (FSNPs) enable rapid detection of pathogens like *Mycobacterium tuberculosis*, *Salmonella typhimurium*, and *Staphylococcus aureus* within hours (Qin et al., 2007; Shangguan et al., 2015; Q. Y. Wang & Kang, 2016). Metal-based NPs also facilitate advanced detection techniques, including colorimetric, fluorescence, mass spectrometry, and electrochemical assays. Surface plasmon resonance (SPR) technology, for instance, identifies DNA/RNA sequences, proteins, and infection-related analytes (Pedrosa & Baptista, 2015).

Recent innovations include quantum dot-based nanosensors for sensitive HIV detection from minimal sample volumes (El-Sayed & Kamel, 2020) and fluorescent immunosensors capable

of diagnosing *Salmonella spp.* at low concentrations using cellulose-based swabs, providing rapid and accurate results (Tang et al., 2016).

2.1. Nano-based biomedical devices

2.1.1. Nanobiosensors

A nanosensor is a device that leverages nanostructures to detect physical, chemical, or biological stimuli with high sensitivity. In biosensors, this sensitivity is enhanced by integrating biological elements such as DNA strands, antibodies, enzymes, or whole cells, which specifically bind to the target analyte (X. Huang et al., 2021).

Electrochemical nano biosensors are particularly effective, combining a biological detection element with an electrode-based transducer. Nanostructures like nanoparticles, nanotubes, and nanowires optimize the connection between the bioreceptor and the transducer, significantly improving the sensor's performance and efficiency (X. Huang et al., 2021).

These advancements have led to practical applications such as implantable glucose biosensors that automatically release insulin when glucose levels are elevated, eliminating the need for traditional injections or finger-prick testing. Subcutaneous nanosensors are also used for long-term health monitoring, such as detecting cardiac arrhythmias through an implantable EKG monitor that transmits data to a physician’s device for remote analysis (Ullah et al., 2023).

2.1.2. GlucaGen Hypo Kit

The GlucaGen Hypo Kit, a device leveraging nanotechnology, is employed to address acute hypoglycaemia in diabetic patients. This kit includes a pre-filled syringe containing glucagon, which is stored as a stable, freeze-dried powder. The powder is mixed with water using an integrated needle, and the resulting solution is administered through a subcutaneous injection (Spoială et al., 2021).

2.1.3. Abbott's Freestyle Libre

Abbott's Freestyle Libre is a continuous glucose monitoring (CGM) device that tracks glucose levels in the interstitial fluid using a small sensor attached to the skin. The sensor contains a fine filament embedded with glucose oxidase NPs, an enzyme that reacts with glucose to generate a measurable signal. Research indicates that both adults and children with type 1 diabetes, as well as adults with type 2 diabetes, can achieve effective glycaemic control using this device, whether they are on insulin therapy or not (Cao et al., 2015).

2.1.4. Arterial pressure monitoring

When data is transmitted to a wristwatch-like device, a nanopressure sensor can monitor cardiovascular system pressure. This external device then sends the information to a central remote monitoring station, allowing healthcare professionals to view the data in real-time (Tang et al.,2022).

B. Nanoparticles for cancer therapy

Chemotherapy involves using anti-cancer drugs to target and destroy rapidly dividing tumour cells. Agents like doxorubicin, gemcitabine, and cisplatin disrupt DNA replication by either inhibiting polymerases or forming crosslinks within DNA strands, impairing their function (W. Yang et al., 2016). NPs have been developed to improve chemotherapy by ensuring targeted, sustained drug delivery while minimizing side effects. These advancements extend drug circulation time and enhance therapeutic efficacy (Raheem et al., 2023).

For instance, iron oxide nanoparticles (SPIONs) loaded with Gemcitabine enable controlled release at tumour sites, effectively slowing tumour growth in pancreatic cancer models. Similarly, SPIONs carrying paclitaxel (PTX) have been used in magnetic therapy for glioblastoma, offering targeted and pH-responsive delivery with proven effectiveness in preclinical studies. Other examples include EGFRvIII-modified iron oxide nanoparticles that concentrate in brain tumours, improving treatment outcomes, and DOX-loaded silver nanoclusters (AgNCs) that enhance drug transport into glioma cells under acidic conditions. Innovations such as dendritic glycopeptide-lipid-coated magnetic nanoparticles have also demonstrated improved liver tumour targeting, highlighting the versatility of nanoparticles in chemotherapy (Raheem et al., 2023).

Radiotherapy, another key cancer treatment, uses ionizing radiation to destroy tumour cells. Nanoparticles have emerged as valuable tools in this field, enhancing radio-sensitization and enabling image-guided therapies. Metal nanoparticles with high atomic numbers, such as gold and platinum, improve radiation absorption and generate particles like Compton and Auger electrons, which increase their tumour-targeting efficiency (Jin et al., 2020; Kwatra et al., 2013).

In gene therapy, nanoparticles also show great promise. SPION-based therapeutic complexes have been used for MRI-guided silencing of the PLK1 gene, inhibiting pancreatic cancer progression. Silica nanoparticles combined with hyperthermia and the HSV-TK/ganciclovir system have enabled magnetically targeted suicide gene therapy for liver cancer (Mahajan et al., 2016; Z. Wang et al., 2018). Furthermore, lipid-polymer hybrid nanoparticles functionalized with cyclic RGD peptides have successfully delivered the CRISPR/Cas9 system across the blood-brain barrier, overcoming drug resistance in preclinical models (Q. Yang et al., 2021).

Nanoparticles are also advancing cancer immunotherapy. Immuno-nanoparticles (iNPs) conjugated with AcDEX have effectively stimulated immune responses, while lipid-protamine-DNA nanoparticles carrying pCCL2 have outperformed commercial CCL2 antibodies in enhancing immune function (Fontana et al., 2017; Liu et al., 2021). Additionally, ultrasmall iron nanoparticles functionalized with iRGD have been utilized in ferroptosis therapy, boosting immune memory and proving effective in therapeutic applications.

C. Nanoparticle-based theragnostics

Cancer remains a leading global cause of mortality, with an annual incidence of approximately 14 million new cases and nearly 8 million deaths in 2019 (Liu et al., 2019). Standard therapies such as surgery, chemotherapy, radiotherapy, immunotherapy, and hormonal treatments often cause severe side effects and may not achieve optimal outcomes.

To address these challenges, theragnostic nanoparticles (NPs) have been developed, integrating diagnostic and therapeutic capabilities within a single system. These NPs enable personalized treatment by facilitating precise drug delivery and monitoring therapeutic responses through imaging (Roma-Rodrigues et al., 2019). Various NPs, including liposomes, micelles, nanoemulsions, nanogels, and polymeric nanoparticles, offer advantages like biocompatibility, reduced toxicity, and targeted drug delivery (Maurya et al., 2019; Chenthamara et al., 2019). Functionalized NPs, such as those with transferrin, integrins, or folic acid, enhance cancer targeting by leveraging the enhanced permeability and retention (EPR) effect, which allows NPs to accumulate in tumours due to their leaky vasculature (Krais et al., 2014; Patel et al., 2018).

Theragnostic NPs serve multiple functions: they assist in diagnosing the disease, pinpointing its location, determining the stage, and monitoring treatment response. Moreover, these NPs can deliver therapeutic agents directly to the diseased area, achieving the required concentrations through molecular or external triggers (Johnson et al., 2015; Ullah et al., 2023). Beyond oncology, theragnostics are also being explored in cardiovascular diseases for imaging and treating atherosclerosis, in neurology for diagnosing and targeting neurodegenerative conditions such as Alzheimer's and Parkinson's disease, and in infections management, particularly for real-time detection and treatment of bacterial and viral infections. In each of these domains, theragnostic systems enable precise, targeted treatment while simultaneously providing critical feedback on treatment efficacy, helping to reduce side effects and improve patient outcomes (Raheem et al., 2023).

NPs also aid in overcoming biological barriers to deliver drugs directly to the TME. Common imaging techniques, including computed tomography (CT), fluorescence imaging (FI), and magnetic resonance imaging (MRI), are used to detect and monitor cancer. Contrast agents, like magnetic nanoparticles, improve imaging accuracy by distinguishing tumours from healthy tissue during MRI scans (Cai et al., 2019; Fathi Karkan et al., 2017).

As drug carriers, NPs improve solubility, extend circulation time, and allow for controlled or stimuli-responsive drug release. For instance, pH-sensitive carriers release drugs in acidic tumour environments, while temperature-responsive carriers respond to hyperthermia. Advanced formulations like glutathione-PEGylated liposomes enhance brain cancer therapy by improving blood-brain barrier penetration, as seen with methotrexate-loaded liposomes in mice (Y. Hu et al., 2019a). Gold nanoparticles (AuNPs) also facilitate targeted drug delivery and induce apoptosis in various cancer types (Y. Hu et al., 2019b; Tran et al., 2017).

Emerging techniques such as imaging biomarkers and radiomics further expand the potential of NPs in non-invasive diagnostics. NPs can detect tumour-specific biomarkers, crucial for early diagnosis. For example, nanogenosensors amplify miRNA-21 signals, a breast cancer biomarker, while gold nanoclusters (AuNCs) provide tunable photoluminescence for label-free imaging in cancers like ovarian carcinoma (Sun et al., 2022a; Salahandish et al., 2018). Silver nanoparticles (AgNCs) formed in situ also enable targeted imaging in cervical cancer using near-infrared fluorescence (Gao et al., 2014).

In imaging applications, gold nanoparticles (AuNPs) are particularly effective as CT contrast agents due to their high X-ray attenuation, while PEGylation enhances their biocompatibility and clearance. Superparamagnetic iron oxide nanoparticles (SPIONs) are valuable in MRI, offering strong magnetic properties for T2-weighted imaging. They also support photodynamic and ferroptosis therapies, allowing visualization and therapeutic monitoring (Bouché & Cormode, 2022; G. Zhang et al., 2020).

Photoacoustic imaging (PA), which uses ultrasonic waves generated by tissue photoacoustic effects, benefits from inorganic NPs like AuNPs, copper sulphide nanoparticles (CuSNPs), and silica nanoparticles. These materials enhance PA imaging due to their photothermal stability, biocompatibility, and strong light absorption, particularly in the case of AuNPs, which produce robust PA signals (Dulińska-Litewka et al., 2019; Fei et al., 2020; Chen et al., 2021).

D. Optimization techniques for enhanced efficacy and safety

While nanocarriers are showing significant promise as drug delivery vehicles and diagnostic systems, several challenges still limit their full potential. In fact, NPs are promptly eradicated by immune mechanisms and since they lack the ability to actively sense and focus on the pathological site, accumulation in the lesions remains limited. Key issues include limitations in biocompatibility, drug loading capacity, and targeting specificity. The following section will explore various optimization techniques developed to overcome these obstacles and optimize the efficacy of nanoparticle-based therapies.

1. Improving biocompatibility

Nanomaterials wrapped in cell-derived membranes have emerged as a promising avenue in Bio-inspired particle design. By using biological cell by-products like extracellular vesicles and membranes, these coatings allow nanoparticles to gain the biocompatibility and functions inherent to their source cells. This upwards engineering strategy offers new possibilities for developing therapeutic strategies. A variety of cell membranes, including those from red blood cells, immune cells, platelets, stem cells, macrophages, and cancer cells, can be utilized to coat these nanomaterials. These coated nanomaterials have been effectively used in applications like targeted therapy, vaccination, virus detection, and more (Fang et al., 2018; Tezel et al., 2021).

2. Increasing the targeting efficiency

Micro- and nano-robots integrated with biomacromolecules have emerged as tools for medical diagnosis and therapy, capable of altering shape and performing diverse functions in response to internal or external stimuli (Raza et al., 2019). For example, gold nanoparticles (AuNPs) can polymerize under UV light, increasing their size and photothermal efficiency, which is advantageous for tumour treatments (Cheng et al., 2017). DNA origami technology has enabled the development of nano-robots that precisely target tumours, release therapeutic agents, and inhibit metastasis. These robots utilize a hollow, tubular DNA structure to encapsulate thrombin molecules, which trigger localized coagulation and necrosis upon binding to tumour-associated proteins like nucleolin (N. Wang et al., 2019).

Enhancing nanoparticle (NP) targeting can be achieved by coating them with cell membranes, such as those derived from red blood cells (RBCs) or cancer cells. These coatings help nanoparticles evade immune detection and home in on specific tissues. For instance, RBC membrane-coated NPs benefit from extended circulation times due to the natural properties of RBCs, while cancer cell membrane coatings enable homotypic targeting, ensuring higher accumulation in tumour cells (Fu et al., 2015; Choi et al., 2020). Similarly, platelet membrane-coated NPs have shown promise in enhancing uptake by cancer cells while reducing macrophage-mediated clearance (Rao et al., 2017).

Membrane-coated NPs can also leverage the unique attributes of white blood cells, which actively target inflammation sites and cancer cell markers. Combining multiple membrane types, such as RBC and cancer cell membranes, further enhances their targeting and therapeutic efficiency (De Ávila et al., 2018). However, challenges like the limited availability of cell membranes, low yields during extraction, and potential immune responses must be addressed.

Bacteria also hold potential as "micro-doctors" due to their ability to autonomously navigate to tumour sites and respond to environmental cues. Hybrid systems combining bacterial propulsion with electromagnetic guidance have shown promise for precise navigation to targeted locations (Li et al., 2015).

Stimuli-responsive delivery systems enable controlled drug release triggered by specific internal or external signals. Tumour microenvironment (TME)-specific triggers, such as pH, redox gradients, and enzymatic activity, allow for localized drug release. External stimuli like light, temperature, and magnetic fields provide additional control. For instance, magnetic nanoparticles convert magnetic fields into heat, which can trigger drug release, while light-sensitive systems use specific wavelengths to activate drug release (Slimani et al., 2015; Amreddy et al., 2018).

Ultrasound is another tool for enhancing NP-mediated drug delivery. Curcumin-loaded nanodroplets exposed to ultrasound demonstrated improved growth inhibition of breast cancer cells (Baghbani & Moztarzadeh, 2017). Similarly, redox-sensitive systems using modified nanoparticles have shown enhanced retention and efficacy in tumour models compared to traditional formulations (Ye et al., 2021).

Enzyme-responsive NPs made from biodegradable polymers, such as L-tyrosine-derived poly(ester-urethane)s, enable targeted drug release within cells. These systems increase

therapeutic efficacy while reducing toxicity to healthy tissues (Aluri & Jayakannan, 2017). pH-sensitive systems also show promise in overcoming multidrug resistance (MDR) by releasing drugs in acidic tumour environments (Almeida et al., 2017).

Despite significant progress, many advanced drug delivery systems remain in the preclinical stage. Continued optimization of synthesis, scalability, and safety is essential for transitioning these technologies to clinical applications.

3. Increasing the drug loading efficiency

Optimization of nanoparticles (NPs) for enhanced drug delivery is a pivotal focus in nanomedicine research. By carefully designing NP properties such as size, shape, surface chemistry, and composition, scientists aim to maximize the effective drug load and improve targeted delivery to specific sites within the body. This optimization enhances therapeutic efficacy and minimizes side effects. Research in this area thoroughly examines the drug-carrying potential of various nanoparticle-based delivery systems, including inorganic, organic, and metal-organic framework (MOF) nanoparticles, as well as emerging carrier-free nanomedicine approaches.

Inorganic nanoparticles, particularly those with porous structures, can carry significantly more drug due to their large surface area and ample pore space. Common examples include silica, carbon, magnetic, and titanium dioxide nanoparticles. A key advantage of these inorganic carriers is their ability to enhance drug targeting (Naz et al., 2019). On the other hand, mesoporous silica nanoparticles (MSNPs) possess a robust structure with a symmetrical surface that can be readily modified. Their elongated shape allows for increased surface modification and better accommodation of drug molecules within their porous framework without disrupting the silica structure. With a large surface area and pore volume, MSNPs can hold a significant amount of drug, up to 50% of their weight (Abouaitah & Lojkowski, 2021).

Organic carriers are composed of synthetic polymers like poly(ethylene glycol) (PEG), poly(vinylpyrrolidone) (PVP), and polyoxazoline (POx), as well as natural biopolymers such as proteins, peptides, and nucleic acids. Modifying the surface of PEG-based nanocarriers is especially useful because it minimizes their interaction with plasma proteins and reduces

nonspecific uptake by the reticuloendothelial system, leading to extended circulation time. (Y. Li et al., 2019).

Poly(vinylpyrrolidone) (PVP) has gained attention as a viable alternative to poly(ethylene glycol) (PEG) due to its avoidance of accelerated blood clearance (ABC), a limitation sometimes seen with PEGylated carriers. Amphiphilic spherical polymer micelles, which are formed by the aggregation of hydrophobic segments, feature a hydrophobic core and a hydrophilic shell. This core-shell structure is highly suitable for loading hydrophobic drugs into the micelle core, allowing these drugs to be delivered efficiently in aqueous environments (Jia et al., 2021).

Natural polymers, being endogenous substances, offer superior biocompatibility and safety since they can undergo natural metabolism via physiological routes. For instance, CPT and CUR have been functionalized with 2-acetylphenylboronic acid (2-APBA) and subsequently combined with bovine serum albumin (BSA) through the formation of iminoborate bonds. This approach results in NPs with high drug loading efficiency and excellent colloidal stability (Hao et al., 2021).

The MOF is an innovative hybrid material created through the coordination of a metal core with an organic bridge segment. This integration of organic and inorganic elements offers several distinct advantages, including a large contact surface, high permeability, adjustable pore size, Ease of chemical tailoring, and biological degradation. These properties make MOFs a highly promising platform for drug delivery, allowing for high drug loading efficiency (S. Guo et al., 2020). As a drug carrier, the unique framework structure of MOFs enhances drug loading capacity. However, challenges such as potential toxicity and low biodegradability currently limit their use in clinical applications (Ma et al., 2019).

A significant challenge with carrier-based nanomedicine is the typically low drug-carrying capacity, often less than 10 wt%, which limits the effective accumulation and therapeutic impact of the drugs. Additionally, the inertness of the nanocarriers and the extensive chemical processing involved in their preparation can pose potential risks to the body (Hrdlička et al., 2018; Zhai et al., 2013).

To mitigate these issues, carrier-free nanomedicines have been developed, where the nanomaterial matrix is primarily composed of active pharmaceutical ingredients. These carrier-

free nanomedicines boast a significantly higher drug loading capacity, often exceeding 80 wt% (Shen et al., 2017; Y. Zhang et al., 2020).

Nanocrystalline medicines offer several advantages due to their lack of a carrier. These benefits include high drug loading capacity, freedom from encapsulation rate limitations, and flexibility in adjusting drug dosages. Additionally, the reduction of drugs to the nanoscale enhances the efficacy of poorly soluble drugs in aqueous environments. Furthermore, these nanocrystalline medicines can be formulated into various dosage forms such as capsules, tablets, or injection-type freeze-dried powders, making them suitable for industrial production.

E. Challenges and prospects of nanotechnology applications in healthcare

For a nanoparticle-based medicine to gain market approval, several challenges must be addressed. These include designing well-characterized nanostructures with appropriate properties, ensuring reproducible and scalable manufacturing processes, and employing robust analytical methods. Demonstrating safety and efficacy through clinical trials is also essential (Babes-Bolyai University et al., 2024).

Nanomedicines are intricate three-dimensional systems that depend on precise spatial arrangements of their components. Minor alterations in composition or manufacturing can disrupt their functionality and stability. To meet clinical and regulatory standards, these systems must remain sterile, stable during storage and use, and effective in targeting specific sites (Bernal-Chávez et al., 2021; Jain & Bhattacharya, 2023). Key physicochemical properties, such as size, surface charge, and functional groups, play a critical role in determining nanoparticle stability, immune system evasion, and targeting efficiency. Maintaining particle sizes under 200 nm is particularly important for biological applications (Rahman et al., 2023).

Manufacturing methods, whether "top-down" or "bottom-up," must ensure chemical integrity and minimize impurities. Early in-process testing is essential to maintain consistency, especially during scale-up. Additionally, maintaining sterility and structural integrity during aseptic manufacturing and sterilization processes poses significant challenges (Bernal-Chávez et al., 2021).

Safety concerns extend beyond the product itself to include environmental and immunological risks. Nanoparticles can become airborne, posing inhalation and dermal exposure hazards. Within the body, they may trigger immune responses like opsonization or complement activation, potentially leading to adverse effects such as hemolysis, thrombogenicity, or rapid clearance. Comprehensive preclinical and clinical toxicity evaluations, including immunological assessments, are necessary (Rahman et al., 2023; Yedgar et al., 2022).

Regulatory frameworks for nanomedicines are not yet standardized. Approval processes require rigorous testing of physicochemical properties, safety, and efficacy. Generic nanomedicines face additional challenges in demonstrating bioequivalence to original products (Vikram Singh et al., 2024).

Biomedical nanotechnology offers transformative potential in personalized medicine, gene therapy, regenerative medicine, and medical imaging. Advances include nanorobots for targeted drug delivery, nanoparticle-based vaccine systems, and improved contrast agents for imaging. In gene therapy, nanoparticles enhance DNA delivery and editing accuracy. Similarly, nanomaterial scaffolds support tissue regeneration, and applications in neurodegenerative diseases focus on targeted drug delivery and neuroprotection (Kang et al., 2021; Jiang et al., 2022).

These advancements highlight the vast potential of nanotechnology to revolutionize healthcare by enhancing disease diagnosis, treatment, and prevention, paving the way for innovative and effective medical solutions.

Conclusions

This monograph has thoroughly explored the diverse and rapidly evolving field of nanotechnology applications in drug delivery systems, clinical diagnostics, and therapeutic innovations. The discussion of inorganic nanoparticles, such as silica nanoparticles, quantum dots, and gold nanoparticles, highlighted their unique properties and their significant impact on drug delivery mechanisms. Similarly, lipid-based nanomaterials, including liposomes, niosomes, and lipid nanoparticles, were shown to play a critical role in enhancing the bioavailability and targeting precision of therapeutic agents.

The exploration of nanoparticle-based diagnostic systems and biomedical devices demonstrated the potential of nanotechnology to revolutionize early disease detection and monitoring. Emphasis was placed on nanobiosensors and theragnostic approaches, especially in the context of cancer treatment, where nanoparticles offer unprecedented opportunities for precise diagnosis and targeted therapy.

Moreover, the monograph delved into the optimization techniques employed in nano-based drug delivery systems, focusing on strategies to improve biocompatibility, increase targeting efficiency, and enhance drug loading capacities. These advancements are crucial for maximizing the therapeutic efficacy and safety of nanomedicine.

Finally, the discussion of challenges and prospects in nanotechnology applications in healthcare acknowledged the ongoing hurdles in nanoparticle-based therapeutics, such as scalability, regulatory concerns, and potential toxicity. However, the future of nanotechnology remains promising, with continued research and innovation likely to overcome these challenges and further integrate nanomaterials into mainstream medical practice.

In summary, this monograph underscores the transformative potential of nanotechnology in healthcare, paving the way for more effective, targeted, and personalized medical treatments. The insights and innovations discussed here not only contribute to the current understanding of nanomedicine but also set the stage for future advancements that will continue to shape the future of healthcare.

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