3

Nanoparticles as Nanomedicines: Types of Nanoparticles for Biomedical Applications

Sadia Mughal[1], Ahmed El-Malul[2,3]

[1]Department of Medicine, Bahria University Medical and Dental College (BUMDC), Bahria University, Karachi
[2]Radom University, Radom-Poland
[3]Medical Department, University of Al Zintan, Al Zintan, Libya

Outline

Introduction....37
Types of Nanoparticles....38
Metal-based nanoparticles....39
Carbon Based Nanoparticles....46
Polymer based Nanomaterials....49
Metal Organic Frameworks – MOFs (Hybrid)....53
Liposomes....55
Conclusion....56
References....57

Introduction

In the context of chemotherapy, tumor cell destruction follows first-order kinetics, implying that higher killing of cancerous cells requires higher dose concentration of the drugs [1]. However, their systematic toxicity i.e., toxicity on normal blood cells limits the administered doses. Despite systemic administration, achieving a beneficial drug concentration at the site of the target without causing toxicity on normal cells remains a challenge. Addressing this challenge, antitumor drugs coupled with nanoparticles show significant potential, resulting in reduced tumor growth and delayed drug elimination [2].

This intersection of nanotechnology and medicine, evolving since the early 20th century, has witnessed synergistic developments across diverse fields – biotechnology, cell and molecular biology, chemistry, engineering, and physics [3]. Pioneering work by Paul Ehrlich in targeted drug therapy, such as the development of Salvarsan, influenced subsequent drug synthesis with a focus on specificity [4]. Breakthroughs in biomaterials, DNA structure elucidation, and genetic code investigations provided insights into molecular mechanisms.

Nanotechnology's exploration in medicine, particularly in nanomedicines utilizing nanosized colloidal particles, has led to significant advancements [5]. Initially, naked nanoparticles, i.e., lacking surface functionalization, were researched vastly for their potency and toxicity against a range of cancer cell lines and disease-causing pathogenic bacterial strains. However, with development, drugs, and various functional biomolecules were attached to nanoparticle surfaces to achieve target specificity [6-8].

These nanoparticles, whether encapsulating or attaching drug molecules to their matrix, offer unique advantages such as safeguarding the bioactive molecules against hydrolytic degradation, and enzymatic, thereby prolonging circulation and retention time and enhancing therapeutic aids [9]. Anti-tumor drugs coupled to nanoparticles (either on the surface or trapped inside) have demonstrated extended drug retention time within tumors in contrast to pure drugs, resulting in obvious reductions in tumor progress and extended survival rates in tested animal models [10]. This approach proves promising in cancer chemotherapy, enabling target-specific drug supply to obtain better clinical outcomes and potentially minimizing the need for surgical interventions.

Administering biodegradable nanoparticles via multiple available routes, including intraperitoneal, intravenous, and intra-arterial, may guide intra-tumoral localization [11]. This targeted approach reduces systemic levels of toxic drugs, minimizing adverse side effects. Sustained drug release from nanoparticles allows for prolonged exposure to cytotoxic drugs, with lowered blood flow contributing to drug persistence time at the tumor site [8]. The use of safe and biodegradable nanoparticles facilitates repeated dosing via an in-dwelling hepatic catheter.

A study showed the tenfold retention time of paclitaxel (PTX) loaded over nanoparticles compared to Taxol, which showcases the importance and benefits of drug loading over nanoparticles [10]. The therapeutic potential of nanoparticles and their use for medicinal purposes was pioneered in the late 1980s by Yasuhiro Matsumura and Hiroshi Maeda, making them leaders in the nanomedicine field [12]. Their focus was to develop tumor-specific delivery systems using nanoparticles with the association of anti-tumor drugs since the nanoparticle had enhanced retention and permeability and deposition in the micro-environments.

Types of Nanoparticles

Diverse nanoparticles, spanning zero-valent metals like Fe, Co, Zn, to metal oxides such as FeO, NiO, and ZnO, have undergone extensive biomedical studies [13]. In an attempt to enhance their applications, bimetallic nanoparticles like NiFeO and ZnFeO were synthesized, introducing new challenges regarding their potential toxicity [14]. Despite employing various studies and biological models to predict nano-toxicity, questions persist about the behavior of nanoparticles within a living organism and its immune system. The intricate topic of toxicity will be delved into in a subsequent chapter.

Shifting focus from metal oxide nanoparticles, researchers redirected their attention to organic, carbon-based polymeric nanoparticles after exploring their biomedical applications. This transition presented a novel range of applications with reduced toxicity [7, 15]. However, concerns about nanoparticle accumulation persisted, prompting considerations for reducing nanoparticle dosage. To address these challenges and make nanoparticles more or equally toxic to cancer cell lines, surface functionalization with antibodies and drug molecules became a pivotal strategy. This approach offers two significant benefits: i) a reduced concentration of nanoparticles, mitigating the likelihood of accumulation in body organs, and ii) diminished systemic toxicity of both the nanoparticles and the loaded drug. **Table 3.1** contains information about various nanoparticle types that have been studied for their potential to replace traditional medicines.

TABLE 3.1
Types of nanomaterials or nanoscale structures that can be used for their bio-medicinal properties.

Type of Nanoparticles		Nanoparticles
Stimuli and non-stimuli responsive		
Metal-based	Zero valent	Iron, Nickel, Zinc etc.
	Oxides	Iron Oxide, Chromium Oxide, Aluminum Oxide etc.
Carbon-based		Cabron Nano Tubes, Carbon Quantum Dots, etc.
Lipid		Solid Lipid nanoparticles, drug-loaded lipid nanocarriers etc.
Polymeric		PLGA, Chitosan etc.
Metal-Organic Frameworks (MOFs)		PEGylated Iron Oxide nanoparticles
Hydrogels (nanogels)		
Liposomes		

Each type of nanostructured material is chosen based on the specific application requirements. For laboratory-based studies, naked and unfunctionalized nanoparticles are often employed. These nanoparticles serve as a fundamental and simplified form, allowing researchers to understand their basic properties and behavior. The more advanced studies involve the use of surface-modified nanoparticles/nanocarriers and animal models to mimic real-life scenarios. Surface modification/functionalization is the change in the outermost layer of a nanoparticle, that modification can be with a biomolecule, a drug molecule, a ligand, and/or an antibody, that enhances the interaction of the nanomedicines with the immune system and gives the nanoparticles a

camouflage like properties to bypass the hindrances and reach a specific target to deliver the drug. These types of modifications also result in higher bioavailability and lesser toxicity of the drug.
Whereas certain types of animal model studies may involve the use of both naked (Un-functionalized/un-modified) nanoparticles and surface-modified nanoparticles, to establish a comparative analysis. With the help of such studies and experiments, the researchers can easily establish the advantages of surface-modified nanomaterials over un-modified ones. These advantages can be enhanced bioavailability of loaded drugs, the lesser time needed by the surface-modified nanoparticles to reach the target, ease of moving around the body, and lesser toxicity.
In summary, it falls on the application and kind of the experiment if the surface-modified nanoparticles/nanocarriers/nanomedicines are required or un-modified are required.

Metal-based nanoparticles

Metal and Metal Oxide nanoparticles have been vastly employed for their use as nanomedicines, for the past many years, due to their enhanced properties and diversity. At the nanoscale, the materials exhibit remarkable properties i.e., optical, and physiochemical, which allow them to show extraordinary potential for their use of anti-bacterial, anti-viral, and other medical-based uses. This size reduction not only gives physiochemical and optical properties enhancement to the nanomaterials but also drastically changes their magnetic and other properties, which allows the use of nanoparticles from diagnosis to imaging and to their potential to treat various fatal diseases like cancer, by employing various routes of causing apoptosis inside the cells. As highlighted in Chapter 1, their diminutive size and specific shapes enable them to traverse cell membranes, causing damage to pathogenic bacteria or exposed cancerous cells.
Furthermore, metal oxides and zero-valent metal nanoparticles are the category of nanomaterials that are most employed for their potential biomedical applications. These nanoparticles may include but are not limited to Iron Oxide (IO), Iron (Fe), Nickel Oxide (NiO), Nickel (Ni), Silver (Ag), Silver Oxide (AgO), Zinc Oxide (ZnO), Zinc (Zn), and Gold (Au) nanoparticles, amongst a few examples of these type of nanoparticles. It is now a well-established fact that these nanoparticles range have the potential to treat various infectious diseases and viral diseases. These unique sets of skills of these nanoparticles have made them one of the most favorite candidates for researchers to use these metal oxide and zero valent metal nanoparticles for various medical-based applications and potentials.

Iron Oxide

Hassan et al., conducted a study on IO nanoparticles, the hematite phase, by synthesizing them with the help of plant extracts (flower) of *Callistemon viminalis*. Furthermore, the biogenic IO nanoparticles were evaluated for their physiochemical and structural properties using HR-SEM, HR-TEM, EDS, SAED, FTIR, and XRD. The group found the average diameter of IO nanoparticles to be 22 nm. Biogenic IO nanoparticles were investigated for a diverse range of biomedical applications, including toxicity against 12 pathogenic, water-borne, bacterial strains. The results showed excellent anti-bacterial potential by retaining 93% of bacteria at 1000 µg/mL concentration i.e., the highest employed concentration. furthermore, the synthesized IO nanoparticles were used to evaluate their anti-cancer toxicity against Hepatocellular carcinoma (HepG2) cell lines also known as Liver cancer

and observed ~80% inhibition against the cell lines at the highest concentration of 500 µg/mL. With that, the group also investigated the anti-leishmanial - a pathogenic disease caused by the bite of a sand fly and found them to be quite potent against the pathogen. Although the results were very promising, but still the toxicity of the nanoparticles was still in question and to evaluate this part, the group followed a Hemolytic assay, by exposing the IO nanoparticles against freshly drawn human Macrophages. The results exposed that the biogenic IO nanoparticles are quite safe to use at lower concentrations, whereas even at high concentrations, they were showing a %lysis lower than 30% [16].

Silver and Silver Oxide

Silver is known as the most antibacterial material in the world and has been broadly exploited and researched for biomedical applications [17], it is also known to contain anti-fungal and anti-viral potential and antioxidant properties. The Ag nanoparticles show enhanced antibacterial potency compared to silver ions [18]. Silver and silver oxide (Ag_2O) nanoparticles demonstrate versatility and efficacy across various fields, with notable applications in dentistry, particularly for diverse antibacterial purposes [19]. They are employed as scaffolds in tissue engineering and as materials promoting wound healing. Beyond healthcare, silver-based nanomaterials play a crucial role in a range of industries such as drinking water disinfection, food packaging, and textile manufacturing.
In a study by Alnehia, A., and group synthesized ZnO nanoparticles doped with Ag following a green approach that employed an aqueous leave extract of *Plectranthus barbatus* which acted as a reducing and stabilizing agent for the precursor salts and nanoparticles, respectively. The synthesized nanoparticles underwent comprehensive characterization through various techniques, XRD highlighted the retained hexagonal crystalline structure of the nanoparticles, in antibacterial tests conducted on *Staphylococcus aureus* and *Escherichia coli*, via the disk diffusion method using azithromycin as a control drug, to compare the nanoparticle results with, showed minimal potency against the *E. coli* while selective action was seen against *S. aureus*. The antibacterial activity against S. aureus was sought to be dependent on nanoparticle concentration and concentration of the doping agent, i.e., increased concentrations resulting in enhanced activity [20].
Another study group, led by Said, A., reported on the genesis of Ag nanoparticles using plant extract of *Lawsonia inermis* to study their anti-bacterial potential against *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus arlettae, Acinetobacter baumannii*, and *Proteus mirabilis*. The results explained the higher antibacterial potential of biogenic Ag nanoparticles with the highest activity against *A. baumannii* with a zone of inhibition of ~30mm [21]. **Figure 3.1** shows various ZOI's against respective bacterial strain for the tested Ag nanoparticles. The figure has been adopted from [21], published under open access Creative Commons (*CC*) 4.0 license.
There have been vast investigations on the antimicrobial potency mechanism of Ag nanoparticles. Silver nanoparticles have been found to physically interact with various microorganisms' surfaces [22]. Additionally, silver ions intermingle with the peptidoglycan of bacterial cell wall, inducing structural alterations that enhance permeability across the membrane, ultimately leading to cell mortality [23]. The Ag nanomaterials also retain the DNA replication by interacting with sulfhydryl groups of bacterial proteins [23]. In the case of Ag_2O nanoparticles, they interact with bacterial DNA, impairing replication and causing cell cycle arrest at the G2/M phase [24]. This DNA damage, coupled with oxidative stress, leads to the demise of the bacterial cells. The multifaceted antimicrobial

properties of silver-based nano-compounds make them valuable across a spectrum of applications, contributing to their widespread use in various industries.

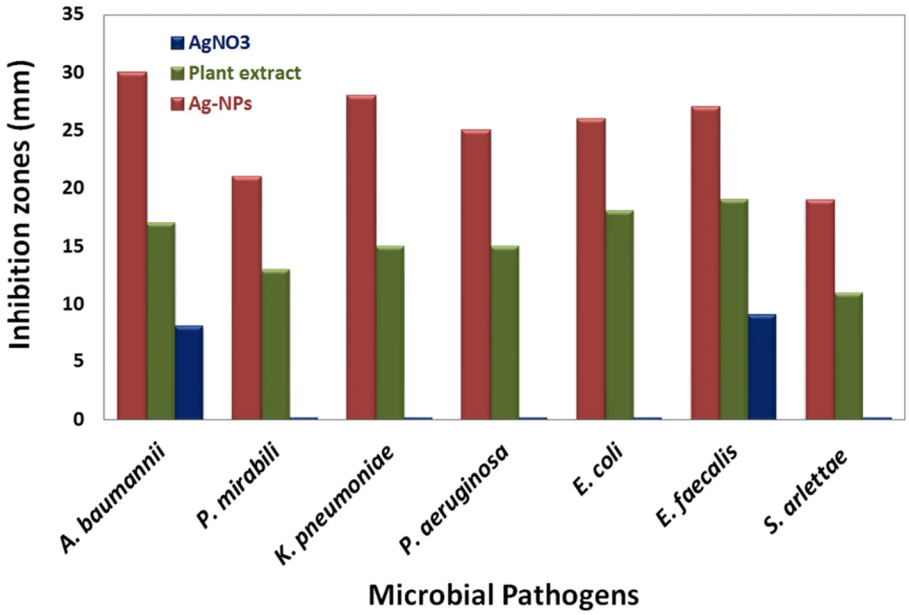

FIGURE 3.1
Antibacterial potential of Ag nanoparticles compared to Ag nitrate salt and plant extract against all bacterial strains (Figure adopted from [21], published under open access Creative Commons (*CC*) 4.0 license).

Zinc Oxide

Zinc oxide nanostructures exhibit broad-spectrum antimicrobial properties, surpassing the bactericidal activity of silver nanoparticles against *S. mutans*, particularly at a concentration of 1% w/w [25]. The proposed nanoparticles' mode of action involves the introduction of reactive oxygen species (ROS) inside the bacteria, triggering bacterial cell death [25, 26]. The Confocal microscopy suggested that ZnO nanoparticles exhibited antimicrobial activity through both ROS production and accumulation on the outer side of the cell membrane or inside the cytoplasm [27]. Further the release of ions (i.e., Zn^{2+}) and nanoparticle connection to the bacterial cell membrane induced physical damage to cell wall [28]. A research group further found that the decrease in nanoparticle size brings out more damage, resulting in enhanced potency and efficacy, although variations may occur among different microorganisms. The isoelectric points of ZnO nanoparticles and +ve surface charges under physiological situations, interact with bacteria through electrostatic forces, promoting phagocytosis, cellular uptake, and bacterial toxicity. ZnO nanoparticles inside sunscreens are known for their potency against *E. coli*, due to their potential of generating ROS, since the ZnO nanoparticles are known for enhanced ROS generation in comparison to their bulk counterparts. The applications of ZnO nanoparticles, attributed to their ROS generation property causing death in bacterial cells, allow their use beyond the cosmetics, covering food industry also, for their use as antibacterial agent

in active food packings. Moreover, they contribute to tissue regeneration and restorative dentistry, imparting antimicrobial activity and enhancing mechanical properties in these contexts [25].

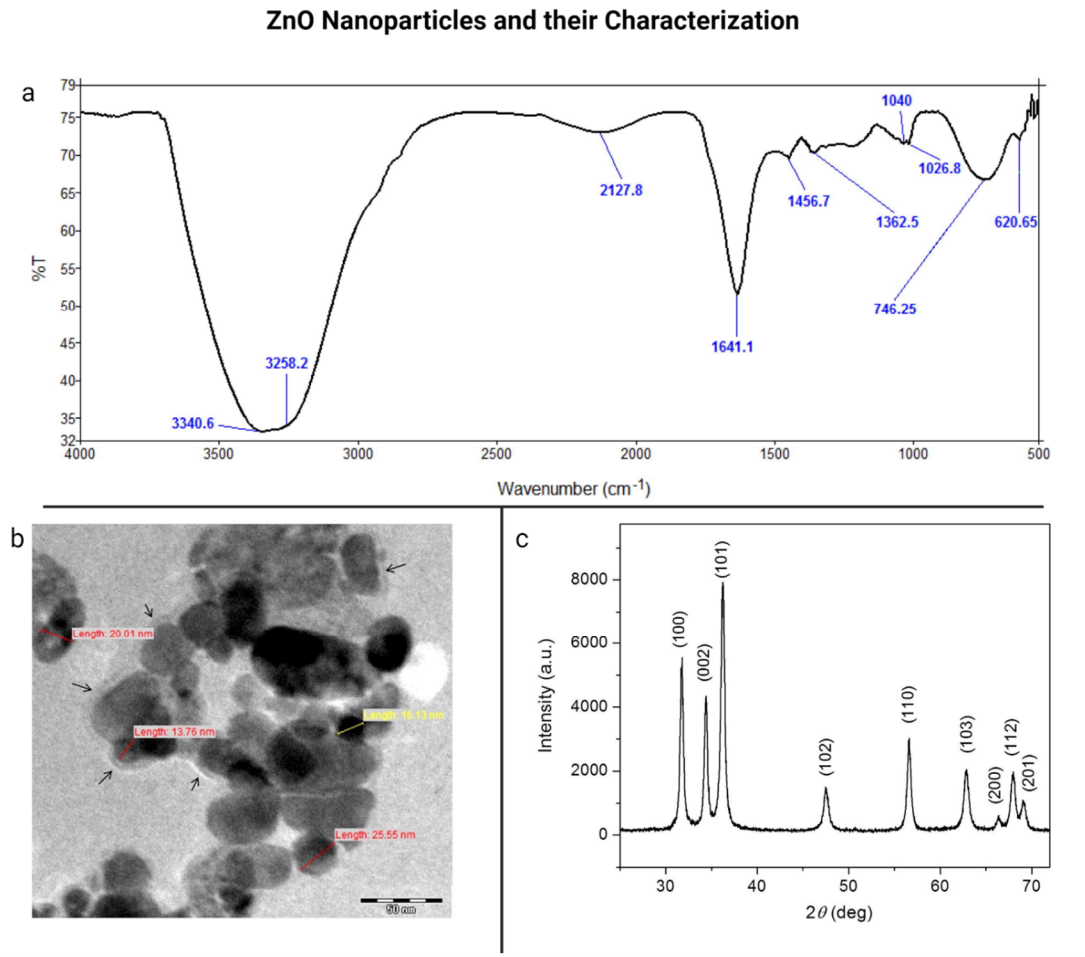

FIGURE 3.2
Characterizations of Biogenic ZnO nanoparticles. (a) FTIR spectrum (b) TEM image with possible size distribution and (c) XRD pattern for the synthesized ZnO nanoparticles. (Figure adopted from [30], published under Open Access Creative Commons (*CC*) License).

A study by S. Vijayakumar and group, successfully produced Zinc Oxide nanoparticles through the utilization of *Atalantia monophylla* plant's leaf extract. The biosynthesized nanoparticles underwent characterization using Fluorescence spectrometer (PL), and UV-Vis spectrophotometer (UV-Vis) with identified mounds at 410 and 352nm, respectively. The morphological analysis of the biogenic ZnO nanoparticles was done by employing EDAX and TEM, while purity and crystal nature of biogenic ZnO nanoparticles was investigated with employment of XRD. Additionally, the information about the functional groups responsible for synthesis, capping and stabilization of ZnO nanoparticles was

gathered with the help of FTIR spectroscopy. The antimicrobial efficacy of the synthesized ZnO nanoparticles was evaluated using the well diffusion (on agar) method against bacteria and fungi. Results implied enhanced and elevated fungal and bacterial inhibition compared to standard drugs and pure plant extract. The group established that biogenically synthesized ZnO nanoparticles have added functional groups on their surface, giving them more powerful antimicrobial potential and the group recommends them for their potential use against microbes like fungi and bacteria [29]. Furthermore, P. Jamdagni, and group followed green synthesis approach for ZnO nanoparticles was employed, utilizing the aqueous flower extract of (night flowering Jasime (*Nyctanthes arbortristis*) as the biological reduction agent. The flower extract played a crucial role in reducing zinc acetate dihydrate to synthesize the nanoparticles. Optimization of synthesis conditions aimed at achieving both maximal yield and a lower size distribution of the ZnO nanoparticles. The resulting ZnO nanoparticles underwent thorough characterization through various analytical techniques, including XRD, UV-Vis, TEM, and DLS. The biogenic ZnO nanoparticles were stored and displayed highly stable nature, even after 4 months. The size distribution of the nanoparticles, synthesized under optimal conditions, was determined to be 12–32 nm, computed with the help of TEM images. Furthermore, FTIR displayed the information of added functional groups, came from plant extract, while TEM showed crystalline nature and fringes from that crystalline nature gave away the size distribution of the nanoparticles. **Figure 3.2 (a-c)** contains characterization results for synthesized ZnO nanoparticles (Figure adopted from [30], published under Open Access Creative Commons (*CC*) License). The antifungal assessment resulted ZnO nanoparticles to be quite potent towards the test 5 fungal strains and the group explained that ZnO nanoparticles were highly toxic to fungal stains even at lower concentrations which is exemplified by the Minimum Inhibitory Concentration (MIC) recorded to be 16 µg/mL. This study highlights a straightforward and efficient green synthesis approach for antifungal ZnO nanoparticles genesis [30].

Nickel and Nickel Oxide

Nickel (Ni) and Nickel Oxide (NiO) nanoparticles have been broadly appreciated for their anti-bacterial potency against the multidrug-resistant *E. coli* and *K. pneumonia*. These nanoparticles find applications in food packaging, water purification from pathogens, medicine, and the textile industry [31]. While Ni-based nanoparticles exhibit weaker antimicrobial abilities compared to those derived from Ag and Si, they surpass Au nanoparticles in this regard [32]. Recent advancements in manufacturing and novel surface modification techniques have led to Ni nanomaterials demonstrating antibacterial properties comparable to those of Ag. To elevate the Ni and NiO nanoparticles' synthesis, owing to their extra-ordinary properties, the scientists have developed ways to shift the synthesis methods from traditional – non-environmentally friendly (i.e., chemical coprecipitation, physical, solgel etc.) to bio- and eco-friendly synthesis methods – biochemical synthesis (i.e., by employing some micro-organisms or by using a plant extract), [33] as explained in chapter 1.

Surface modification of Ni nanoparticles with polymers, such as Ni nanoparticle-loaded chitin nanogels, enhances antibacterial properties and cytocompatibility. These nanogels modified Ni nanoparticles, for instance, exhibit elevated potency at lower concentrations to bacterial strains in comparison to naked Ni NPs. These nanogel loaded Ni NPs are further regarded as non-toxic in nature, shown in their *in vitro* cytocompatibility against L929 and A549 cancerous cell lines [34]. It is

further to consider also that the source, from where polymer, metal, microbial strain and cancerous strains are collected, may influence the potency and cytocompatibility of nanoparticles [35].
Furthermore, as discussed in Chapter 1, the size, shape and dimensions of the nanoparticles may also influence the biocompatibility and cytocompatibility of the Ni and NiO nanoparticles. The form is impacted by the pace of expansion in various crystallographic orientations, managed by modifying experimental parameters like acidity, proportion of metal ion to reducing agent, exposure duration, and manufacturing power. Exploring the utilization of nickel hydroxide nanoparticles or nickel oxide nanoplates, providing greater diversity in shape and dimensions, presents an alternative for enhancement. For instance, innovative platinum-on-flower-like Ni nanoparticles, induced by ultraviolet irradiation to accumulate ROS, exhibit enhanced antibacterial activity [35].
For instance, a study by A. H. Hashem, involved zero valent Ni-NPs synthesized with the help of *Penicillium chrysogenum* and encapsulated into starch obtained from potatoes to build nano-capsules (Ni-NPs@st), aiming to enhance their anticancer, antioxidant, and antimicrobial toxicity. First thing after synthesis of Ni-NPs@st was to characterize them using a range of physiochemical characterization techniques including FTIR, UV-Vis, TEM, TGA and DLS. Results suggested that that Ni-NPs@st show cased higher antimicrobial activity in comparison to Ni-NPs against *Cryptococcus neoformans* ATCC 14116, *E. coli* ATCC25922, *Candida albicans* ATCC90028, *Bacillus subtilis* ATCC605, *A. fumigatus* RCMB 02568, and *Aspergillus ni*ger RCMB 02724. Whereas decent antioxidant potential for Ni NPs and Ni-NPs@st was noted with IC is noted for Ni-NPs@st and Ni-NPs, with IC_{50} values of 97 and 60 µg mL^{-1}, respectively. While for to evaluate the cytotoxicity, the group exposed Ni NPs and Ni-NPs@st against Vero cells and found the IC_{50} values of 238 and 204 µg mL^{-1}, respectively. Furthermore, in evaluating the cyto-toxicity against the MCF-7 Breast Cancer cell lines, the group computed IC50 values of 217 and 118 µg mL^{-1} for Ni NPs and Ni-NPs@st, respectively. This study underscores the potential biomedical activities of Ni-NPs@st nano-capsules, suggesting their use in treating chronic infections [36].
Breast cancer stands as the most widespread form of cancer in women, and its curability reaches up to 80% in the majority of cases when identified and addressed at an early stage (non-metastatic). Nanotechnology has significantly advanced potential chemotherapeutic approaches, especially in tumor treatment, offering pharmaceutical applications. Chitosan, a natural polymer derived from chitin, has undergone extensive exploration for its diverse applications, including its anticancer properties in medicine.
A study by S. Mickymaray and group focused on the synthesis and characterization of NiO-TiO2-Farnesol hybrid nanomaterials encapsulated in Chitosan – (CNTF HNMs) using numerous techniques, Including FE-SEM, UV-Vis, TEM, FTIR, RDX, PL, XRD and DLS. The results revealed the nanoparticles to be 34.8nm in diameter with face centered crystallographic structure. Furthermore, the synthesized hybrid nanoparticles were studied for their anti-cancer potential against Multi Drug Resistant MB-231 cell lines by employing MTT assay. The findings showcased the robust antibacterial effectiveness of CNTF HNMs against multi-drug resistant extended-spectrum beta-lactamases (ESBL)-producing gram-negative bacterial pathogens and reference strains. The research revealed an elevation in reactive oxygen species (ROS), changes in mitochondrial membrane potential ($\Delta\psi m$), and the onset of apoptosis. These results suggest substantial promise for CNTF HNMs, showcasing their dual potential as both antibacterial and anticancer agents [37].

Gold

Gold nanomaterials exhibit great promise for biomedical applications due to characteristics such as rapid and straightforward preparation, coupled with inherent biological inertness that imparts outstanding bioconjugation potential to these nanomaterials. The integration of Au nanostructures into biomedical devices capitalizes on their antimicrobial efficacy. For instance, introducing Au nanoparticles into a poly(methyl) methacrylate (PMMA)-based bone cement enhances the physical strength properties of the polymeric matrix and diminishes *S. aureus* biofilm formation [38]. Being the most frequently employed metallic nanomaterials in the biomedical field, understanding the interaction between nanoparticles and biological systems, especially in terms of cytotoxicity, is imperative for evaluating their enduring effects on human health. Historical reports from the 1990s suggest that gold colloid-based monolayers exhibit selective toxicity towards specific cells, notably red blood cells. However, this toxicity is dependent on factors such as the size, composition, and surface properties of the gold colloid films [35]. Recent research indicates the presence of 18 mm diameter Au nanoparticles in the spleen and liver, illustrating internalization by macrophages [39]. Au nanoparticles and nanoclusters have demonstrated in vivo toxicity, leading to a reduction in red blood cell count and damage to organs such as the spleen, liver, and kidney [40]. Rigorous biosafety investigations are essential to enhance public acceptance of nanotechnology-derived products. A recent study highlighted improved antimicrobial activity against both Gram-ve and Gram+ve bacteria by reducing the size of gold nanoparticles, less than 2nm to the nanocluster range. Importantly, this reduction increased intracellular ROS levels without imposing additional cytotoxic and genotoxic burdens on host cells [41].

A unique characteristic of Au nanomaterials is their ability to absorb, amplify electromagnetic light, scatter, and fluoresce. These attributes enable the elimination of bacteria through photothermal treatment, a process achieved by exposing gold-based nanostructures to near-infrared radiation. Modifying the shape of Au nanomaterials, such as to nanospikes or nanorods, has been proposed to broaden the absorption wavelength to the near-infrared region, thereby improving the efficiency of light-to-heat conversion [42]. These nanomaterial structures show potential for use in water sterilization due to their swift heating and efficient light-to-heat conversion under low-powered infrared laser exposure. These groundbreaking nanotechnologies could have future applications in enhancing the efficiency of bacteria elimination through photothermal or photodynamic therapy [43]. Utilizing probes demonstrated to be effective in DNA microarrays, pure gold nanoparticles can also serve for bacteria identification [43]. Additionally, the ability to modify the surface chemistry of gold nanoparticles by binding appropriate ligands to create hybrid nanoparticles holds significant promise in antimicrobial therapy [44]. Recent progress involves the synthesis of peptide-coated gold nanoparticles through a one-pot process. These peptide-conjugated antimicrobial nanoparticles not only maintain their antibacterial efficacy but also demonstrate a notable enhancement in stability against trypsin digestion. This improvement leads to a remarkable extension of their functional life and antibacterial properties [45].

Demonstrating the utilization of a hybrid assembly involving gold nanoparticles for both surface-enhanced Raman scattering (SERS) imaging and near-infrared photodynamic antimicrobial therapy, this study focuses on live strains of vancomycin-resistant enterococci [46]. A plasmonic nanoparticle core emitting SERS signals is established using initially silver-coated 60-nanometer-diameter gold nanoparticles (Au@AgNPs). A silica shell is then constructed over the Au@AgNP, facilitating conjugation with silicon 2,3-naphthalocyanine dihydroxide (Nc) and further attachment of

vancomycin (Van). This configuration serves as a platform for near-infrared photodynamic antimicrobial therapy, enhancing the antimicrobial activity of vancomycin. The hydrophilic silica shell not only improves the biocompatibility and stability of the nanoparticle complexes but also enables the aggregation of vancomycin-functionalized NP@Nc-Van nanoparticles on the surface of Enterococcus faecium (Van A) and E. faecalis (Van B). This aggregation serves as a potential probe for live bacterial Raman imaging. The incorporation of Nc and Van enables the light illumination-induced killing of these bacterial strains in a concentration-dependent manner while leaving eukaryotic cells unharmed. The minimal inhibitory concentration of NP@Nc-Van (>20 nm for Van A and Van B) is found to be 100-fold lower than that of Van (>88 × 10^{-6} m for Van A and 44 × 10^{-6} m for Van B), indicating the significantly more potent antimicrobial activity of the Au nanoparticle hybrid. In vivo mouse infection assays conducted to evaluate VRE lethality demonstrate that this hybrid nanomaterial, upon near-infrared light irradiation, leads to substantial infection regression or complete eradication compared to the non-treated groups in a murine model of vancomycin-resistant Enterococci subcutaneous infection [35].

Carbon Based Nanoparticles

The abundance of diverse carbon allotropes, spanning well-known phases like amorphous carbon, graphite, and diamonds to recently discovered and promising carbon nanotubes (CNTs), graphene quantum dots (GQDs), graphene oxide (GO), and fullerene, has bestowed significant value upon carbon-based materials in recent times[47]. Each affiliated allotrope within the carbon family boasts unique features and has found extensive applications across various biological fields, including biosensing, drug delivery, tissue engineering, imaging, diagnosis, and cancer therapy [48]. **Figure 3.3** shows different types of available Cabron allotrops and their uses for biomedical purposes (Figure adopted from [49], published under open access Creative Commons (*CC*) license).

CNTs – the hollow cylinders, composed of graphitic sheets, were catalogued into either multi-walled carbon nanotubes (MWCNT) or single-walled carbon nanotubes (SWCNT) form. The SWCNTs, can be formed, by cylindrical rolling of a single graphitic sheet, with diameter in nanoscale range, with a high aspect ratio, while their multi-walled counterparts can be obtained with the cylindrical rolling of multiple sheets of graphite stacked over each other with an interlayer spacing of 3.4Å [49, 50]. Due to their exceptional electrical, structural, and mechanical, they parade higher electrical conductivity, flexibility, and strength towards numerous biological objects, proving valuable for detection, prognosis and treatment of variety of medical diseases [51].

The remarkable properties of graphene, including its ease of functionalization for precise and choosy detection of biological sections, chemical purity, an extraordinarily large surface area, and the presence of free π electrons, position it as an ideal applicant for drug delivery [52]. Moreover, its compatibility with a range of therapeutic agents, fluorescent dyes, and biomaterials establishes it as a commonly utilized material for *in vivo* cancer diagnosis, imaging, and treatment. Additionally, newly established and charismatic biomaterial within the carbon family is graphene quantum dots (GQDs), which are also known as Zero-dimensional dots of graphene sheets, in the nanoscale range, consisting of 3 to 5 graphene sheets (roughly) [53]. During the revolution from two-dimensional graphene sheets to zero dimensional GQDs, they present excellent photoluminescence attributed to quantum confinement [54].

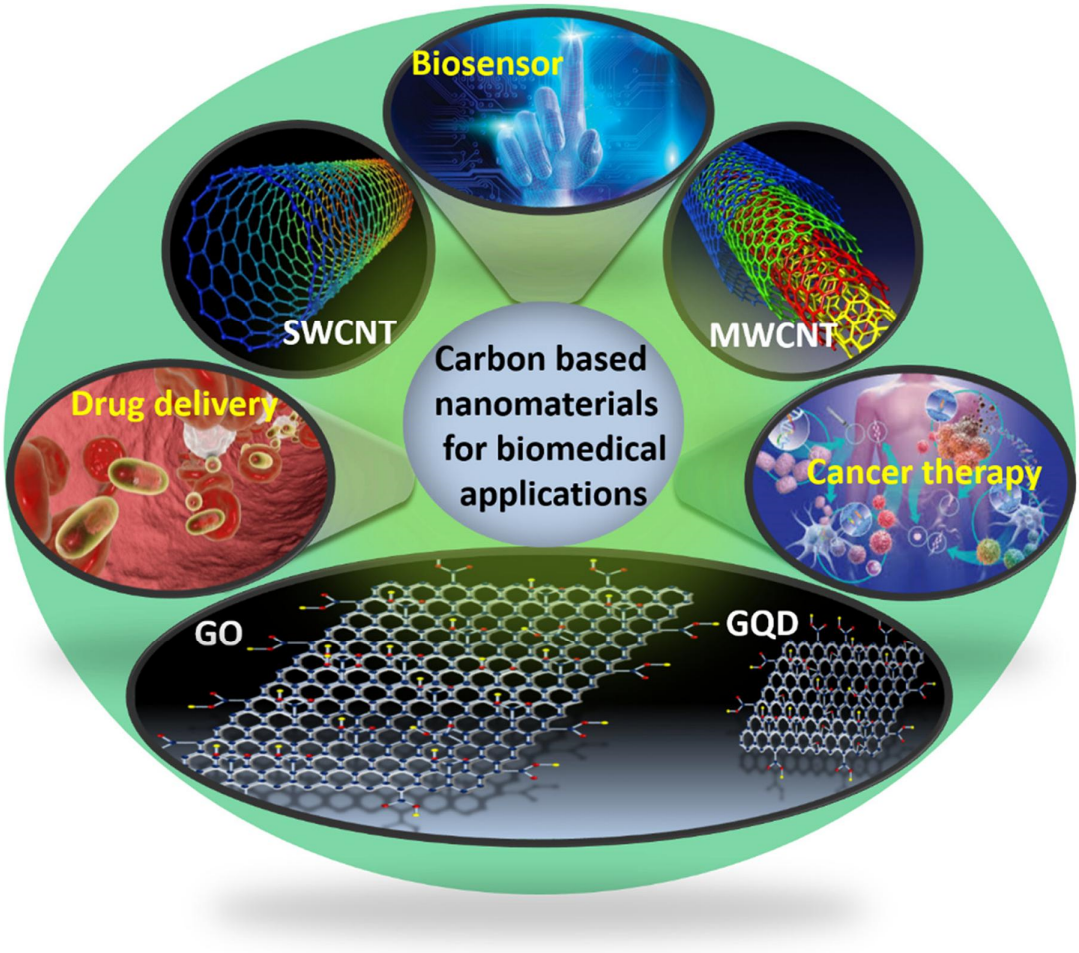

FIGURE 3.3
Various allotropes and biological applications of carbon (Figure adopted from [49], published under open access Creative Commons (*CC*) license).

For example, in T. A. Salaheldin, and groups research, Graphene nanosheets (rGO), magnetite nanoparticles (Fe3O4), and G/Fe3O4 were synthesized through chemical methods and thoroughly characterized using various techniques. The cytotoxicity of these nanomaterials was assessed through a colorimetric Sulforhodamine B cell viability assay at 24 and 48 hours, revealing altered cell morphology and toxic effects on DNA with a concentration of 400 µg/mL for rGO, Fe3O4, and G/Fe3O4 after 24 hours. The evaluation of cytotoxic effects indicated mRNA expression in β-actin and Bax apoptotic genes, with no expression of caspase-3 mRNA after 24 hours, implying an intrinsic apoptotic caspase-independent pathway. Additionally, G/Fe3O4 exhibited a photothermal effect upon irradiation of HepG2 cells. A significant decrease in cell viability, ranging from 40% to 5%, was observed with 10 and 50 µg/mL G/Fe3O4 after 48 hours of cell treatment. These findings suggest the potential of G/Fe3O4 in inducing cytotoxicity and a photothermal effect in HepG2 cells [55]. **Figure**

3.4 shows %cell viability of HepG2 cell lines when exposed to Graphene and composite materials at various time scales (Figure adopted from [55], published under open access Creative Commons (*CC*) License).

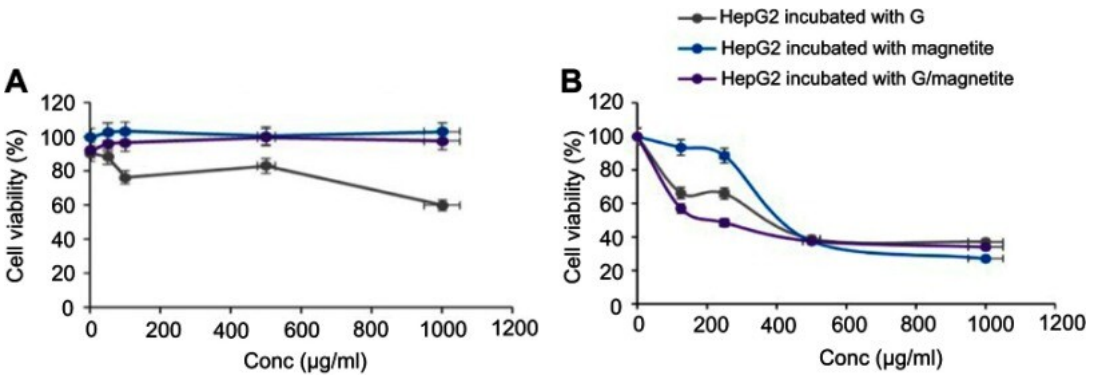

FIGURE 3.4
%cell viability of HepG2 (liver cancer) cell lines when exposed to Graphene, Magnetite and Graphene/Magnetite composite materials (a) after 1 day of incubation period and (b) after 2 days of incubation period.

Further ahead, M. Zoghi, and group worked on curcumin encapsulated with Fe2O3/Chitosan/CQDs synthesis, using the double emulsion method (W/O/W) to exploit its anticancer properties while overcoming its limitations, presenting a promising solution for targeted drug delivery. Chitosan, a hydrophilic biopolymer, forms an adhesive pH-sensitive matrix, enabling the entrapment of the hydrophobic drug and ensuring controlled drug release in cancerous environments. Carbon quantum dots provide luminescence and water solubility, facilitating drug release tracing, bioimaging, and enhancing biocompatibility. Fe2O3 contributes to chemical stability, bioavailability, and anti-cancer properties. XRD and FTIR analyses confirmed both physical interaction and chemical bonding within the nanocomposite. FESEM analysis affirmed the matrix and spherical structure of the fabricated drug. DLS analysis determined a mean size of approximately 227.2 nm, and the zeta potential indicated perfect stability. Various kinetic models, including Korsmeyer-Peppas' model, were fitted to experimental data, highlighting its predominant role in cancerous environments. In vitro studies employing flow cytometry and MTT assay demonstrated significant cytotoxicity against MCF-7 cell lines. The findings suggest that the curcumin-loaded CS/CQDs/Fe2O3 nanocomposite is a promising alternative for targeted drug delivery [56]. Furthermore, novel drug carriers utilizing multi-walled carbon nanotubes functionalized with naringenin were designed by R. P. Morais, to enhance lung cancer treatment efficacy. Characterization of the nanocarrier employed various techniques, including FTIR, RAMAN, DSC (Differential Scanning Calorimetry), XPS and TEM). In vitro drug release rates were determined through the dialysis method. Cytotoxicity evaluations were performed utilizing the MTT assay, contrasting a normal human skin cell line (hFB) as a representative for normal cells and an adeno-carcinomic human alveolar basal epithelial (A569) cell line as an in vitro model for lung cancer. Findings highlighted the presence of non-covalent associations in the functionalization of carbon nanotubes with naringenin. The release patterns demonstrated pH-responsive characteristics, suggesting extended release within the tumor pH environment. The carbon nanotubes functionalized with naringenin exhibited reduced cytotoxic effects on non-malignant cells

(hFB) in contrast to free naringenin, showcasing an improved anticancer impact on malignant lung cells (A549), serving as an in vitro representation of lung cancer [57]. The results obtained from this study are represented in Figure 3.5 (figure adopted from [57], published under open access Creative Commons (*CC*) License).

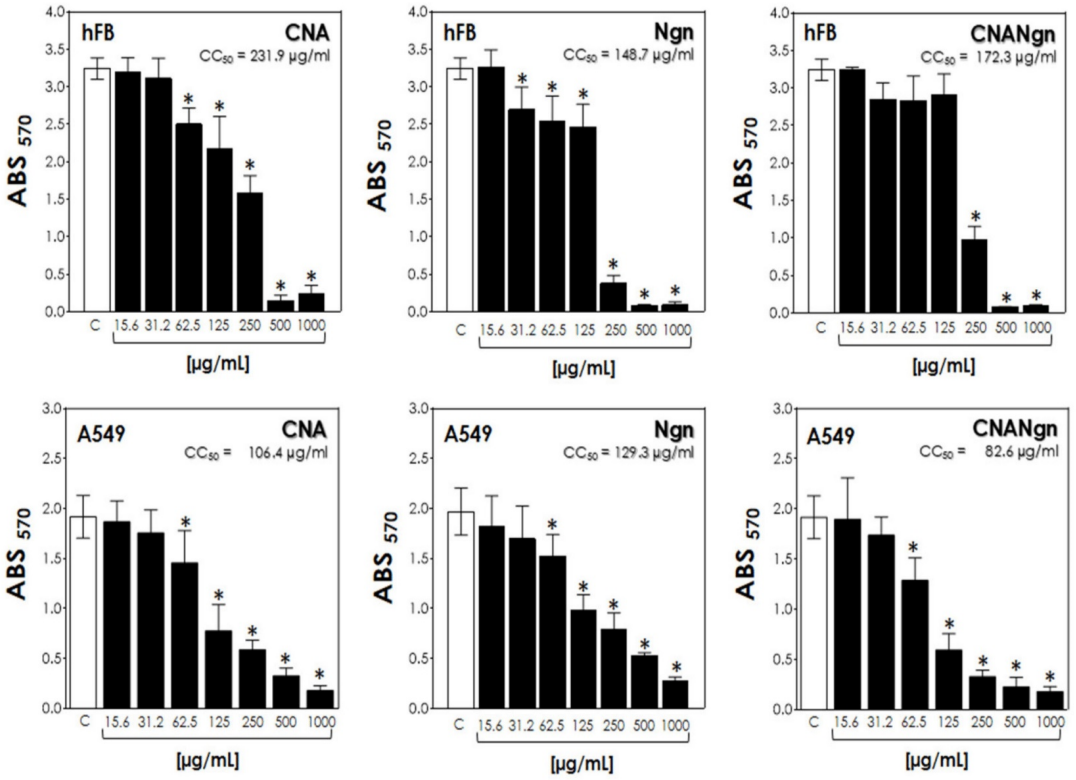

FIGURE 3.5
Cell viability of hFB (human skin cell line, top) and A549 (adenocarcinomic human alveolar basal epithelial cell line, bottom) cells treated with CNA (CNT after acid treatment), Ngn (naringenin), and CNANgn (CNA treated with naringenin) at various concentrations after a 48-hour incubation. The presented data illustrates the mean values with standard deviations (n = 3), and significant differences between different concentrations are denoted by * ($p < 0.05$). (Figure adopted from [11], published under open access Creative Commons (CC) License).

Polymer based Nanomaterials

Polymeric nanoparticles, comprising active pharmaceutical ingredients adsorbed on macromolecular substances, have been in development for an extended period as drug delivery systems (DDS). They present numerous benefits as DDS, such as being generally biocompatible, biodegradable, easily manufacturable, non-toxic, non-immunogenic, and enabling site-specific targeting of particular organs or tissues [54]. The majority of nanomedicines approved by the U.S. Food and Drug

Administration (FDA) are polymeric nanoparticles, including liposomes and drug-bound albumin [58, 59]. Noteworthy examples are Doxil (PEGylated Liposomal doxorubicin) and Abraxane (Albumin-particle bound paclitaxel), approved by the U.S. FDA in 1995 and 2005, respectively [7, 60]. Since the introduction of the first nanomedicine, an increasing array of polymeric nanomedicines has undergone translation, clinical testing, and approval by both the U.S. FDA and the European Medicines Agency (EMA) [61]. FDA-approved nanomedicines span various areas, encompassing several cancer types, transthyretin-mediated amyloidosis, hepatitis A vaccine, and more [62]. Advancements in nanotechnology facilitate the delivery of chemical drugs, protein/antibody drugs, and gene therapy [63]. Moreover, nanoparticles devoid of loaded drugs can effectively treat diverse diseases [64]. The swift progress in nanotechnology has spurred the development of more inventive drugs aimed at enhancing the pharmacokinetic properties of existing drugs or addressing rare diseases [7].

For example, S. Bhattacharya, and group's investigation centered on enhancing gemcitabine (GTB) delivery through cationic polymeric nanoparticles to evaluate their toxicity against ovarian cancer, aiming to facilitate efficient restricted delivery and sustained drug preservation during biological ejection. The group initiated their research by generating four samples of GTB functionalized polymeric nano-carriers: chitosan & polysarcosin nanoparticles (CS-PSar-NPs), chitosan nanoparticles (CS-NPs), poly-l-lysine & polysarcosin nanoparticles (PLL-PSar-NPs), and polysarcosin nanoparticles (PSar-NPs). By assessing initial particle characteristics such as encapsulation efficiency, size, zeta potential, release profiles, DSC, surface morphology, and cellular internalization studies utilizing Nile red and rhodamine 123 fluorescent markers, the hypothesis was formulated that CS-PSar-NPs represented the optimal cationic formulation, demonstrating robust bio-cytotoxicity towards the OVCAR-8 ovarian cancer cell line and biocompatibility against the normal cell lines. Further, to increase the toxicty, bioavailability and cellular penetration, in terms of *iv* vitro studies, epidermal growth factor receptor variation III (EGFRvIII) was attached to surfaces of all four polymeric nanoparticles. Confocal imaging revealed that polymeric nanoparticles of cationic GTB, conjugated with EGFRvIII, demonstrated dual internalization capabilities and enhanced cellular uptake compared to their unconjugated counterparts, with a time-dependent cell entry. GTB nanocarriers loaded with attached EGFRvIII displayed enhanced potential for penetrating OVCAR-8 cells within the initial incubation hour. FTIR and TEM analysis endorsed the functionalization of EGFRvIII on the surface of CS-PSar-NP (non-target specific), rendering CS-PSar-NPS-EGFRvIII as a safer and target oriented candidate for ovarian cancer therapy in terms of drug delivery [65]. While, in a research endeavor conducted by X. Song and group, multifunctional thiolated chitosan derivatives (DCA-CS-PEG-FA-NAC) were synthesized, and arsenic trioxide (ATO) was incorporated onto the derivatives via glutathione (GSH)-responsive AsIII-S bonds. Stable CS-ATO nanodrugs were then fabricated using a straightforward self-assembly method. The adjustment of the thiol substitution degree in CS led to a significant enhancement in the drug loading capacity of the nanodrugs, reaching up to 20 ATO molecules per CS molecule (DCA10.7-CS-PEG3.1-FA-NAC20.2-ATO). In vitro release studies distinctly revealed minimal ATO leakage under physiological conditions, with over 95% of ATO released after 24 hours under GSH. Both in vitro and in vivo investigations showcased that the DCA10.7-CS-PEG3.1-FA-NAC20.2-ATO nanodrug markedly increased the intracellular accumulation of ATO in tumors, mitigated the toxic and side effects of ATO on healthy organs, and amplified the therapeutic efficacy of ATO in a HepG2 mice tumor model (achieving a tumor inhibition rate of 86.4%). These findings suggest the potential clinical application of ATO in the treatment of liver cancer [66]. In an investigation led by D. Jafari-Gharabaghlou, FA-PLGA-PEG NPs were synthesized

using the W1/O/W2 technique, and their physicochemical attributes were assessed through FE-SEM, TEM, FTIR, and DLS methodologies. The cytotoxic effects of both free and Nano-encapsulated drugs were scrutinized using the MTT technique. Additionally, the RT-PCR technique was employed to gauge the expression levels of apoptotic and anti-apoptotic genes. Results revealed that Met-loaded FA-PLGA-PEG NPs demonstrated dose-dependent cytotoxic effects, displaying more pronounced cytotoxicity compared to other groups, as indicated by the MTT assay. The treated MDA-MB-231 cells with Met-loaded FA-PLGA-PEG NPs exhibited a noteworthy down-regulation of hTERT and Bcl-2, along with an up-regulation of Caspase7, Caspase3, Bax, and p53 gene expression. In conclusion, Folate-Functionalized PLGA-PEG Nanoparticles are recommended as a suitable strategy to enhance the anticancer properties of Met, thereby improving the efficacy of breast cancer cell treatment [67].

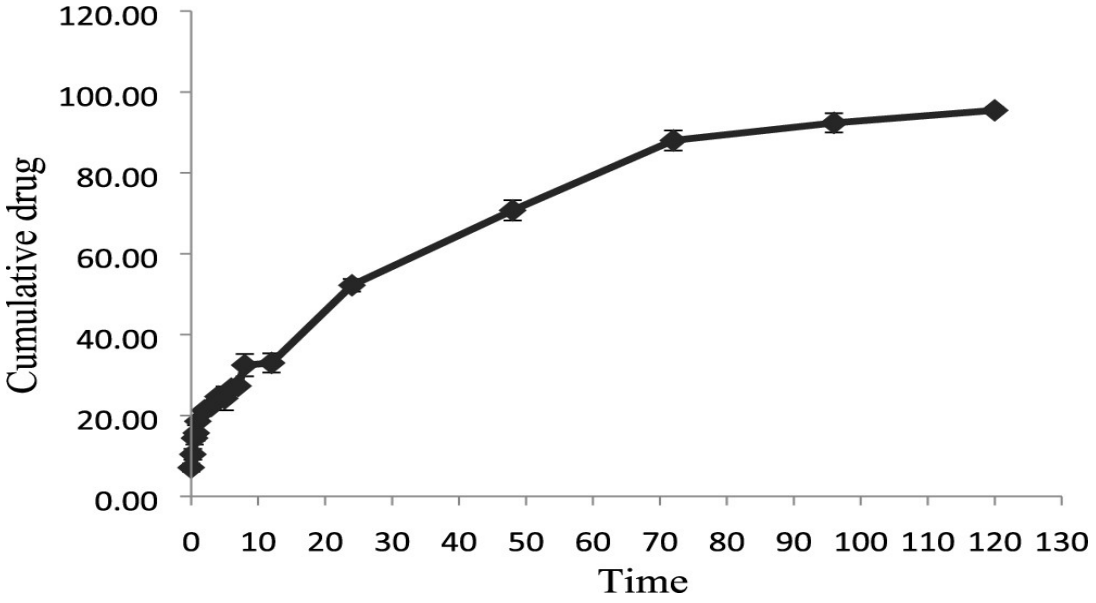

FIGURE 3.6
Graph of %Cumulative drug release vs. Time (Figure adopted from [68], published under open access, Creatives Commons (*CC*) License).

In a further investigation, A. Wadhawan et. al., present the evaluation of cytotoxicity exhibited by polymeric nanoparticles containing a innovative biosurfactant against MDA-MB-231 cells. The polymeric nanoparticles surface modified with biosurfactant, were formulated using PLA–PEG copolymers through the double emulsion solvent evaporation method. Folic acid (FA) used, served as a steering ligand to dynamically deliver the anticancer drug/cargo at the cancer site. The %E.E of the nanoparticles was computed to be 84.9%, and the delivery of the biosurfactant followed a Fickian diffusion kinetic model. The 100% drug release was obtained within the 3 days, at pH 7.4. **Figure 3.6** shows the graph of cumulative drug release with respect to time (figure adopted from [68], published under open access, Creatives Commons (*CC*) License.). The PLA–PEG copolymer nanoparticles demonstrated controlled delivery of the biosurfactant. Moreover, FA caused the enhancement of biosurfactant loaded polymeric nanoparticles and cause biotoxicity inside the MDA-MB-231 cell lines with elevated cellular uptake, in comparison to plain polymeric nanoparticles with biosurfactant

loaded without FA. The nanoparticles induced apoptosis in cancer cells, suggesting their potential as an anticancer agent. This study introduces a promising candidate for further efficiency analysis and as an alternative tool in cancer treatment [68].

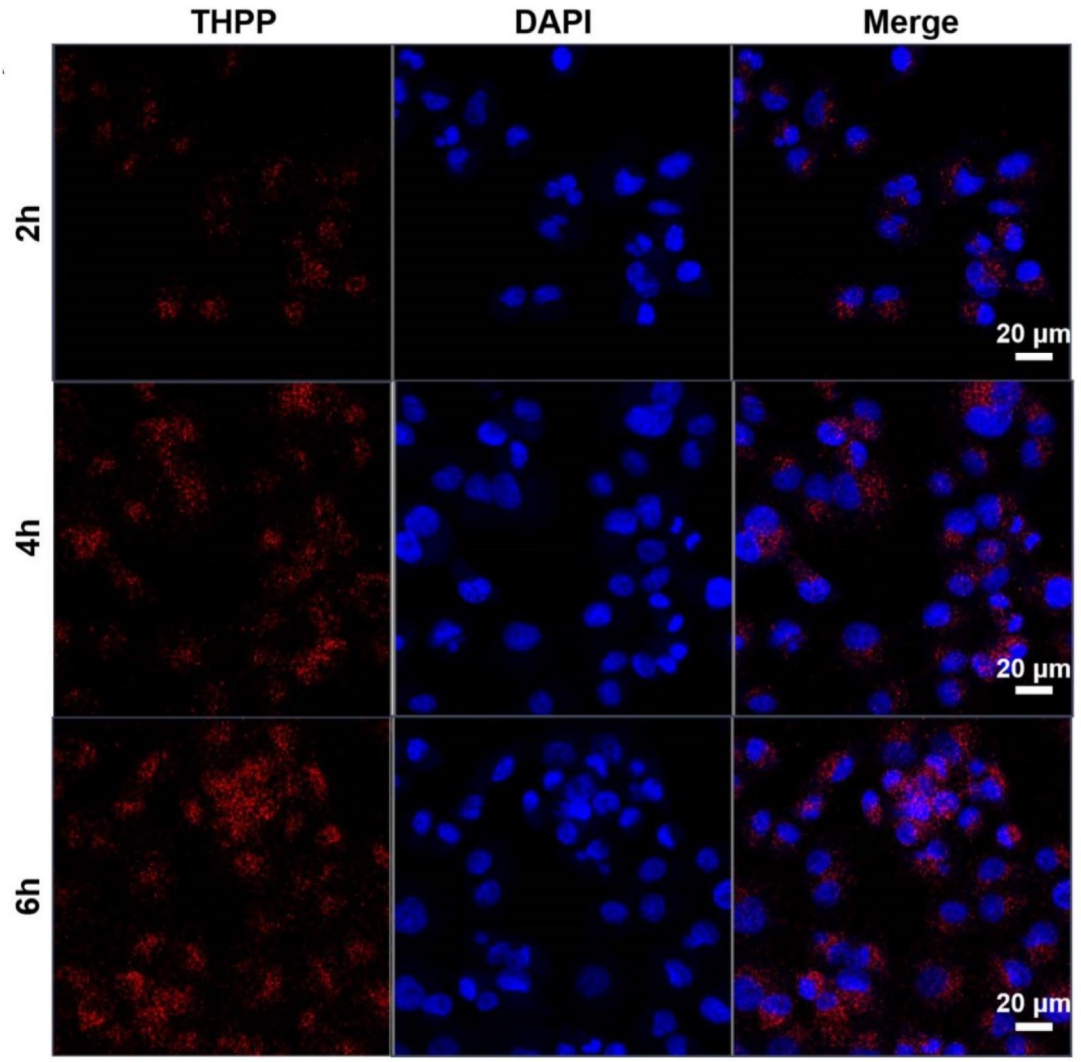

FIGURE 3.7
Confocal Scanning LASER Microscopic images of Cancerous Cells with nanoparticle uptake after 2, 4 and 6 hours (adopted from [70], published under open access, Creative Commons (*CC*) License).

Whereas, S. Ilbeigi and group conducted the synthesis and characterization of selenium-polyethylene glycol-curcumin nanoparticles (Se-PEG-Cur NPs) designed to function as a novel sono-sensitizer capable of absorbing ultrasonic waves. A comparative natured toxicity study was conducted to test the Se-PEG-Cur and GEM, to find a better standard medication for treatment of pancreatic (PAN)

cancer. The Se-PEG-Cur NPs and HEM were introduced to ASPC1 cell line individually and in combination, with and without ultrasonic (US) radiation. The influence of US radiation on the GEMs' and Se-PEG-Cur NPs cytotoxicity, and their combination was assessed by examining ROS levels, cell viability, and evaluating apoptosis. The outcomes implied that sonodynamic therapy (SDT) using GEM, Se-PEG-Cur NPs, and their use in combination primarily led to the production of ROS that encouraged apoptosis in ASPC1 cells. The combined effects of GEM and Se-PEG-Cur NPs effectively inhibited the spread of ASPC1 cells *(in vitro)*, attributable to an improved toxicity triggered by US radiation. The study suggests that Se-PEG-Cur NPs hold promise as a innovative and inexpensive sonosensitizer for SDT in the therapy of PAN cancer [69].

In another study Q. Wu and group addressed the challenges posed by the aggressive and refractory nature of pancreatic carcinoma by establishing a steady nanoplatform that integrates photodynamic therapy (PDT), and chemotherapy to enhance therapeutic efficacy. Initially, a PTX-based prodrug was designed and synthesized, which was then amalgamated with GEM and the photosensitizer THPP with optimized ratios. This mixture was consequently poured dropwise to an DSPE-PEG (amphiphilic polymer) water solution, forming micelles consisting of TPG NPs. The TPG NPs, containing and average diameter of 135 nm, exhibited a remarkable DTT-inspired release of GEM and PTX. Furthermore, the TPG NPs demonstrated efficient uptake by PANC-1 (PAN cancer) cells, leading to effective cell killing, particularly when combined with 650 nm laser irradiation. The NPs showed elevated anti-tumor activity with improved cellular uptake and circulation, when they were bombarded with a Laser having λ = 650nm, in the mouse model with introduced PANC-1 cell lines. To conclude, the formulated TPG nanoparticles showcase considerable promise in simultaneously delivering the paclitaxel prodrug, GEM, and THPP, enabling a synergistic approach involving chemo-photodynamic therapy for cancer treatment. The controlled release of the PTX prodrug and GEM not only refines tumor cell targeting but also amplifies the therapeutic impact on cancer cells. All in all, the TPG nanoparticles emerge as a favorable contender for addressing pancreatic cancer [70]. Figure 3.7 (adopted from [70], published under open access, Creative Commons (*CC*) License) shows CSLM images of nanoparticles uptake by cancer cells after 2, 4 and 6 hours.

Metal Organic Frameworks – MOFs (Hybrid)

Nanotechnology holds considerable promise in the realm of cancer immunotherapy by employing nanoparticles as efficient carriers for carrying immunostimulatory agents i.e., adjuvants and antigens [71]. This utilization allows for the protection of these agents from deprivation and offers them extended presence inside the body, thereby enhancing their therapeutic impact [72]. Additionally, the adept integration of nanotechnology into nanomedicines enables selective targeting of crucial sites, including immune cells, tumors, and lymph node locations, leading to an augmentation of medicinal efficacy [73].

Consequently, diverse nanocarriers have emerged for this purpose, encompassing nanosized metal-organic frameworks (nMOFs), liposomes [74], mesoporous silica nanoparticles [75], and micelles [76]. **Figure 3.8** shows schematics of Metal Organic Framework. Particularly, nMOFs stand out as hybrid porous materials fabricated from organic bridging ligands, and metal ions, gaining substantial popularity since the past 20 years [77]. Their application has extended from catalysis, energy storage, gas separation, and to drug delivery [78], showcasing their versatile potential in advancing cancer immunotherapy.

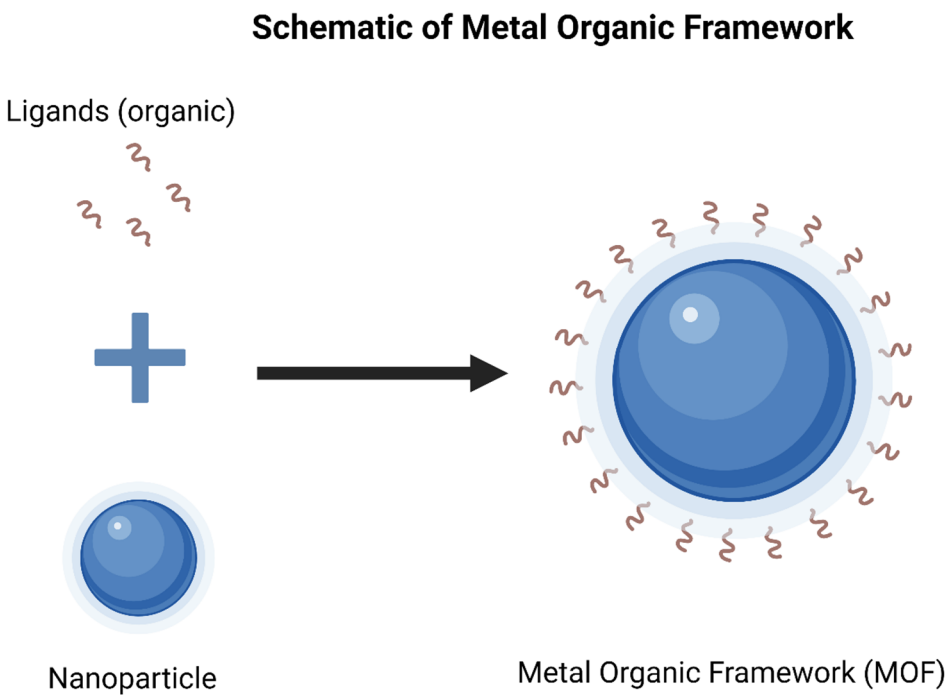

FIGURE 3.8
Schematic of Metal Organic Framework (MOF) structure.

In a study conducted by Q. Zhao and research group, an innovative lysosome-targeting nanoparticle (LYS-NP) had been devised, incorporating a mineralized metal–organic framework (MOF) coupled with a lysosome-targeting aptamer (CD63-aptamer). The primary objective was to amplify the antitumor efficacy of T cells. The MOF, synthesized from Zn^{2+} and dimethylimidazole, exhibited favorable protein encapsulation and acid sensitivity, rendering it an optimal vector for lysosomal delivery. To induce MOF mineralization, calcium carbonate ($CaCO_3$) was employed, enhancing the stability of the composite material in encapsulating therapeutic proteins and providing calcium ions with synergistic effects. Prior to mineralization, therapeutic proteins crucial for T-cell activity against tumors, namely perforin and granzyme B, were preloaded into the MOF. Moreover, prior to lysosomal reprogramming, T cells underwent pretreatment with processed tumor-specific antigens, activating or generating memory. This sequence facilitated the liberation of therapeutic proteins through the T cell receptor (TCR). The utilization of T cells restructured by LYS-NPs demonstrated a significant advancement in controlling breast cancer, validating the heightened efficacy of this method. The LYS-NPs efficiently housed perforin and granzyme B within the lysosomes of artificial T cells (ATVs), with the therapeutic proteins being released upon the binding of the T cell receptor (TCR) of ATVs to tumor cells. Concurrently, the breakdown of $CaCO_3$ into Ca^{2+} within the lysosome synergistically augments the effects of perforin and granzyme B. These elements collectively

manifest potent capabilities in inducing cell apoptosis and fostering target cell lysis within immunological synapses [79].

Liposomes

Liposomes, tiny vesicles characterized by lipid bilayers, present themselves as potential drug carriers due to their unique attributes. These include the capacity to direct drugs to precise sites of action, protect drugs from degradation, and alleviate toxicity or side effects [80]. In 1964, Bangham and colleagues presented the first microscopic image of lipid bilayered spherical vesicles surrounded by an amphiphilic phospholipid membrane. The film hydration technique stands out as the most commonly employed method for liposome synthesis. The preparation of an ideal liposomal system containing levocetirizine hydrochloride, achieved through film hydration followed by sonication, has been elucidated [81]. Phosphatidylethanolamine and phosphatidylcholine constitute the fundamental ingredients for synthesizing these vesicles. The surface charge of liposomal vesicles is dictated by the charge present on the phospholipid, whether it is positive or negative. Introducing cholesterol is suggested to adjust the rigidity of the lipid membrane. The effectiveness of lipid vesicles administered orally is compromised by their vulnerability to enzymatic and chemical destabilization, resulting in concerns related to inadequate storage stability, low encapsulation efficiency, and the swift release of water-soluble drugs into the bloodstream [80]. In addressing these challenges, solid lipid nanoparticles have surfaced as a feasible alternative, providing enhanced stability when compared to conventional liposomes [82].

In an investigation, Z. Tang and the research group developed RGD-modified liposomes (LPs) loaded with gemcitabine (GEM) using the emulsification-solvent evaporation method, and their antitumor activity was assessed both *in vitro* and *in vivo*. The physicochemical characteristics of the LPs, encompassing parameters such as particle size, zeta potential, and in vitro drug release, underwent comprehensive examination. The influence of RGD-GEM-PEG LPs on ovarian cancer was also subject to investigation. RGD-PEG3500-DSPE GEM LPs displayed a consistent spherical morphology, with an average particle size of 106.7 nm and a polydispersity index of 0.13. The encapsulation rate (ER%) and drug loading (DL%) of the formulation were determined to be 79.6±3.1% and 6.1±1.4%, respectively. In comparison to the free drug, RGD-modified GEM LPs demonstrated sustained-release properties in vitro. In vivo, the DiD-RGD-PEG3500-DSPE GEM LPs group exhibited noticeable fluorescence intensity in the mice's tumor at all times, whereas free DiD-GEM and DiD-GEM LPs displayed no discernible fluorescence intensity, indicating a lack of tumor-targeting functionality for ordinary liposomes and drugs. Figure 3.9 shows the Fluorescence images captured at the difference of 12 hours for 1 day (Figure adopted from [83], published under open access, Creative Commons (CC) License). RGD-PEG3500-DSPE GEM LPs displayed superior antiproliferative effects on SKOV3 cells and exhibited enhanced antitumor efficacy in vivo compared to non-modified LPs.

While liposomes and polymeric nanoparticles are commonly used in nanomedicine, there are numerous other types of nanomedicines currently in use. The ongoing progress in the field of nanomedicine continues to yield new innovations regularly. However, it is crucial to note that despite their widespread use, nanoparticles and nanomedicines are still under thorough investigation for their potential toxicity. Scientists are working diligently to comprehensively understand, and address questions related to the safety and potential adverse effects of these nanomaterials, given that detailed answers regarding their toxicity are not yet available.

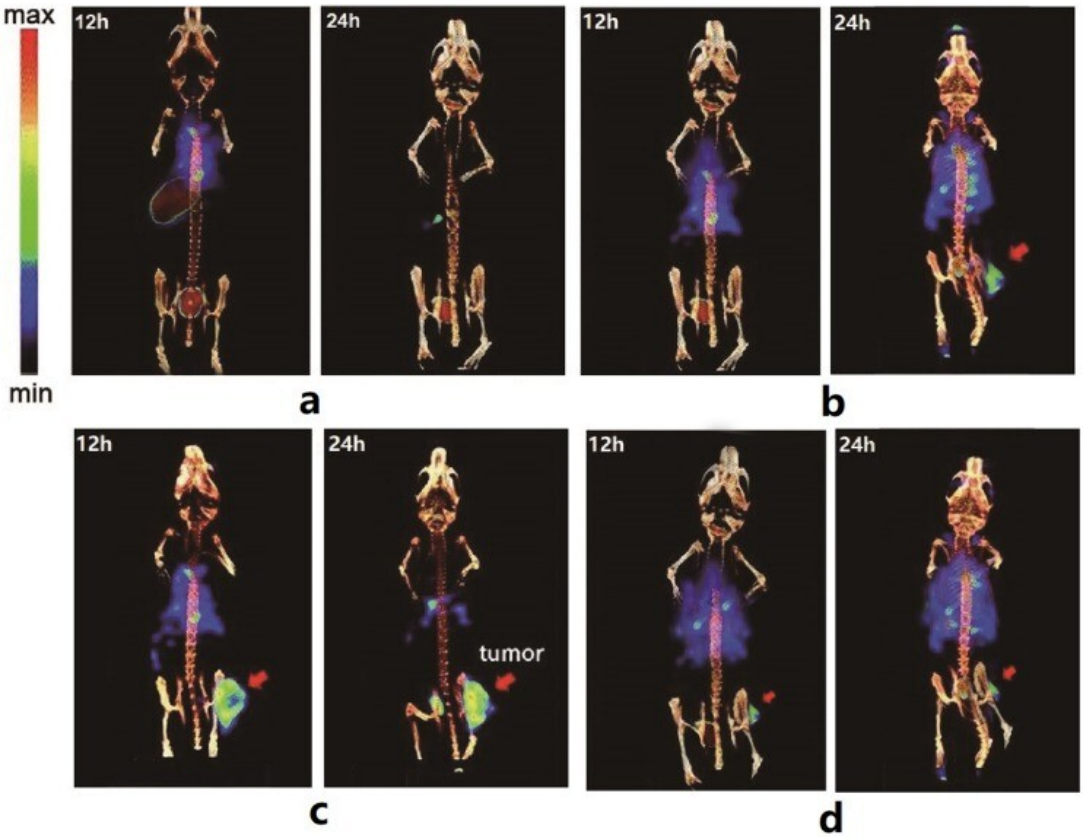

FIGURE 2.5
Schematics of a CLSM, Figure adopted from [29], published under open access, Creative Commons (*CC*) 4.0.

Conclusion

Nanotechnology is the science of latest trends and nanomaterials are very well known for their biomedical properties. Various types of nanomaterials have been kwon for these properties, including Iron, Nickel, Zinc, and many more from metal nanoparticles, Iron Oxide, Zinc Oxide, Nickel Oxide, and many more from metal oxide nanoparticles, liposomes, PEG and others from polymeric nanoparticles and PEGylated Iron Oxide and other from hybrid nanoparticles have already been studied for their potential biomedical applications. Other than that, various carbon-based nanomaterials have also been studied i.e., Carbon dots, CNTs, MWCNT. Although the nanomaterials have shown enhanced and promising biomedical properties, but there are a lot of studies needed to find out how nanoparticles would be killing a cell and what are the factors that could limit their use.

References

1. Gupta, P., C. Hung, and F. Lam, *Applications of particulate carriers in intratumoral drug delivery.* Drugs and the pharmaceutical sciences, 1993. **61**: p. 135-164.
2. Henry-Michelland, S., et al., *Attachment of antibiotics to nanoparticles: preparation, drug-release and antimicrobial activity in vitro.* International Journal of Pharmaceutics, 1987. **35**(1): p. 121-127.
3. Egan, M., et al., *Toward Interdisciplinary Synergies in Molecular Communications: Perspectives from Synthetic Biology, Nanotechnology, Communications Engineering and Philosophy of Science.* Life, 2023. **13**(1): p. 208.
4. Valent, P., et al., *Paul Ehrlich (1854-1915) and His Contributions to the Foundation and Birth of Translational Medicine.* Journal of Innate Immunity, 2016. **8**(2): p. 111-120.
5. Hassan, D., et al., *Physiochemical properties and novel biological applications of Callistemon viminalis-mediated α-Cr2O3 nanoparticles.* Applied Organometallic Chemistry, 2019. **33**(8): p. e5041.
6. Sani, A., et al., *Floral extracts-mediated green synthesis of NiO nanoparticles and their diverse pharmacological evaluations.* Journal of Biomolecular Structure and Dynamics, 2021. **39**(11): p. 4133-4147.
7. Pourmadadi, M., et al., *Novel epirubicin-loaded nanoformulations: Advancements in polymeric nanocarriers for efficient targeted cellular and subcellular anticancer drug delivery.* Inorganic Chemistry Communications, 2023. **155**: p. 110999.
8. Hassan, D., A. Sani, and D.I. Medina, *Limitations of Nanocarriers Such as Cell and Tissue Toxicity, Genotoxicity, Scale-Up of Nanomaterials*, in *Nano Drug Delivery for Cancer Therapy: Principles and Practices*, F.A. Khan, Editor. 2023, Springer Nature Singapore: Singapore. p. 149-171.
9. Narvekar, M., et al., *Nanocarrier for Poorly Water-Soluble Anticancer Drugs—Barriers of Translation and Solutions.* AAPS PharmSciTech, 2014. **15**(4): p. 822-833.
10. Couvreur, P., et al., *Biodegradable polymeric nanoparticles as drug carrier for antitumor agents*, in *Polymeric nanoparticles and microspheres*. 2018, CRC Press. p. 27-94.
11. Perez-Potti, A., et al., *Nanoparticle-based immunotherapeutics: From the properties of nanocores to the differential effects of administration routes.* Advanced Drug Delivery Reviews, 2023. **197**: p. 114829.
12. Awasthi, R., B. Bhushan, and G.T. Kulkarni, *Chapter 9 - Concepts of nanotechnology in nanomedicine: From discovery to applications*, in *Targeting Chronic Inflammatory Lung Diseases Using Advanced Drug Delivery Systems*, K. Dua, et al., Editors. 2020, Academic Press. p. 171-209.
13. Vagena, I.-A., et al., *Functionalized ZnO-Based Nanocomposites for Diverse Biological Applications: Current Trends and Future Perspectives.* Nanomaterials, 2024. **14**(5): p. 397.
14. Idris, D.S. and A. Roy, *Synthesis of Bimetallic Nanoparticles and Applications—An Updated Review.* Crystals, 2023. **13**(4): p. 637.
15. Pourmadadi, M., et al., *Recent advancements in the targeted delivery of Gemcitabine: Harnessing nanomedicine for enhanced cancer therapy.* OpenNano, 2023. **13**: p. 100177.
16. Hassan, D., et al., *Biosynthesis of pure hematite phase magnetic iron oxide nanoparticles using floral extracts of Callistemon viminalis (bottlebrush): their physical properties and*

novel biological applications. Artificial Cells, Nanomedicine, and Biotechnology, 2018. **46**(sup1): p. 693-707.
17. Jiang, H., et al., *Metal-based nanoparticles in antibacterial application in biomedical field: Current development and potential mechanisms.* Biomedical Microdevices, 2024. **26**(1): p. 12.
18. Kumar-Krishnan, S., et al., *Chitosan/silver nanocomposites: Synergistic antibacterial action of silver nanoparticles and silver ions.* European Polymer Journal, 2015. **67**: p. 242-251.
19. Manikandan, V., et al., *Green synthesis of silver oxide nanoparticles and its antibacterial activity against dental pathogens.* 3 Biotech, 2017. **7**(1): p. 72.
20. Alnehia, A., et al., *Phyto-mediated synthesis of silver-doped zinc oxide nanoparticles from Plectranthus barbatus leaf extract: optical, morphological, and antibacterial properties.* Biomass Conversion and Biorefinery, 2023.
21. Said, A., et al., *Antibacterial Activity of Green Synthesized Silver Nanoparticles Using Lawsonia inermis Against Common Pathogens from Urinary Tract Infection*. Applied Biochemistry and Biotechnology, 2023.
22. Le Ouay, B. and F. Stellacci, *Antibacterial activity of silver nanoparticles: A surface science insight.* Nano Today, 2015. **10**(3): p. 339-354.
23. More, P.R., et al., *Silver Nanoparticles: Bactericidal and Mechanistic Approach against Drug Resistant Pathogens.* Microorganisms, 2023. **11**(2): p. 369.
24. Xu, L., et al., *Silver nanoparticles: Synthesis, medical applications and biosafety.* Theranostics, 2020. **10**(20): p. 8996-9031.
25. Hernández-Sierra, J.F., et al., *The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide, and gold.* Nanomedicine: Nanotechnology, Biology and Medicine, 2008. **4**(3): p. 237-240.
26. Abdal Dayem, A., et al., *The Role of Reactive Oxygen Species (ROS) in the Biological Activities of Metallic Nanoparticles.* International Journal of Molecular Sciences, 2017. **18**(1): p. 120.
27. Babayevska, N., et al., *ZnO size and shape effect on antibacterial activity and cytotoxicity profile.* Scientific Reports, 2022. **12**(1): p. 8148.
28. da Silva, B., et al., *Relationship Between Structure And Antimicrobial Activity Of Zinc Oxide Nanoparticles: An Overview.* International Journal of Nanomedicine, 2019. **Volume 14**: p. 9395-9410.
29. Vijayakumar, S., et al., *Green synthesis of zinc oxide nanoparticles using Atalantia monophylla leaf extracts: Characterization and antimicrobial analysis.* Materials Science in Semiconductor Processing, 2018. **82**: p. 39-45.
30. Jamdagni, P., P. Khatri, and J.S. Rana, *Green synthesis of zinc oxide nanoparticles using flower extract of Nyctanthes arbor-tristis and their antifungal activity.* Journal of King Saud University - Science, 2018. **30**(2): p. 168-175.
31. Mirhosseini, M., et al., *Antibacterial activity of nickel and nickel hydroxide nanoparticles against multidrug resistance K. pneumonia and E. coli isolated urinary tract.* Nanomedicine Journal, 2018. **5**(1): p. 19-26.
32. Zhang, W., et al., *Photogeneration of reactive oxygen species on uncoated silver, gold, nickel, and silicon nanoparticles and their antibacterial effects.* Langmuir, 2013. **29**(15): p. 4647-4651.

33. Wu, M., et al., *Oxygen-Evolution Activity of p–n Heterojunction NiO–SnO2 Ceramic on Ti Substrate Fabricated Using a Simple Layer-by-Layer Method.* ACS Omega, 2020. **5**(35): p. 22652-22660.
34. Li, S., et al., *Antibacterial Hydrogels.* Advanced Science, 2018. **5**(5): p. 1700527.
35. Makvandi, P., et al., *Metal-Based Nanomaterials in Biomedical Applications: Antimicrobial Activity and Cytotoxicity Aspects.* Advanced Functional Materials, 2020. **30**(22): p. 1910021.
36. Hashem, A.H., et al., *Synthesis of Nanocapsules Based on Biosynthesized Nickel Nanoparticles and Potato Starch: Antimicrobial, Antioxidant, and Anticancer Activity.* Starch - Stärke, 2022. **74**(1-2): p. 2100165.
37. Mickymaray, S., et al., *Chitosan-encapsulated nickel oxide, tin dioxide, and farnesol nanoparticles: Antimicrobial and anticancer properties in breast cancer cells.* International Journal of Biological Macromolecules, 2023. **248**: p. 125799.
38. Russo, T., et al., *Preliminary focus on the mechanical and antibacterial activity of a PMMA-based bone cement loaded with gold nanoparticles.* Bioactive Materials, 2017. **2**(3): p. 156-161.
39. Semmler-Behnke, M., et al., *Biodistribution of 1.4- and 18-nm Gold Particles in Rats.* Small, 2008. **4**(12): p. 2108-2111.
40. Sheikhzadeh, E., V. Beni, and M. Zourob, *Nanomaterial application in bio/sensors for the detection of infectious diseases.* Talanta, 2021. **230**: p. 122026.
41. Zheng, K., et al., *Antimicrobial Gold Nanoclusters.* ACS Nano, 2017. **11**(7): p. 6904-6910.
42. Han, S., et al., *Photothermal Cellulose-Patch with Gold-Spiked Silica Microrods Based on Escherichia coli.* ACS Omega, 2018. **3**(5): p. 5244-5251.
43. Ma, H., et al., *A gold nanoparticle based fluorescent probe for simultaneous recognition of single-stranded DNA and double-stranded DNA.* Microchimica Acta, 2018. **185**(2): p. 93.
44. Carvalho, R.S., et al., *Antibacterial and antifungal activities of phenolic compound-enriched ethyl acetate fraction from Cochlospermum regium (mart. Et. Schr.) Pilger roots: Mechanisms of action and synergism with tannin and gallic acid.* South African Journal of Botany, 2018. **114**: p. 181-187.
45. Wadhwani, P., et al., *Antibiotic gold: tethering of antimicrobial peptides to gold nanoparticles maintains conformational flexibility of peptides and improves trypsin susceptibility.* Biomaterials Science, 2017. **5**(4): p. 817-827.
46. Zhou, Z., et al., *Multifunctional nanocomplex for surface-enhanced Raman scattering imaging and near-infrared photodynamic antimicrobial therapy of vancomycin-resistant bacteria.* Colloids and Surfaces B: Biointerfaces, 2018. **161**: p. 394-402.
47. Mostofizadeh, A., et al., *Synthesis, Properties, and Applications of Low-Dimensional Carbon-Related Nanomaterials.* Journal of Nanomaterials, 2011. **2011**: p. 685081.
48. Hong, G., et al., *Carbon Nanomaterials for Biological Imaging and Nanomedicinal Therapy.* Chemical Reviews, 2015. **115**(19): p. 10816-10906.
49. Maiti, D., et al., *Carbon-Based Nanomaterials for Biomedical Applications: A Recent Study.* Frontiers in Pharmacology, 2019. **9**.
50. Odom, T.W., et al., *Atomic structure and electronic properties of single-walled carbon nanotubes.* Nature, 1998. **391**(6662): p. 62-64.
51. Shanbhag, V.K.L. and K.S. Prasad, *Graphene based sensors in the detection of glucose in saliva – a promising emerging modality to diagnose diabetes mellitus.* Analytical Methods, 2016. **8**(33): p. 6255-6259.

52. Pattnaik, S., K. Swain, and Z. Lin, *Graphene and graphene-based nanocomposites: biomedical applications and biosafety.* Journal of Materials Chemistry B, 2016. **4**(48): p. 7813-7831.
53. Song, E., et al., *Hyaluronic Acid-Decorated Graphene Oxide Nanohybrids as Nanocarriers for Targeted and pH-Responsive Anticancer Drug Delivery.* ACS Applied Materials & Interfaces, 2014. **6**(15): p. 11882-11890.
54. Wang, J., et al., *Theoretical Investigations of Optical Origins of Fluorescent Graphene Quantum Dots.* Scientific Reports, 2016. **6**(1): p. 24850.
55. Salaheldin, T.A., et al., *IR-enhanced photothermal therapeutic effect of graphene magnetite nanocomposite on human liver cancer HepG2 cell model.* International Journal of Nanomedicine, 2019. **14**(null): p. 4397-4412.
56. Zoghi, M., et al., *Synthesis and characterization of chitosan/carbon quantum dots/Fe2O3 nanocomposite comprising curcumin for targeted drug delivery in breast cancer therapy.* International Journal of Biological Macromolecules, 2023. **249**: p. 125788.
57. Morais, R.P., et al., *Naringenin-Functionalized Multi-Walled Carbon Nanotubes: A Potential Approach for Site-Specific Remote-Controlled Anticancer Delivery for the Treatment of Lung Cancer Cells.* International Journal of Molecular Sciences, 2020. **21**(12): p. 4557.
58. Jia, Y., et al., *Approved Nanomedicine against Diseases.* Pharmaceutics, 2023. **15**(3): p. 774.
59. Liu, Q., et al., *Current research trends of nanomedicines.* Acta Pharmaceutica Sinica B, 2023. **13**(11): p. 4391-4416.
60. Arshad, R., et al., *Nanomaterials as an advanced nano-tool for the Doxorubicin delivery/ Co-Delivery—A Comprehensive Review.* Journal of Drug Delivery Science and Technology, 2023. **83**: p. 104432.
61. Thapa, R.K. and J.O. Kim, *Nanomedicine-based commercial formulations: current developments and future prospects.* Journal of Pharmaceutical Investigation, 2023. **53**(1): p. 19-33.
62. Anselmo, A.C. and S. Mitragotri, *Nanoparticles in the clinic: An update.* Bioengineering & Translational Medicine, 2019. **4**(3): p. e10143.
63. Patra, J.K., et al., *Nano based drug delivery systems: recent developments and future prospects.* Journal of Nanobiotechnology, 2018. **16**(1): p. 71.
64. Aschner, M., *Chapter 8 - Nanoparticles: Transport across the olfactory epithelium and application to the assessment of brain function in health and disease*, in *Progress in Brain Research*, H.S. Sharma, Editor. 2009, Elsevier. p. 141-152.
65. Bhattacharya, S., M.M. Anjum, and K.K. Patel, *Gemcitabine cationic polymeric nanoparticles against ovarian cancer: formulation, characterization, and targeted drug delivery.* Drug Delivery, 2022. **29**(1): p. 1060-1074.
66. Song, X., et al., *Thiolated chitosan nanoparticles for stable delivery and smart release of As2O3 for liver cancer through dual actions.* Carbohydrate Polymers, 2023. **303**: p. 120462.
67. Jafari-Gharabaghlou, D., et al., *Potentiation of Folate-Functionalized PLGA-PEG nanoparticles loaded with metformin for the treatment of breast Cancer: possible clinical application.* Molecular Biology Reports, 2023. **50**(4): p. 3023-3033.
68. Wadhawan, A., et al., *Anticancer Biosurfactant-Loaded PLA–PEG Nanoparticles Induce Apoptosis in Human MDA-MB-231 Breast Cancer Cells.* ACS Omega, 2022. **7**(6): p. 5231-5241.

69. Ilbeigi, S., et al., *Sonodynamic therapy of pancreatic cancer cells based on synergistic chemotherapeutic effects of selenium-PEG-curcumin nanoparticles and gemcitabine.* Applied Physics A, 2023. **129**(2): p. 82.
70. Wu, Q., et al., *Co-Delivery of Paclitaxel Prodrug, Gemcitabine and Porphine by Micelles for Pancreatic Cancer Treatment via Chemo-Photodynamic Combination Therapy.* Pharmaceutics, 2022. **14**(11): p. 2280.
71. Mustafa, G., et al., *Advances in nanotechnology versus stem cell therapy for the theranostics of Huntington's disease.* Journal of Drug Delivery Science and Technology, 2023. **87**: p. 104774.
72. Smith, D.M., J.K. Simon, and J.R. Baker Jr, *Applications of nanotechnology for immunology.* Nature Reviews Immunology, 2013. **13**(8): p. 592-605.
73. Jiang, H., et al., *Turning the Old Adjuvant from Gel to Nanoparticles to Amplify CD8+ T Cell Responses.* Advanced Science, 2018. **5**(1): p. 1700426.
74. Qiao, C., et al., *Enhanced non-inflammasome mediated immune responses by mannosylated zwitterionic-based cationic liposomes for HIV DNA vaccines.* Biomaterials, 2016. **85**: p. 1-17.
75. Li, W.A., et al., *The effect of surface modification of mesoporous silica micro-rod scaffold on immune cell activation and infiltration.* Biomaterials, 2016. **83**: p. 249-256.
76. Zeng, Q., et al., *Tailoring polymeric hybrid micelles with lymph node targeting ability to improve the potency of cancer vaccines.* Biomaterials, 2017. **122**: p. 105-113.
77. Wu, M.-X. and Y.-W. Yang, *Metal–Organic Framework (MOF)-Based Drug/Cargo Delivery and Cancer Therapy.* Advanced Materials, 2017. **29**(23): p. 1606134.
78. Rui, K., et al., *Dual-Function Metal-Organic Framework-Based Wearable Fibers for Gas Probing and Energy Storage.* ACS Applied Materials & Interfaces, 2017. **10**.
79. Zhao, Q., et al., *Target Reprogramming Lysosomes of CD8+ T Cells by a Mineralized Metal–Organic Framework for Cancer Immunotherapy.* Advanced Materials, 2021. **33**(17): p. 2100616.
80. Zhong, X.-f. and X. Sun, *Nanomedicines based on nanoscale metal-organic frameworks for cancer immunotherapy.* Acta Pharmacologica Sinica, 2020. **41**(7): p. 928-935.
81. Hammerich, L., A. Binder, and J. Brody, *In situ vaccination: Cancer immunotherapy both personalized and off-the-shelf.* Molecular Oncology, 2015. **9**.
82. Lawler, S.E., et al., *Oncolytic Viruses in Cancer Treatment: A Review.* JAMA Oncology, 2017. **3**(6): p. 841-849.
83. Tang, Z., et al., *Gemcitabine-loaded RGD modified liposome for ovarian cancer: preparation, characterization and pharmacodynamic studies.* Drug Design, Development and Therapy, 2019. **13**(null): p. 3281-3290.