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Review Article

Advancements in the Utilization of Metal Nanoparticles for Breast Cancer Treatment: An *In Vivo* Studies Update

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ABSTRACT

Breast cancer continues to pose a significant threat to women's health around the globe, requiring continuous research and innovation in treatment. In recent years, metal nanoparticles have emerged as a promising means of treating breast cancer with greater precision and efficiency. The *in vivo* studies have indicated that metal nanoparticles, such as gold, silver, and platinum, have demonstrated a remarkable ability to selectively target breast cancer cells while sparing healthy tissue. These nanoparticles' size, shape, and surface chemistry can be altered to enhance their biocompatibility, stability, and drug-loading capacity. They are also highly versatile for therapeutic applications due to their unique physicochemical properties, such as drug delivery, photothermal therapy, and imaging. This review focuses on recent *in vivo* studies evaluating metal nanoparticles' safety and efficacy in treating breast cancer. Several studies have demonstrated that metal nanoparticles can trigger apoptosis, inhibit tumor growth, and reduce metastasis in cancer cells. Furthermore, using these nanoparticles with traditional chemotherapy and radiotherapy has demonstrated a synergistic effect, enhancing treatment efficacy. This review also examines the challenges and concerns associated with the clinical translation of metal nanoparticles. Factors like biocompatibility, pharmacokinetics, and long-term safety profiles are discussed in the context of regulatory approval and patient-specific considerations. In conclusion, this review highlights the evolving landscape of breast cancer treatment with the development of metal nanoparticles, as evidenced by recent *in vivo* studies. In addition to their therapeutic versatility, these nanoparticles can potentially improve patient outcomes and decrease the burden of breast cancer on society.

1. Introduction

Breast cancer's persistent and relentless nature has seriously challenged individuals and healthcare systems worldwide[1]. Millions of women are affected by breast cancer each year, making it the second most common cause of cancer deaths among women[2]. Considering breast cancer as a complex disease with different subtypes and highly individualized responses to treatment interventions, innovative and effective treatment approaches must be developed to control the disease as effectively as possible[3-5]. Despite its global impact, breast cancer affects people across continents and socio-economic levels[6]. Patients and families who suffer from breast cancer face a challenging road full of treatments, surgeries, and emotional hardship[7]. The high demand for advanced diagnostics, treatments, and supportive care makes breast cancer a substantial financial burden for healthcare systems[8].

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Nanotechnology has revolutionized human and animals treatment recently, bringing about a transformative and promising paradigm shift[9,10]. Nanoparticles have emerged as key protagonists in the modern therapeutic revolution because of their minuscule size and extraordinary properties[11]. In addition to these nanoparticles, metal nanoparticles have gotten much attention for their remarkable attributes, making them ideal for cancer cell targeting[12]. Metal nanoparticles are crucial in cancer therapy due to their inherent flexibility[13]. In addition to delivering therapeutic agents accurately and efficiently, these attributes allow precision imaging and controlled drug release[14]. All these characteristics make metal nanoparticles indispensable for medical research. Another feature of metal nanoparticles that have attracted great interest is their ability to accumulate within tumor tissues while sparing healthy cells selectively[15]. This selective accumulation stems from the enhanced permeability and retention (EPR) effect, a phenomenon often observed in tumors due to their leaky vasculature and limited lymphatic drainage[16]. In order to minimize unintended damage to healthy tissues, this differential accumulation is imperative since it reduces the debilitating side effects usually associated with traditional chemotherapy and radiotherapy[17]. Therefore, metal nanoparticles have great potential as precision weapons against cancer, as they aim at the cause of the disease while minimizing collateral damage[18].

This review aims to provide an overview of the utilization of metal nanoparticles in treating breast cancer, emphasizing insights from in-vivo studies. It seeks to provide an in-depth understanding of their applications, potential synergies with conventional treatments, and the associated challenges. Moreover, this review emphasizes the importance of ongoing research, clinical trials, and regulatory efforts to maximize the therapeutic potential of metal nanoparticles.

2. Physicochemical properties and customization

The therapeutic potential of metal nanoparticles in breast cancer therapy depends on precisely manipulating their physicochemical properties[19]. Nanoparticles can be tailored to meet specific therapeutic objectives based on their size, shape, surface charge, and surface chemistry[20].

2.1. Size

The size of metal nanoparticles is a key factor influencing their biological behavior. For breast cancer therapy, nanoparticles between 1-100 nanometers are optimal[21]. Their small size range allows them to navigate quickly through the complex terrain of biological barriers. Due to their relatively small size, they are easily absorbed by tumor tissues and, importantly, can enter cancer cells[22]. It is particularly important to have this attribute when seeking to deliver drugs intracellularly since nanoparticles must traverse cell membranes to reach their target[23]. Moreover, these nanoparticles capitalize on the enhanced permeability and retention (EPR) effect in tumor tissues[24]. A nanoparticle can selectively accumulate in the tumor microenvironment due to the EPR effect[25]. In cancerous tissues, lymphatic drainage is limited due to increased leakiness of the tumor's blood vessels[26]. As a result, therapeutic payloads are concentrated within the tumor, reducing exposure to healthy cells and, thus, reducing undesirable side effects[27,28].

2.2. Shape

Metal nanoparticles are versatile due to their shape[29]. These nanoparticles can take different forms, such as spheres, rods, or even more complex structures[30]. In biological systems, each shape confers distinct characteristics[31]. Different shapes, for instance, have different surface-to-volume ratios, which affect their circulation time in the bloodstream[32]. Additionally, the electrostatic properties of these shapes play an important role in their uptake by cells and their distribution within tumors[33]. Therefore, researchers can choose the most appropriate nanoparticle shape based on the intended application and pharmacokinetics of the drug[34]. Customizing the shape allows greater control over how the nanoparticles interact with the body[35].

2.3. Surface charge

An underappreciated aspect of the design of metal nanoparticles is their zeta potential or surface charge[36]. As a result of this electrostatic property, their stability, and interactions with biological molecules are influenced[37]. Positively charged nanoparticles can exhibit an affinity for negatively charged cell membranes[38]. As a result of this interaction, the therapeutic agent can be absorbed into the breast cancer cells, which is a crucial step in the delivery of therapeutic agents[39]. Conversely, nanoparticles with neutral or negatively charged surfaces tend to be more stable in biological fluids, reducing aggregation and prolonging circulation[40,41]. Using surface charge to customize nanoparticles ensures that they behave optimally in biological environments[42,43].

2.4. Surface chemistry

The term surface chemistry refers to the functional groups and molecules attached to the surface of metal nanoparticles[44]. This aspect provides a wide range of customization options. Surface chemistry can be modified to introduce specific moieties that allow the attachment of targeting ligands, drugs, or imaging agents[45,46]. As a result of this customization, nanoparticles are able to selectively bind to breast cancer cells or to specific receptors within cells, enhancing their specificity[47]. By enhancing specificity, the risk of unintended interactions with healthy cells is minimized, which is especially important when developing precision therapies for breast cancer[48]. Therefore, customized surface chemistry enables nanoparticles to deliver therapeutic payloads to their intended targets via

the bridge between nanoparticles and their targets[49].

3. Metal nanoparticles in breast cancer therapy

Metal nanoparticles have emerged as promising frontrunners in cancer therapy as the relentless search for more innovative approaches continues[50]. They are versatile and potent precision medicine platforms due to their small size and exceptional physicochemical properties[51]. Gold, silver, and platinum nanoparticles are centered in the spotlight, each of which has its own unique characteristics and applications in the fight against breast cancer[52,53].

3.1. Gold nanoparticles

Gold nanoparticles have drawn the attention of both researchers and clinicians due to their exceptional characteristics[54,55]. Their small size allows them to penetrate biological barriers, infiltrating cancer cell bodies[56,57]. The use of gold nanoparticles can be skillfully engineered to deliver therapeutic agents directly to breast cancer cells, such as chemotherapeutic drugs or small interfering RNA (siRNA)[58]. In addition, their surfaces can be precisely modified to enhance biocompatibility and stability, which is crucial for safely and effectively transporting therapeutic substances[59,60]. It is fascinating to explore the role gold nanoparticles play in photothermal therapy. The ability to convert light energy into heat is demonstrated when they are exposed to near-infrared light[61]. As a result of this phenomenon, cancer cells can be selectively destroyed while healthy tissues are left unaffected[62]. These dual functions as a drug delivery vehicle and a photothermal therapy agent make gold nanoparticles attractive as potential treatment options for localized tumors[63,64].

3.2. Silver nanoparticles

Nanoparticles of silver have also been successful in treating breast cancer[65]. In the context of breast cancer therapy, silver nanoparticles are particularly notable for their inherent antibacterial properties[66]. Silver nanoparticles, with their natural antibacterial prowess, offer a potential solution to infections at the surgical site after breast cancer surgery[67]. These versatile particles can have their properties tailored to suit specific therapeutic purposes *in vivo*[68]. Targeting ligands can be applied to their surfaces, increasing their specificity for cancer cells and offering hope for precise cancer treatment[69]. In addition to their potential as drug delivery systems, they can act synergistically with existing treatments, amplifying their effectiveness in fighting breast cancer[70].

3.3. Platinum nanoparticles

Among the most powerful advocates for battling breast cancer are platinum nanoparticles, which are compatible with cisplatin, a widely used chemotherapy drug[71]. Cisplatin can be controlled by these nanoparticles by exploiting their surface properties and petite size. Targeted delivery minimizes collateral damage associated with off-target toxicity while also enhancing cancer-eradication efficacy[72,73]. Additionally, platinum nanoparticles serve as valuable contrast agents in medical imaging. Providing insights into breast cancer can significantly impact patient care by enabling early detection and therapy[74,75]. Platinum nanoparticles provide new dimensions in breast cancer management, improving targeted therapies (Figure 1)[75-77].

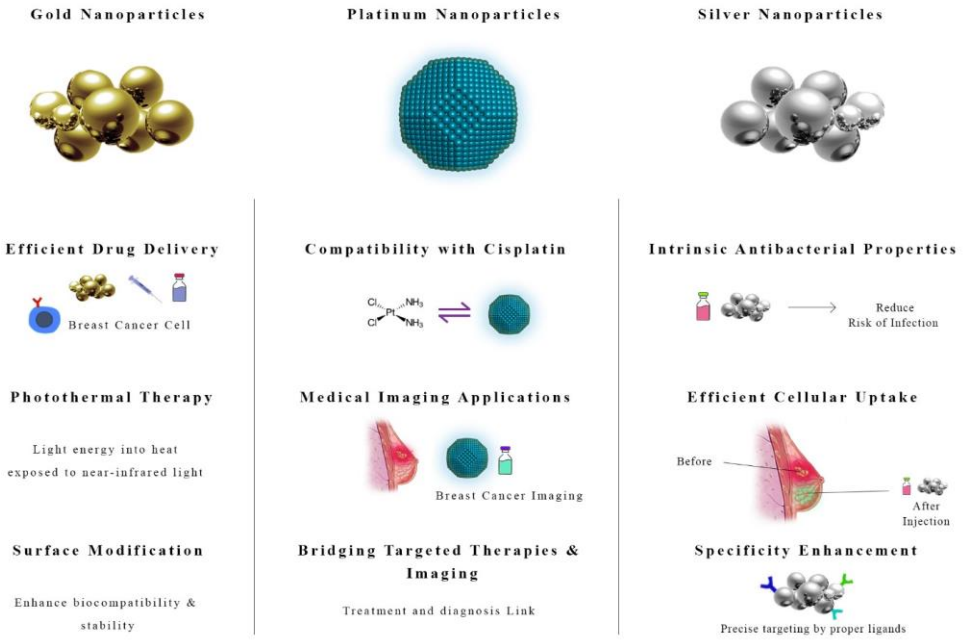

Figure 1. Metal Nanoparticles in Breast Cancer Therapy. This figure highlights the pivotal roles of three types of metal nanoparticles (gold, silver, and platinum) in breast cancer therapy.

4. *In vivo* studies update

Recently, researchers conducted an *in-vivo* study to investigate the potential of silver nitroprusside nanoparticles (AgNNPs) as cancer therapeutics[78]. The AgNNPs were modified slightly to enhance their effectiveness as wedded to cancer cells. The nanoparticles were found to be biocompatible in normal cell lines and cytotoxic in various cancer cell lines. Further, they showed antiangiogenic properties and significantly inhibited the growth of breast tumors in mouse models, increasing the survival of tumor-bearing mice. The study suggests that AgNNPs could be a promising nanomedicine for breast and other cancers, pending further biosafety evaluation.

Researchers have developed a method to encapsulate curcumin in monodispersed isoreticular nanoscale metal-organic framework (NMOF-3) nanoparticles for selective drug delivery in triple-negative breast cancer (TNBC)[79]. These nanoparticles, specifically folic acid-conjugated curcumin-loaded IRMOF-3 (IRMOF-3@CCM@FA), demonstrated cytotoxicity against TNBC cells. Apoptosis was promoted by IRMOF-3@CCM@FA by upregulating Bax, downregulating Bcl-2, and upregulating JNK and p53 in human TNBC cells. Based on in-vivo studies of mice with TNBC, targeted delivery of curcumin using IRMOF-3@CCM@FA increased survival and reduced tumor volume compared to non-targeted delivery, suggesting its potential as a therapeutic agent.

A rapid, cost-effective, and environmentally friendly method has been developed for producing titanium dioxide (TiO2) nanoparticles by combining an aqueous leaf extract of *Zanthoxylum armatum* with an aqueous leaf extract of Magnesium oxide in order to reduce the particles[80]. The safety and anti-tumor efficacy of these TiO2 nanoparticles were investigated, compared to doxorubicin (DOX), a commonly used breast cancer treatment known for its cardiotoxicity. TiO2 nanoparticles have been found to be small, spherical, and crystalline, and they have shown strong cytotoxic properties both *in vitro* and *in vivo*, with a notable reduction in tumor volume. This effect was partially mediated by generating reactive oxygen species (ROS). In contrast to DOX, the TiO2 nanoparticles did not induce cardiotoxicity or alter body weight in the mice tested. These findings suggest that *Zanthoxylum armatum*-derived TiO2 nanoparticles are a cost-effective, efficient, and safer alternative to DOX for breast cancer therapy, prompting the potential for clinical trials.

As part of a recent study, researchers developed a multifunctional nanoprobe composed of Raman reporter (DTTC)-coupled Agcore@Aushell nanostars (Ag@Au-DTTC), which has an enhanced capability for surface-enhanced Raman scattering (SERS) imaging and NIR-triggered photothermal therapy (PTT) for breast cancer[81]. In this two-step coupling of DTTC, the Au nanostars are coated onto Ag nanoparticles, which resulted in a significant improvement in the SERS signal and a decrease in nanoparticle cytotoxicity. The Ag@Au-DTTC nanostars displayed high photostability and efficient photothermal performance with a conversion efficiency of 79.01% under 808 nm laser irradiation. Both in-vitro and in-vivo measurements of SERS revealed clear Raman peaks, making them helpful in imaging MCF-7 cells and tumor-bearing mice. Tumors in mice treated with Ag@Au-DTTC nanostars and irradiated with 808 nm lasers almost disappeared after 14 days. In this study, Ag@Au-DTTC nanostars are demonstrated to be effective multifunctional agents for enhancing SERS imaging and safe NIR-triggered PTT of breast cancer.

In an athymic nude mouse model, a novel technique was developed to functionalize gold nanorods (GNRs) as *in vivo* targets for breast cancer tumors[82]. This involved covalently attaching Herceptin (HER), a monoclonal antibody for recognizing specific tumor-associated antigens, and poly(ethylene glycol) (PEG) to GNRs, which helped evade the body's reticuloendothelial system. In vitro tests confirmed the stability and functionality of these particles (Her-PEG GNRs) in blood, and subsequent *in-vivo* experiments in breast cancer-bearing nude mice demonstrated successful accumulation of functionalized gold nanorods within HER2/neu overexpressing breast tumors. These findings support the potential use of GNRs for molecular tumor imaging.

A recent *in-vivo* study investigated Zeolitic imidazole frameworks (ZIF-90) as potential cancer treatments[83]. Nano ZIF-90 was synthesized with superior biocompatibility, mitochondrial targeting, and in vivo survival compared to nano ZIF-8. A Y1 receptor ligand was conjugated to doxorubicin-encapsulated nano ZIF-90 (AP-ZIF-90) to enhance its cancer treatment capabilities. The approach led to an 80% survival rate in MDA-MB-231 tumor-bearing mice after 40 days, with minimal liver and renal side effects. The combination of nano ZIF-90 and Y1 receptor ligands shows promise for treating triple-negative breast cancer *in vivo*.

Recent *in vivo* research modified copper oxide nanoparticles with folic acid to enable targeted delivery[84]. Physicochemical properties of both copper oxide nanoparticles (CuO NPs) and folic acid-conjugated copper oxide nanoparticles (CuO–FA NPs) were characterized. The study demonstrated the targeting efficacy of CuO–FA NPs using folate receptor-positive and folate receptor knockdown in human breast cancer cells (MCF7). Flow cytometry, ROS generation, and apoptotic protein expression indicated that CuO–FA NPs induced apoptosis in MCF7 cells. In an in vivo experiment using Dalton's lymphoma-induced tumors in mice, CuO–FA NPs effectively destroyed tumor cells and decreased tumor size significantly after 15 days, suggesting their anti-cancer properties.

A recent study has demonstrated the selective efficacy of silver nanoparticles (AgNPs) in treating triple-negative breast cancer (TNBC) cells without harming non-malignant breast epithelial cells, both *in vitro* and *in vivo*[85]. According to the research, AgNPs, regardless of size, shape, or stabilizing agent, exhibit high cytotoxicity against TNBC cells while sparing non-malignant breast cells. Due to the specific nanoparticle formulation, there

is selective cytotoxicity. Although AgNPs are internalized by both TNBC and non-malignant breast cells, they are rapidly degraded only within TNBC cells, depleting antioxidants and stressing the endoplasmic reticulum. Moreover, AgNPs cause DNA damage to TNBC tumor nodules without disrupting normal breast cells. When administered systemically to mice, AgNPs effectively reduced the growth of TNBC tumor xenografts, suggesting that AgNPs may be used in TNBC treatment that is safe and specific.

In a recent *in-vivo* study, gold nanoparticles (AuNPs) were synthesized using an aqueous extract from the endophytic Cladosporium sp. isolated from *Commiphora wightii*, resulting in MycoAuNPs[86]. These nanoparticles are spherical and have an average 5-10 nanometers size. MycoAuNPs exhibited anti-cancer activity against the MCF-7 breast cancer cell line, inducing apoptosis, and were effective against tumor growth in mouse models, increasing lifespan and reducing ascites volume and body weight. MycoAuNPs showed no adverse effects on normal mice and displayed photocatalytic activity in the presence of sunlight for dye degradation. This study highlights the multifaceted therapeutic and catalytic applications of biosynthesised MycoAuNPs.

Various biosynthesized inorganic nanoparticles of different sizes and shapes, including silver and selenium nanomaterials, were tested for safety, toxicity, and efficacy in an *in vivo* study[87]. These bioinspired nanomaterials, all under 35 nm and exhibiting hexagonal and spherical shapes, were characterized using spectroscopic and microscopic techniques. Hemolysis and endotoxin tests showed low hemolytic effects, and no endotoxins were detected. A hematological, biochemical, histological, and DNA damage study conducted in Swiss mice revealed minimal adverse effects. Moreover, these nanoparticles demonstrated significant anti-tumor potential in a DMBA-induced breast cancer model in female rats, reducing tumor volume. Based on their low toxicity and high therapeutic effectiveness, these biologically synthesized nanocomposites may help manage life-threatening conditions.

An *in vivo* study recently used a novel approach that uses modified metal-organic frameworks (MIL-101(Fe)) containing selenium/ruthenium nanoparticles to deliver pooled small interfering RNAs (siRNAs)[88]. This strategy aimed to enhance therapy efficacy by silencing multidrug resistance (MDR) genes and interfering with microtubule (MT) dynamics in Taxol-resistant MCF-7/T cells. Due to the presence of coordinatively unsaturated metal sites, selenium/ruthenium nanoparticles strongly interacted with MIL-101(Fe). These nanoparticles, loaded with MDR gene-silencing siRNAs, increased siRNA protection, cellular uptake, and escape from endosomes/lysosomes, leading to MDR gene silencing and enhanced cytotoxicity via apoptosis induction and disruption of MT dynamics. The nanoparticles were proven to reduce the toxicity of cancer therapy while improving efficacy in vivo in mice with MCF-7/T xenografts.

Porphyrin-based metal-organic framework (MOF) materials were investigated for their potential in treating breast cancer in an in-vivo study. By loading doxorubicin hydrochloride (DOX) into these MOFs, they examined the synergistic effect of DOX carriers and photodynamic therapy on breast cancer. The study found that MOFs prolonged DOX residence in tumor tissues, facilitated DOX endocytosis by tumor cells, and, when combined with photodynamic therapy, resensitized breast cancer tumors to DOX, enhancing its chemotherapy effect. It provides valuable insights into countering chemotherapy resistance and improving breast cancer treatment outcomes (Figure 2).

5. Challenges and future

Numerous complex challenges and critical concerns exist on the path toward the clinical adoption of metal nanoparticles in breast cancer therapy as their promising potential continues to unfold[89]. These challenges deserve meticulous consideration, including biocompatibility, toxicity, pharmacokinetics, and biodistribution[90]. Biocompatibility is one of the foremost concerns when using metal nanoparticles for breast cancer therapy[91-94]. Although these nanoparticles show great promise, they may provoke immune responses and adverse reactions within the complex body milieu[95]. There is concern regarding the immune system's response to these foreign entities, including potential inflammation and activation of immune cells[96]. Moreover, these nanoparticles may be highly toxic due to their metallic composition[97,98]. Since metal ions can be released into the systemic circulation and accumulate in vital organs, stringent safety tests are required[99]. Careful design and surface modification are vital to ensure the safety of metal nanoparticles used in breast cancer treatment[100]. The complex pharmacokinetics and biodistribution of metal nanoparticles pose another complex challenge. The mechanics of how these nanoparticles move through the bloodstream, accumulate in tumor tissues, and eventually leave the body is a complex puzzle[101]. Factors such as nanoparticle size, shape, surface charge, and surface chemistry all influence these dynamics[102]. Due to the intricate relationship between these factors, as well as nanoparticles' potential to undergo transformations or degradation in the body, rigorous pharmacokinetic studies are essential[103]. Additionally, nanoparticles are potentially retained in vital organs for long periods of time and may accumulate off-target in healthy tissues[104]. A delicate balance must be struck between tumor targeting and minimal off-target effects. In the field of *in vivo* studies, the future holds a tapestry of promising threads, each weaving a story of potential breakthroughs and technological advancements in breast cancer research[105]. The combination of metal nanoparticles and molecular imaging promises to redefine early breast cancer detection and monitoring[106]. Future breast cancer treatment will transcend standardized approaches, delivering tailored and precise interventions that reduce side effects while maximizing therapeutic efficacy[107].

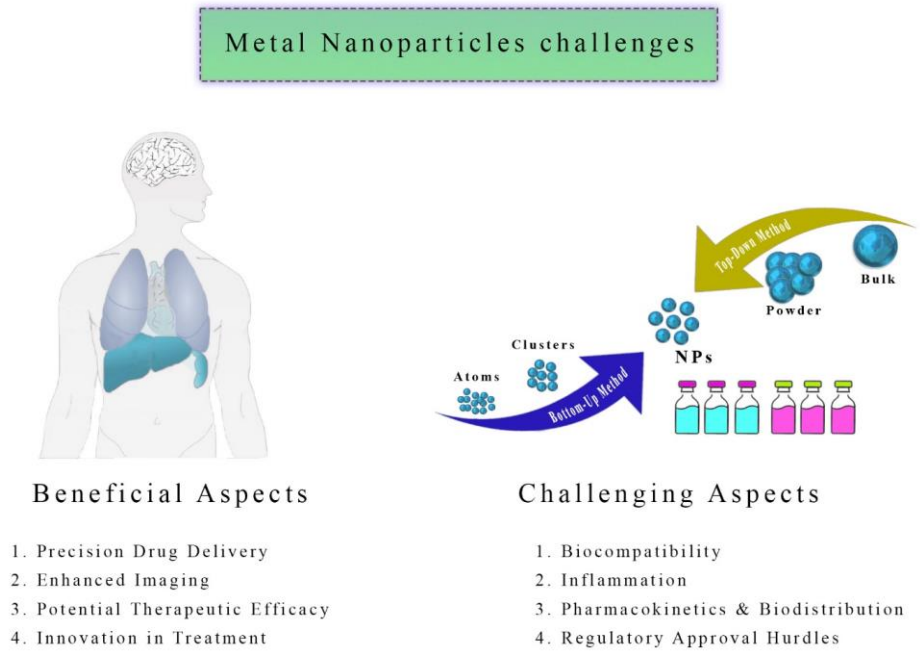

Figure 2. The multifaceted landscape of utilizing metal nanoparticles in breast cancer therapy. Beneficial aspects include their potential for precise drug delivery, enhanced imaging capabilities, and innovative treatment options, such as controlled drug release. However, challenges exist in terms of biocompatibility and immune responses, complex pharmacokinetics and biodistribution, regulatory approval hurdles, and the integration of metal nanoparticles into clinical practice. Careful consideration of these aspects is essential to harness the full potential of metal nanoparticles in the fight against breast cancer.

6. Conclusion

In summary, *in vivo* studies have demonstrated significant progress and promising prospects in harnessing the full potential of metal nanoparticles in breast cancer therapy. Adaptable nanoscale entities have illuminated a path toward more precise, effective, and patient-centric treatment options. Clinical trials and ongoing research initiatives are at the forefront of this endeavor, which offers the potential for translating laboratory findings into tangible benefits for actual patients. Individualized strategies for breast cancer treatment, meticulously tailored to the specific profiles of individual patients, point to a future in which therapeutic approaches will exhibit unparalleled precision, thereby minimizing adverse effects and increasing therapeutic effectiveness. Furthermore, it is vital to emphasize that metal nanoparticles have the potential to influence patient outcomes in a significant way. The use of these innovative therapies not only holds the key to managing disease but has also been shown to improve the quality of life of breast cancer patients.

Declarations

Competing interests

The authors declare no conflict of interest.

Authors' contributions

The conceptualization of the project was primarily carried out by Ghazale Ahmadi, while the initial writing and preparation of the original draft involved the collaborative efforts of Mahdiyeh Rahdari, Homa Sadat Hashemi, Seyed Mohamad Ali Hashemi, Ali Nadjafi-Semnani, Saeid Jamalie, and Mohammad Hossein Sakhaee. Subsequently, the project underwent thorough review and editing, with contributions from Fariba Zabihi, Seyed Ali Shariat Razavi, and Masoumeh Taghdisi Khaboushan. The project was self-funded, and supervision throughout its development was provided by Ghazale Ahmadi. All authors checked and approved the final version of the manuscript for publication in the present journal

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Ethical considerations

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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