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Silver Nanoparticles in Breast Cancer Targeting: A Comprehensive Review

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Abstract

Breast cancer remains a significant global health concern, necessitating advancements in therapeutic strategies. Recent scientific investigations have focused on the potential of silver nanoparticles (AgNPs) as an innovative tool for targeted breast cancer therapy. AgNPs have demonstrated unique properties including a high surface area-to-volume ratio, tunability of size and shape, and superior biocompatibility, rendering them advantageous for drug delivery applications. Importantly, their inherent cytotoxic properties against cancer cells add an extra dimension to their therapeutic efficacy. This review article discusses the synthesis of AgNPs, their potential anticancer effects, and their ability to be utilized as targeted drug delivery systems for breast cancer treatment. It explores the mechanisms by which AgNPs exert their antitumor effects, including the induction of oxidative stress, modulation of signal transduction pathways, and interference with DNA replication. The potential of functionalizing AgNPs with various ligands for targeted delivery to breast cancer cells is also examined, with a particular emphasis on ligands targeting overexpressed receptors on breast cancer cells. The use of AgNPs for co-delivery of chemotherapeutic agents and genes is further discussed. Additionally, the review outlines the current challenges in translating AgNPs into clinical applications, including issues related to their toxicity, biodistribution, and pharmacokinetics. This comprehensive review underscores the promise of AgNPs in revolutionizing breast cancer therapy, while also emphasizing the need for more research to realize their full therapeutic potential.

Keywords: Silver nanoparticles, breast cancer, targeting, drug delivery, silver nanoparticles synthesis, usnic acid, CD44.

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INTRODUCTION

Breast cancer remains one of the most commonly diagnosed cancers worldwide, significantly affecting both developed and developing nations alike. The World Health Organization (WHO) reported over 2 million new cases of breast cancer in 2020, with the disease being the most prevalent among women globally (World Health Organization, 2020). The high incidence of breast cancer, coupled with the heterogeneous nature of the disease, continually necessitates the exploration of novel therapeutic approaches to improve patient outcomes. Breast cancer manifests in several subtypes, each possessing distinct morphological and molecular characteristics, which further complicate disease management (Perou, Sørlie, Eisen et al., 2000). The heterogeneity within breast cancer types often results in variability in response to treatment, hence highlighting the need for personalized therapeutic strategies. The development and application of silver nanoparticles (AgNPs) in breast cancer treatment are some of the recent advancements that cater to these needs.

The field of nanotechnology has revolutionized the healthcare industry in the past few decades, with nanoparticle (NP)-based drug delivery systems showing promise for the treatment of a wide range of diseases, including cancer. Nanoparticles are particles with dimensions in the nanometer scale (1-100 nm). Due to their small size and high surface area-to-volume ratio, NPs can effectively target and penetrate tumor tissues, overcoming the limitations of traditional cancer therapies, such as poor specificity and systemic toxicity (Peer, Karp, Hong et al., 2007).

Among various types of NPs, AgNPs have been the subject of intense research due to their unique physicochemical properties and biological activities. They exhibit potent antimicrobial properties and are used in a broad spectrum of applications, including wound dressings, medical device coatings, and water purification systems (Burdușel, Gherasim, Grumezescu et al., 2018). Notably, the cytotoxic effects of AgNPs against a range of cancer cell types have recently been explored, with many studies showing promising results. The mechanisms underlying the anticancer effects of AgNPs are multifaceted and not fully understood. AgNPs are believed to interact with cellular components and induce cytotoxic effects through various mechanisms, including reactive oxygen species (ROS) generation, mitochondrial dysfunction, cell membrane damage, and DNA damage, among others (AshaRani, Low Kah Mun, Hande, & Valiyaveettil, 2009). The potential of AgNPs to target and kill cancer cells, while sparing normal cells, suggests their suitability as a novel therapeutic agent in cancer treatment.

In the context of breast cancer, the utility of AgNPs is being extensively studied, with a focus on their ability to target specific subtypes of breast cancer cells selectively. In vitro studies demonstrate that AgNPs can induce apoptosis in breast cancer cells, impair cancer cell migration, and potentially overcome drug resistance (Sriram, Kanth, Kalishwaralal, & Gurunathan, 2010). Despite these promising results, further research is necessary to fully comprehend the biological behavior of AgNPs and optimize their therapeutic effects. To summarize, this introductory section presents an overview of the ongoing health crisis that is breast cancer, highlighting the pressing need for novel therapeutic approaches. It outlines the promising role of nanotechnology in this context, focusing on the potential of AgNPs for targeted cancer therapy. The following

sections will delve deeper into the synthesis and characterization of AgNPs, their interactions with cancer cells, their application in breast cancer treatment, and future directions in the field.

SYNTHESIS OF SILVER NANOPARTICLES AND CHARACTERIZATION PARAMETERS

The burgeoning interest in silver nanoparticles (AgNPs) in cancer therapy has stimulated extensive research into the synthesis and characterization of these nanomaterials. The objective is to achieve control over the physicochemical properties (such as size, shape, and surface chemistry) of the AgNPs, as these characteristics largely determine their biological activity and toxicity.

Synthesis Methods

The synthesis of AgNPs can be broadly categorized into three main methods: physical, chemical, and biological (green) synthesis.

Physical synthesis: Physical methods, including evaporation-condensation and laser ablation, are generally utilized to produce AgNPs. The evaporation-condensation process usually occurs in a tube furnace at atmospheric pressure, wherein silver is evaporated into vapor and then cooled to form particles (Iravani, Korbekandi, Mirmohammadi & Zolfaghari, 2014). Laser ablation, on the other hand, involves irradiating a silver metal target submerged in a liquid medium with a laser, leading to the generation of AgNPs (Zeng, Du, Guo, & Li, 2012). These physical methods offer advantages like high purity of the product but are often energy-intensive and require complex equipment.

Chemical synthesis: This method remains the most popular for AgNP synthesis due to its simplicity, high yield, and the ability to control nanoparticle size and shape. It typically involves the reduction of a silver salt (often silver nitrate) in the presence of a reducing agent, such as sodium borohydride, and a stabilizer to prevent nanoparticle aggregation, such as citrate or polyvinylpyrrolidone (PVP) (Kumar, Yadav, Yadav, & Yadav, 2010). However, chemical synthesis often involves the use of toxic chemicals, which could pose environmental and health risks.

Biological (green) synthesis: Given the concerns related to the use of toxic chemicals in traditional synthesis methods, green synthesis using biological agents has emerged as an eco-friendly alternative. Green synthesis employs a range of biological entities, such as plant extracts, bacteria, fungi, or yeast, to reduce silver ions to AgNPs (Mittal, Kaur, & Tavanandi, 2017). For example, the use of plant extracts is well-documented, as they are abundant, safe, and contain a variety of reducing and capping agents. Shankar, Ahmad, & Sastry (2003) reported the synthesis of AgNPs using the reduction capability of the tannic acid present in Geranium leaf extracts.

Characterization Techniques

Once synthesized, AgNPs must be characterized to evaluate their physicochemical properties. These properties are crucial determinants of AgNPs' biological activities. The various characterization parameters are illustrated in Figure 1.

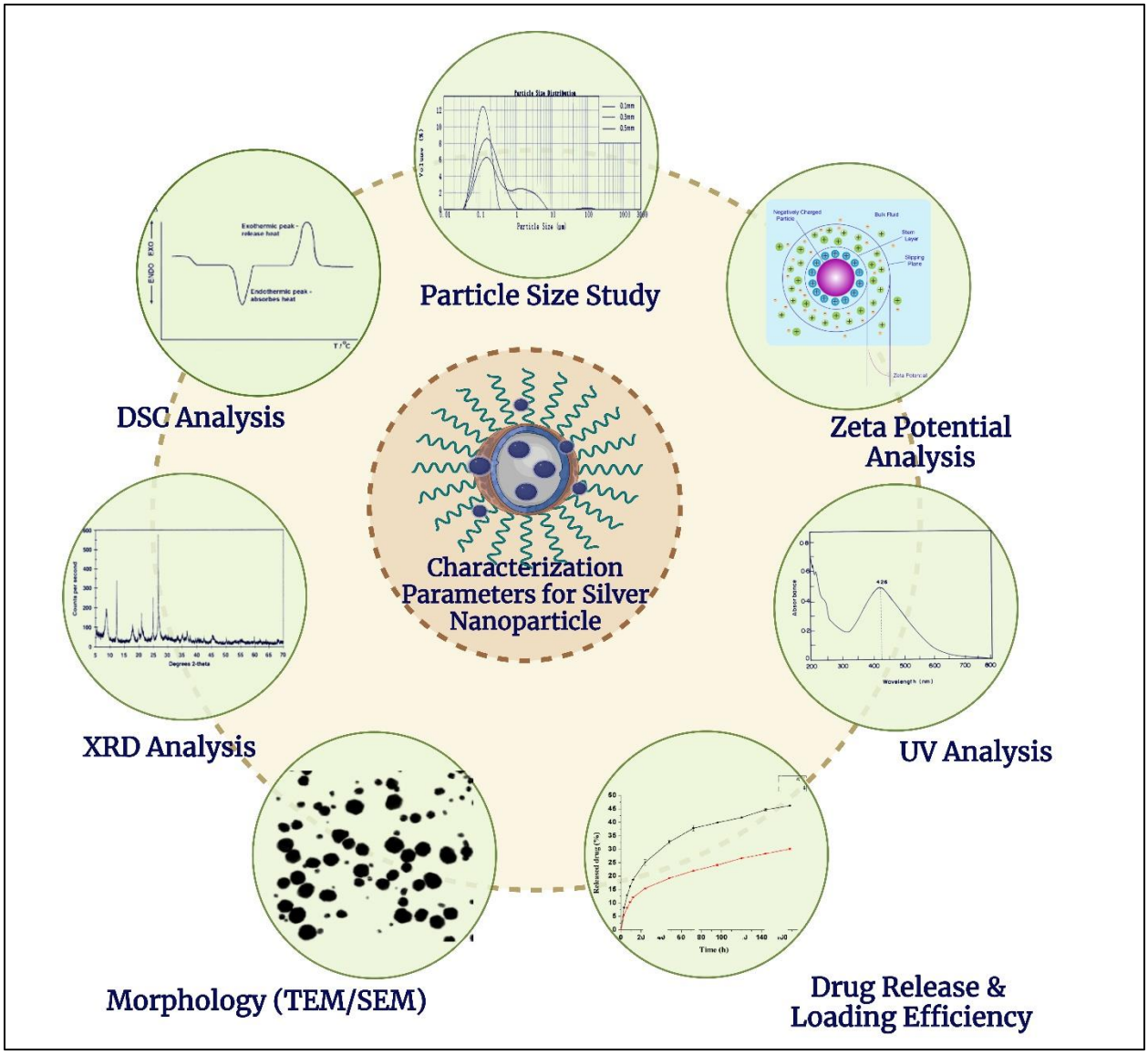

Figure 1. Characterization parameters utilized to assess AgNPs.

UV-Visible Spectroscopy (UV-Vis): This technique is used to verify the formation of AgNPs, relying on the fact that AgNPs exhibit a characteristic surface plasmon resonance (SPR) peak in the UV-Visible range (Amendola & Meneghetti, 2009). Typically, the SPR peak for AgNPs is located around 400-420 nm, although it can vary depending on the size and shape of the nanoparticles.

Transmission Electron Microscopy (TEM): TEM offers high-resolution images that allow for the analysis of the size, shape, and distribution of the nanoparticles (Williams & Carter, 2009). By irradiating a thin specimen with a beam of electrons, TEM enables the visualization of individual nanoparticles and the assessment of their structural characteristics.

Dynamic Light Scattering (DLS) and Zeta Potential Measurement: DLS is commonly used to determine the hydrodynamic size and size distribution of nanoparticles in suspension (Berne &

Pecora, 2000). Zeta potential measurements provide information on the surface charge of the nanoparticles, which is critical for understanding their stability in suspension, as particles with high zeta potential values (positive or negative) are less likely to aggregate due to mutual repulsion (Honary & Zahir, 2013).

X-Ray Diffraction (XRD): XRD analysis can confirm the crystalline nature of the nanoparticles and the absence of impurities. The XRD pattern of AgNPs typically exhibits peaks corresponding to the (111), (200), (220), and (311) planes, indicating the formation of face-centered cubic (fcc) crystalline silver (Borchert, Shevchenko, Robert, Mekis, Kornowski, Grübel, & Weller, 2005).

Fourier-Transform Infrared Spectroscopy (FTIR): FTIR analysis can provide information about the functional groups present on the nanoparticle surface, which is crucial for understanding their interaction with biological entities (Stuart, 2004).

Overall, the synthesis and characterization of AgNPs play a pivotal role in leveraging their potential in various applications, including breast cancer therapy. Careful selection of synthesis method and thorough characterization are crucial to optimize AgNPs' therapeutic efficacy while minimizing potential toxicity.

Importance of DSC and XRD Analysis of Anticancer Drug-Loaded Silver Nanoparticles: Drug-loaded silver nanoparticles (AgNPs) are a prominent research area in the development of targeted cancer therapeutics. Characterizing these particles is critical to understanding their behavior, stability, and efficacy. Two common characterization techniques are differential scanning calorimetry (DSC) and X-ray diffraction (XRD), both of which provide valuable information about the physical and structural properties of the drug-loaded nanoparticles.

Differential Scanning Calorimetry (DSC):

Differential scanning calorimetry is a thermoanalytical technique that measures the difference in the amount of heat required to increase the temperature of a sample and a reference. It is commonly used to determine the melting point, crystallinity, and thermal stability of materials. In the context of drug-loaded AgNPs, DSC can provide information on the physical state of the loaded drug and the interaction between the drug and the nanoparticles. For example, a shift in the melting point or a decrease in the heat of fusion of the drug in the DSC thermogram could indicate the transformation of the drug from a crystalline to an amorphous state or its molecular dispersion in the nanoparticle matrix. These changes can significantly affect the drug's solubility, dissolution rate, and bioavailability (Vippagunta, Brittain, & Grant, 2001).

B. X-ray Diffraction (XRD):

X-ray diffraction is a technique used for determining the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of incident X-rays to diffract into many specific directions. In the case of drug-loaded AgNPs, XRD can be used to confirm the crystallinity of the nanoparticles and the loaded drug, and to identify any changes in the crystal structure due to drug loading. For instance, the disappearance or reduction of the drug's characteristic XRD peaks in the diffractogram of the drug-loaded nanoparticles could indicate the amorphization or molecular dispersion of the drug, which could enhance its dissolution rate and bioavailability. XRD can also confirm the formation of AgNPs by showing characteristic

diffraction peaks at specific angles (Bragg angles) corresponding to the crystal planes of silver (Raveendran, Fu, & Wallen, 2006).

DSC and XRD analysis are crucial for the successful design and optimization of drug-loaded AgNPs. They can provide insights into the drug loading mechanism, the physical state and stability of the loaded drug, the interaction between the drug and the nanoparticles, and the effect of drug loading on the nanoparticles' crystallinity and structure. This information is vital for understanding and predicting the performance of the drug-loaded nanoparticles in terms of drug release, therapeutic efficacy, and stability during storage.

THE INTERACTION OF SILVER NANOPARTICLES WITH CANCER CELLS

Understanding the interaction between silver nanoparticles (AgNPs) and cancer cells is critical to harness their potential in cancer therapy. This interaction encompasses two major aspects: cellular uptake of AgNPs and the mechanism underlying their cytotoxicity.

Uptake of Silver Nanoparticles Towards Cancer Cell

Cellular uptake of nanoparticles involves complex processes and can occur through various pathways. Generally, AgNPs can enter cells via passive diffusion or active endocytosis, which is energy-dependent and includes several types: phagocytosis, macropinocytosis, clathrin-mediated endocytosis, and caveolin-mediated endocytosis (Kumari, Yadav, & Yadav, 2010). The uptake mechanism primarily depends on the physicochemical characteristics of the nanoparticles, such as size, shape, surface charge, and coating, as well as the type of cell and its physiological state (Chithrani, Ghazani, & Chan, 2006). Most studies have shown that smaller nanoparticles tend to have higher cellular uptake. This is mainly because smaller particles have a higher surface-area-to-volume ratio, facilitating interactions with cell membranes. For instance, in a study conducted by AshaRani et al. (2009), smaller AgNPs (10 nm) showed higher uptake and more pronounced cytotoxic effects on human fibroblast cells and human glioblastoma cells compared to larger particles (50 nm and 100 nm). The shape of nanoparticles is also an important factor influencing uptake. Chithrani et al. (2006) reported that rod-shaped nanoparticles had significantly lower uptake than spherical ones, suggesting that cellular entry is more favorable for nanoparticles that minimize distortion of the cell membrane. The surface charge of AgNPs, usually determined by their coating, influences their interaction with the negatively charged cell membrane. AgNPs with a neutral or slight positive charge are often more readily taken up by cells due to the attractive electrostatic interaction with the cell membrane (Kumari et al., 2010).

Mechanism of Silver Nanoparticles' Cytotoxicity

The cytotoxicity of AgNPs against cancer cells is primarily attributed to their ability to induce oxidative stress, leading to cell death through various pathways. The induction of oxidative stress results from the generation of reactive oxygen species (ROS), which cause damage to cellular components, including lipids, proteins, and DNA (AshaRani et al., 2009). AgNPs can interact with the cell membrane, causing physical disruption and altering its permeability. This interaction facilitates the internalization of AgNPs into the cells, leading to further intracellular damage. Once inside the cell, AgNPs can interact with various organelles, including

mitochondria and the endoplasmic reticulum, leading to the generation of ROS, mitochondrial dysfunction, and endoplasmic reticulum stress (Gliga, Skoglund, Wallinder, Fadeel, & Karlsson, 2014). Upon entry into the nucleus, AgNPs can interact with DNA, leading to DNA damage and the activation of DNA damage response pathways. The resulting genomic instability can lead to cell cycle arrest, apoptosis, or necrosis (AshaRani et al., 2009). In addition, AgNPs have been shown to interfere with the mitotic apparatus, leading to abnormalities in cell division and the generation of aneuploid cells (Ferrari et al., 2020). It is also worth noting that the release of silver ions (Ag+) from AgNPs contributes significantly to their cytotoxic effects. These ions can readily interact with thiol (-SH) or amine (-NH2) groups present in proteins, disrupting protein function and leading to cell death (Xiu, Zhang, Puppala, Colvin, & Alvarez, 2012). Understanding these interactions and cytotoxic mechanisms provides valuable insights into the design and optimization of AgNPs for therapeutic applications in cancer. However, it's also critical to evaluate potential toxicity to normal cells and take necessary measures to enhance the selectivity of AgNPs towards cancer cells.

SILVER NANOPARTICLES IN BREAST CANCER

Targeting Mechanism

One of the primary mechanisms through which silver nanoparticles (AgNPs) target breast cancer cells is based on their ability to preferentially accumulate in tumor tissue - a phenomenon referred to as the enhanced permeability and retention (EPR) effect. The EPR effect results from the unique pathophysiological characteristics of tumors, including leaky vasculature and poor lymphatic drainage, which allows nanoparticles to accumulate in tumor tissues more than in normal tissues (Maeda, 2015). This preferential accumulation provides a passive targeting mechanism for AgNPs and other nanoparticles in cancer therapy. In addition to passive targeting, active targeting strategies have been explored to enhance the specificity and effectiveness of AgNPs against breast cancer cells. Active targeting involves the modification of the AgNP surface with ligands that can bind specifically to receptors overexpressed on the surface of breast cancer cells. For instance, the overexpression of human epidermal growth factor receptor 2 (HER2) is observed in approximately 20-30% of breast cancers and is associated with aggressive disease and poor prognosis (Slamon et al., 1987). In a study by Dreaden et al. (2012), AgNPs were conjugated with trastuzumab, a monoclonal antibody against HER2, to target HER2-positive breast cancer cells. The trastuzumab-conjugated AgNPs showed enhanced binding and uptake by HER2-overexpressing cells, leading to increased cytotoxicity compared to non-targeted AgNPs. Similarly, the folate receptor is another potential target for the active targeting of breast cancer cells, as it is overexpressed in several types of cancers, including breast cancer (Parker et al., 2005). Folate-conjugated AgNPs have been shown to be specifically taken up by cancer cells via receptor-mediated endocytosis, leading to enhanced cytotoxicity (Patra et al., 2007). Once inside the cancer cells, AgNPs exert their cytotoxic effects through several mechanisms. One of the primary mechanisms involves the generation of reactive oxygen species (ROS), leading to oxidative stress, damage to cellular structures, and cell death (AshaRani et al.,

2009). AgNPs can also interact with cellular DNA, causing DNA damage and cell cycle arrest (AshaRani et al., 2009). Furthermore, the release of silver ions (Ag+) from AgNPs can interact with proteins and enzymes, disrupting their functions and contributing to the cytotoxic effects (Xiu et al., 2012). However, while the targeting mechanisms of AgNPs provide promising strategies for breast cancer therapy, it's important to note that they also pose potential challenges. For instance, the EPR effect can vary among different tumors and individuals, and not all tumors display a prominent EPR effect (Maeda, 2015). In terms of active targeting, the heterogeneity of breast cancer and the potential for the development of resistance to targeted therapies pose significant challenges (Turner, Reis-Filho, & Russel, 2010). The application of silver nanoparticles (AgNPs) in breast cancer research has gained considerable attention due to their potential in targeted therapy and drug delivery. The following subsections review existing in vitro studies, in vivo studies, and any clinical trials associated with the use of AgNPs in the treatment of breast cancer.

In vitro Studies: In vitro studies offer insights into the cytotoxic effects of AgNPs on different breast cancer cell lines. They allow for a controlled environment where variables can be manipulated and direct cause-and-effect relationships can be established. Studies conducted on various breast cancer cell lines, including MCF-7, MDA-MB-231, and 4T1, have shown that AgNPs can induce cell death, reduce cell proliferation, and impair migration and invasion of cancer cells. Sriram et al. (2010) explored the antitumor activity of biologically synthesized AgNPs against the MCF-7 breast cancer cell line. Their results demonstrated significant dose-dependent cytotoxicity, with AgNPs inducing apoptosis (programmed cell death) as evidenced by morphological changes, DNA fragmentation, and an increase in the sub-G1 cell population. In a study by Satapathy et al. (2013), AgNPs were found to exert their cytotoxic effects on MCF-7 cells by increasing intracellular reactive oxygen species (ROS) levels, leading to oxidative stress, mitochondrial dysfunction, and, ultimately, cell death. They also observed an increase in the expression of the p53 protein, a crucial regulator of the cell cycle, suggesting that AgNPs might induce cell cycle arrest and apoptosis through the p53 pathway. Another interesting area of investigation is the potential of AgNPs to overcome drug resistance, a major challenge in breast cancer treatment. Research conducted by Franco-Molina et al. (2016) showed that AgNPs could potentiate the cytotoxic effect of doxorubicin on a doxorubicin-resistant MDA-MB-231 breast cancer cell line, providing promising implications for the treatment of drug-resistant breast cancer.

In vivo Studies: While in vitro studies provide valuable insights, they do not fully mimic the complex biological environment in living organisms. Therefore, in vivo studies using animal models are crucial for validating the findings from in vitro experiments and evaluating the therapeutic efficacy and toxicity of AgNPs. In a study by Zhang et al. (2016), mice bearing 4T1 murine breast cancer cells were treated with AgNPs. The results demonstrated that AgNPs effectively inhibited tumor growth without causing significant body weight changes or organ damage, suggesting their potential as safe antitumor agents. However, despite the promising results from in vitro and in vivo studies, it's important to note that AgNPs can also exhibit

toxicity to normal cells and tissues. Studies have shown that AgNPs can induce liver and kidney damage in rats, likely due to the generation of ROS and oxidative stress (Rahman, Siddiqui, & Khan, 2019). These findings underscore the need for careful consideration of the potential toxicity of AgNPs when designing them for therapeutic applications.

CONJUGATED SILVER NANOPARTICLES FOR CANCER TARGETING

Designing nanoparticles for targeted cancer therapy involves surface modification with various biological and synthetic materials. One such approach involves the coating of silver nanoparticles (AgNPs) with biocompatible polymers and bioactive molecules such as chondroitin sulfate (CS) and hyaluronic acid (HA).

Role of Polymers in Nanoparticle Modification

Polymers, due to their versatility and diverse range of properties, are often used as surface modifiers for nanoparticles. They can enhance the stability of nanoparticles, prevent their aggregation, prolong their circulation time in the body, and provide functional groups for further modification (Kolate, Baradia, Patil, Vhora, & Kore, 2014). Commonly used polymers for nanoparticle modification include polyethylene glycol (PEG), polylactic-co-glycolic acid (PLGA), chitosan, and others.

Chondroitin Sulfate (CS)

Chondroitin sulfate is a sulfated glycosaminoglycan composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid). It is found widely in the extracellular matrix of animal tissues, especially in cartilage. CS is biocompatible, biodegradable, non-toxic, and has been used in various biomedical applications, including drug delivery, tissue engineering, and regenerative medicine (Volpi, 2007). In cancer therapy, CS has shown promise as a targeting agent due to its ability to bind specifically to various receptors overexpressed on cancer cells. For instance, CS can bind to CD44, a cell surface glycoprotein that is overexpressed in several types of cancer and plays a critical role in cancer cell proliferation, migration, and angiogenesis (Yu et al., 2008).

Hyaluronic Acid (HA)

Hyaluronic acid, another naturally occurring glycosaminoglycan, has been widely used in nanoparticle surface modification due to its biocompatibility, biodegradability, and non-immunogenicity. Similar to CS, HA can bind to CD44, as well as other receptors like the receptor for hyaluronic acid-mediated motility (RHAMM) and the hyaluronan receptor for endocytosis (HARE), making it a promising ligand for targeted cancer therapy (Heldin, Basu, Olofsson, & Porsch, 2013). The modification of AgNPs with HA has shown promising results in enhancing their selectivity and uptake by cancer cells. For instance, in a study by Lee et al. (2011), HA-modified AgNPs were shown to be preferentially taken up by CD44-overexpressing cancer cells and induced significant cytotoxicity.

The combination of polymers, CS, and HA for the surface modification of AgNPs can provide several advantages. The polymer can enhance the stability and circulation time of AgNPs, while CS and HA can improve their selectivity towards cancer cells. This combined

approach could potentially enhance the therapeutic efficacy of AgNPs, reduce their potential toxicity to normal cells, and overcome the barriers encountered by nanoparticles in the body. However, the design and optimization of polymer/CS/HA-modified AgNPs require careful consideration of several factors, such as the size, shape, and surface charge of the nanoparticles, as well as the type and density of the targeting ligands. Furthermore, in vitro and in vivo evaluations are necessary to assess their targeting efficiency, cytotoxic effects, and potential toxicity. In conclusion, the use of polymers, CS, and HA for the surface modification of AgNPs provides a promising strategy for targeted cancer therapy. However, further research is necessary to fully exploit the potential of these modified nanoparticles and translate them into clinically viable therapeutic agents. Figure 2 explained the methodology of synthesis of polymer modified silver nanoparticles loaded with anticancer drugs and its mechanism of action towards the breast cancer cell.

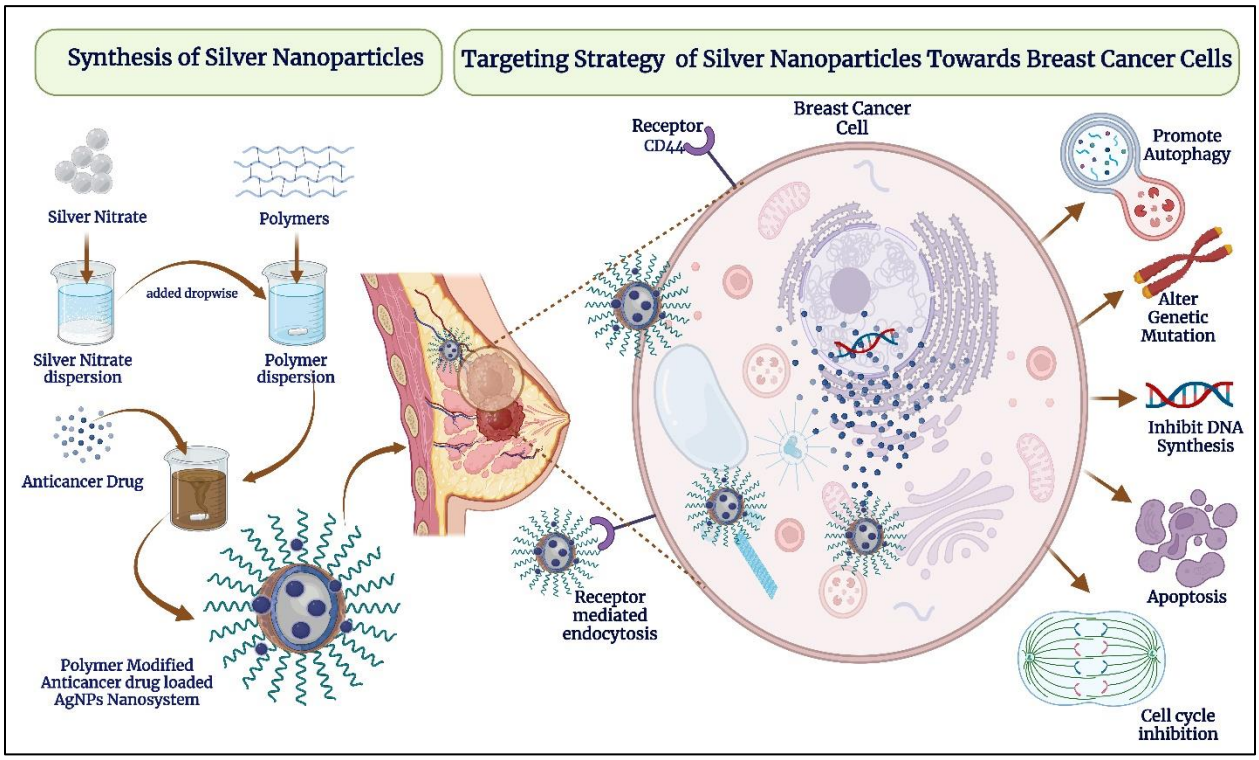

Figure 2. Methodology of synthesis of polymer modified silver nanoparticles loaded with anticancer drugs and its mechanism of action towards the breast cancer cell.

INTERACTION OF SILVER NANOPARTICLES WITH CD44 RECEPTOR AND OTHER RECEPTORS ON BREAST CANCER CELL SURFACES

The interaction of nanoparticles with specific cell surface receptors plays a pivotal role in nanoparticle-based targeted cancer therapy. The CD44 receptor, among others, is frequently overexpressed on the surface of cancer cells, including breast cancer cells, making it an attractive target for nanoparticles such as silver nanoparticles (AgNPs).

CD44 Receptor

The CD44 receptor is a transmembrane glycoprotein involved in a variety of cellular processes, including cell-cell and cell-matrix adhesion, cell migration, and signal transduction (Ponta, Sherman, & Herrlich, 2003). CD44 is expressed in many types of cells but is particularly upregulated in several types of cancer, including breast cancer. Importantly, CD44 has been identified as a marker of cancer stem cells, a subpopulation of cancer cells believed to be responsible for cancer initiation, progression, metastasis, and resistance to therapy (Al-Hajj, Wicha, Benito-Hernandez, Morrison, & Clarke, 2003).

Interaction of AgNPs with CD44 Receptor

The interaction of AgNPs with the CD44 receptor can be enhanced by surface modification of the nanoparticles with ligands that can bind specifically to CD44. Hyaluronic acid (HA) and chondroitin sulfate (CS) are two such ligands, as they are natural ligands for CD44 and can be readily conjugated onto the surface of AgNPs (Banerjee, Mitra, Singh, & Maitra, 2012). Once the HA- or CS-modified AgNPs bind to CD44, they can be internalized by the cancer cells through receptor-mediated endocytosis. This specific binding and uptake of AgNPs can enhance their cytotoxic effects against cancer cells by increasing the intracellular concentration of silver ions, which can interact with cellular proteins and DNA, leading to cell death (AshaRani, Low, Hande, & Valiyaveettil, 2009).

Other Receptors on Breast Cancer Cell Surfaces

In addition to CD44, other receptors are overexpressed on the surface of breast cancer cells and can be targeted by AgNPs. For instance, the human epidermal growth factor receptor 2 (HER2) is overexpressed in approximately 20-30% of breast cancers and is associated with aggressive disease and poor prognosis (Slamon et al., 1987). AgNPs can be conjugated with antibodies or peptides that bind specifically to HER2, enhancing their selectivity and cytotoxicity towards HER2-overexpressing breast cancer cells (Dreaden, Alkilany, Huang, Murphy, & El-Sayed, 2012).

The estrogen receptor (ER) and progesterone receptor (PR) are also important targets in breast cancer. Hormone therapy, which involves drugs that block the effects of estrogen or lower estrogen levels, is a common treatment for ER-positive or PR-positive breast cancers. Interestingly, a study by Shahverdi et al. (2007) suggested that AgNPs might have anti-estrogenic effects, indicating potential interactions between AgNPs and hormone receptors in breast cancer.

While the interaction of AgNPs with specific receptors provides a promising strategy for targeted breast cancer therapy, several considerations need to be taken into account. The heterogeneity of breast cancer, with varying expression levels of different receptors among patients and even among different cells within a tumor, poses a significant challenge to receptor-targeted therapies (Turner, Reis-Filho, & Russel, 2010). Furthermore, the potential for the development of resistance to targeted therapies and the potential toxicity of AgNPs to normal cells highlight the need for further research to optimize the design and application of AgNPs in breast cancer therapy.

In conclusion, the interaction of AgNPs with CD44 and other receptors on the surface of breast cancer cells offers exciting opportunities for targeted therapy. Ongoing research in this area could lead to more effective and safer therapeutic options for patients with breast cancer.

DRUG ENTRAPMENT/LOADING IN SILVER NANOPARTICLES FOR TUMOR TARGETING

Silver nanoparticles (AgNPs) are promising vehicles for targeted drug delivery due to their unique properties such as high surface area, tunable size, and ease of surface modification. Entrapping or loading anticancer drugs in AgNPs can provide several benefits, including enhanced drug stability, controlled drug release, and improved therapeutic efficacy.

Mechanism of Drug Loading

Drug loading onto AgNPs can be achieved either by encapsulation during nanoparticle synthesis (in situ loading) or by adsorption onto the nanoparticle surface after synthesis (post-loading).

In Situ Loading: In this method, the drug is introduced during the synthesis of the nanoparticles. The drug molecules can interact with silver ions (Ag+) and be incorporated into the nanoparticles as they form. In situ loading can provide high drug loading efficiency and prevent drug leakage during storage and transportation. However, this method may not be suitable for all drugs, especially those that can interfere with the nanoparticle synthesis process (Zhang et al., 2008).

Post-Loading: This method involves the adsorption or conjugation of the drug onto the surface of pre-formed nanoparticles. The drug can be physically adsorbed onto the nanoparticle surface through electrostatic, hydrophobic, or van der Waals interactions, or chemically conjugated to functional groups on the nanoparticle surface. Post-loading allows for more control over the amount of drug loaded and can be used for a wide range of drugs. However, the drug loading efficiency can be lower compared to in situ loading, and the loaded drug may be susceptible to premature leakage (Patel et al., 2012).

Advantages of Drug-Loaded AgNPs in Tumor Targeting

Enhanced Drug Stability: Drug-loaded AgNPs can protect the entrapped drug from degradation in the physiological environment, enhancing its stability and prolonging its half-life in the body.

Controlled Drug Release: AgNPs can provide controlled and sustained release of the loaded drug. The drug release rate can be tuned by controlling the nanoparticle size, surface charge, and the type and density of the polymer coating on the nanoparticle surface (Mura, Nicolas, & Couvreur, 2013).

Improved Therapeutic Efficacy: Drug-loaded AgNPs can increase the therapeutic efficacy of the drug by enhancing its delivery to the tumor site. This can be achieved through passive targeting based on the enhanced permeability and retention (EPR) effect, or active targeting by conjugating tumor-targeting ligands onto the nanoparticle surface (Maeda, 2015).

Several studies have demonstrated the potential of drug-loaded AgNPs in tumor targeting. For instance, Zhang et al. (2014) developed doxorubicin-loaded AgNPs and demonstrated their enhanced cytotoxicity against breast cancer cells compared to free doxorubicin. In another study,

Rani et al. (2017) reported the synthesis of curcumin-loaded AgNPs and showed their potent anticancer effects against cervical cancer cells.

While drug-loaded AgNPs hold promise for tumor targeting, several challenges need to be addressed. These include optimizing the drug loading efficiency, controlling the drug release rate, minimizing drug leakage, and ensuring the biocompatibility and safety of the nanoparticles. Further research is necessary to fully exploit the potential of drug-loaded AgNPs and translate them into clinically viable therapeutic agents.

Encapsulation or Adsorption of Anticancer Drugs in Silver Nanoparticles

The incorporation of anticancer drugs into silver nanoparticles (AgNPs) can enhance the therapeutic potential of the drugs, providing controlled drug release, increased stability, and targeted delivery. Three notable anticancer drugs that have been encapsulated or adsorbed in AgNPs are curcumin, doxorubicin, and paclitaxel.

Curcumin-Loaded AgNPs

Curcumin, a natural compound derived from turmeric, has shown promising anticancer properties. However, its poor solubility and low bioavailability limit its therapeutic potential. To overcome these limitations, curcumin can be encapsulated or adsorbed into AgNPs.

In a study by Shankar et al. (2019), curcumin was loaded into AgNPs using a green synthesis approach, where the curcumin served as both a reducing and stabilizing agent. The curcumin-loaded AgNPs showed enhanced cytotoxicity against various cancer cell lines compared to free curcumin, highlighting the benefits of AgNPs in improving the therapeutic efficacy of curcumin.

Doxorubicin-Loaded AgNPs

Doxorubicin is a widely used chemotherapy drug. However, it has significant side effects, including cardiotoxicity, and many cancer cells develop resistance to it. Loading doxorubicin into AgNPs can help to improve its therapeutic index and overcome drug resistance.

Zhang et al. (2014) developed doxorubicin-loaded AgNPs using a two-step process. First, AgNPs were synthesized using a chemical reduction method. Then, doxorubicin was loaded onto the AgNPs through physical adsorption. The doxorubicin-loaded AgNPs showed enhanced cytotoxicity against breast cancer cells compared to free doxorubicin, suggesting that AgNPs can enhance the anticancer effects of doxorubicin.

Paclitaxel-Loaded AgNPs

Paclitaxel is a potent anticancer drug, but it is poorly soluble in water and can cause severe side effects, including neuropathy. The encapsulation of paclitaxel in AgNPs can improve its solubility and reduce its toxicity to normal cells.

In a study by Mitra et al. (2016), paclitaxel was loaded into AgNPs using a solvent displacement technique. The paclitaxel-loaded AgNPs showed controlled drug release and enhanced cytotoxicity against lung cancer cells compared to free paclitaxel.

The encapsulation or adsorption of anticancer drugs into AgNPs provides a promising strategy for improving the therapeutic efficacy of the drugs. However, several challenges need to be addressed, including optimizing the drug loading efficiency, controlling the drug release rate, and ensuring the biocompatibility and safety of the nanoparticles. Further research is needed to

fully exploit the potential of drug-loaded AgNPs and translate them into clinically viable therapeutic agents.

Encapsulation/Adsorption of Usnic Acid in Silver Nanoparticles

Usnic acid, a natural compound extracted from lichens, has been shown to possess various pharmacological activities, including antibacterial, antiviral, anti-inflammatory, and antitumor activities. However, similar to many other natural compounds, the therapeutic application of usnic acid is limited by its poor solubility and bioavailability. Encapsulating or adsorbing usnic acid into silver nanoparticles (AgNPs) can enhance its therapeutic potential. The encapsulation or adsorption of usnic acid into AgNPs involves the incorporation of usnic acid either during the synthesis of AgNPs (in situ loading) or after their synthesis (post-loading). The usnic acid molecules can interact with the silver ions and be entrapped within the nanoparticles as they form, or they can be adsorbed onto the surface of pre-formed nanoparticles. The usnic acid-loaded AgNPs can provide several advantages. First, they can enhance the solubility and bioavailability of usnic acid, thus improving its pharmacological effects. Second, the AgNPs can provide controlled and sustained release of usnic acid, optimizing its therapeutic effects. Third, the AgNPs can facilitate the delivery of usnic acid to specific target sites, improving its therapeutic efficacy and reducing its side effects. Moreover, the combination of usnic acid and AgNPs can provide synergistic effects. Both usnic acid and AgNPs have been shown to possess antimicrobial and anticancer activities. Therefore, the usnic acid-loaded AgNPs can have enhanced antimicrobial and anticancer effects compared to usnic acid or AgNPs alone. In a study by Fernandes et al. (2016), usnic acid was loaded into AgNPs using a simple one-pot method. The usnic acid-loaded AgNPs showed enhanced antibacterial activity compared to free usnic acid, highlighting the potential of AgNPs in enhancing the therapeutic efficacy of usnic acid.

However, further research is necessary to fully exploit the potential of usnic acid-loaded AgNPs. Factors such as the size, shape, surface charge, and stability of the nanoparticles, as well as the loading efficiency and release rate of usnic acid, need to be optimized to achieve the desired therapeutic effects.

Usnic Acid as an Anticancer Drug Candidate and its Use in Silver Nanoparticles:

Usnic acid, a naturally occurring compound found in several lichen species, has drawn considerable interest in recent years due to its broad spectrum of biological activities, including antimicrobial, anti-inflammatory, and antiviral properties. Importantly, several studies have indicated that usnic acid possesses potential anticancer activity, suggesting its promise as an anticancer drug candidate.

Usnic acid has been found to exhibit cytotoxic activity against a variety of cancer cells, including lung, breast, and colon cancer cells. The anticancer activity of usnic acid has been attributed to its ability to interfere with multiple cellular pathways involved in cell proliferation and survival. For instance, studies have shown that usnic acid can induce apoptosis, a form of programmed cell death that is often deregulated in cancer cells (Liu, Zou, Lu, & Zeng, 2018). Usnic acid has been reported to trigger apoptosis by altering the balance of pro-apoptotic and anti-apoptotic proteins, leading to the activation of caspases, a family of proteins that play a

crucial role in executing apoptosis. Moreover, usnic acid has been shown to inhibit angiogenesis, the formation of new blood vessels that tumors need for growth and metastasis (Feng, Jiang, Yang, Li, & Li, 2019). Usnic acid can downregulate the expression of angiogenic factors, including vascular endothelial growth factor (VEGF), and disrupt the vascular network in tumors. Despite its promising anticancer activity, the therapeutic potential of usnic acid is limited by its poor solubility and bioavailability, as well as its potential hepatotoxicity at high doses. To overcome these limitations, usnic acid can be incorporated into nanocarriers such as silver nanoparticles (AgNPs).

AgNPs have been extensively investigated as drug delivery systems due to their unique properties, including their high surface area, tunable size, and ease of surface modification. Encapsulating or adsorbing usnic acid into AgNPs can enhance its therapeutic potential.

The usnic acid-loaded AgNPs can provide several advantages. First, they can enhance the solubility and bioavailability of usnic acid, improving its pharmacokinetics and pharmacodynamics. Second, the AgNPs can provide controlled and sustained release of usnic acid, optimizing its therapeutic effects. Third, the AgNPs can facilitate the delivery of usnic acid to specific target sites, such as tumors, improving its therapeutic efficacy and reducing its side effects.

Furthermore, the combination of usnic acid and AgNPs can provide synergistic effects. AgNPs themselves have been shown to possess antimicrobial and anticancer activities. Therefore, the usnic acid-loaded AgNPs can have enhanced anticancer effects compared to usnic acid or AgNPs alone. While the potential of usnic acid-loaded AgNPs is promising, further research is necessary to fully exploit their potential. Factors such as the size, shape, surface charge, and stability of the nanoparticles, as well as the loading efficiency and release rate of usnic acid, need to be optimized to achieve the desired therapeutic effects. Moreover, the safety profile of usnic acid-loaded AgNPs needs to be thoroughly evaluated before they can be translated into clinically viable therapeutic agents. Overall, while usnic acid shows promise as an anticancer drug candidate, its full potential can be unlocked by utilizing nanoparticle technology, such as AgNPs, to overcome its limitations and enhance its anticancer activity.

CHALLENGES AND FUTURE DIRECTIONS

While silver nanoparticles (AgNPs) hold great promise in breast cancer treatment, it's important to address potential challenges and discuss future directions. As we move towards the clinical application of AgNPs, it becomes critical to consider their potential toxicity, understand the challenges in drug delivery, and determine the future course of research. ***Toxicity Concerns:*** A significant concern with AgNPs is their potential toxicity to healthy cells and the environment. The same properties that make AgNPs effective against cancer cells (e.g., generation of reactive oxygen species (ROS), interaction with proteins and DNA) can also lead to harm in non-target cells and tissues. Indeed, studies have shown that AgNPs can induce cytotoxicity in a variety of non-cancerous cells, such as human fibroblasts and human mesenchymal stem cells, through mechanisms similar to those observed in cancer cells (AshaRani, Mun, Hande, & Valiyaveettil, 2009). Furthermore, when used in vivo, AgNPs can accumulate in various organs, including the

liver, kidney, and spleen, potentially causing organ damage (Loeschner, Hadrup, Qvortrup, Larsen, Gao, Vogel, Mortensen, Lam, & Larsen, 2011). Therefore, it's crucial to evaluate the systemic toxicity of AgNPs in preclinical animal models and eventually in clinical trials to ensure their safety. AgNPs also pose potential environmental risks. They are increasingly used in a wide range of consumer products due to their antimicrobial properties, leading to their release into the environment (Chen & Schluesener, 2008). This raises concerns about their effects on aquatic and terrestrial ecosystems. Studies have shown that AgNPs can be toxic to various aquatic organisms, including algae, crustaceans, and fish, and can affect plant growth and soil microorganisms (Navarro, Baun, Behra, Hartmann, Filser, Miao, Quigg, Santschi, & Sigg, 2008). Therefore, the environmental impact of AgNPs must be carefully considered and monitored. ***Drug Delivery Challenges:*** Despite the advancements in nanotechnology, efficient and targeted delivery of nanoparticles, including AgNPs, to tumor sites remains a significant challenge. The biological barriers encountered by nanoparticles in the body, such as the mononuclear phagocyte system (MPS), blood vessels, extracellular matrix, and tumor interstitial pressure, can limit their accumulation in tumors (Blanco, Shen, & Ferrari, 2015). One strategy to overcome these barriers is to modify the surface of AgNPs with various ligands that can enhance their stability, prolong their circulation time, and improve their selectivity towards cancer cells. For example, the use of polyethylene glycol (PEG) to form a "stealth" coating on the surface of AgNPs can help evade the MPS and enhance their blood circulation time (Blanco et al., 2015). Also, the attachment of tumor-targeting ligands, such as antibodies or peptides, to the nanoparticle surface can enhance their selectivity and uptake by cancer cells (Bertrand, Wu, Xu, Kamaly, & Farokhzad, 2014). ***Future Directions:*** As research progresses, the focus should be on optimizing the design of AgNPs to maximize their therapeutic efficacy while minimizing their potential toxicity. This includes controlling their physicochemical properties (such as size, shape, and surface chemistry) and exploring different surface modifications for targeted delivery.

Moreover, the combination of AgNPs with other anticancer agents could be a promising strategy for enhancing therapeutic outcomes. Studies have shown that AgNPs can enhance the efficacy of various chemotherapeutic drugs, including doxorubicin and cisplatin, against breast cancer cells (Khan, Khan, & Chen, 2017). The synergy between AgNPs and these drugs could lead to improved treatment efficacy, potentially allowing for dose reduction and minimizing side effects.

Conclusion

In recent years, the use of nanotechnology for cancer treatment has become a promising area of research. Among various nanoparticles, silver nanoparticles (AgNPs) have attracted considerable attention due to their unique properties, such as their high surface-area-to-volume ratio, tunable size and shape, and surface chemistry. AgNPs have been shown to exert potent cytotoxic effects on a variety of cancer cells, including breast cancer cells. Evidence from in vitro and in vivo studies suggests that AgNPs can induce cell death in cancer cells through mechanisms involving the generation of reactive oxygen species (ROS), damage to cellular structures (such as the cell

membrane, mitochondria, and DNA), and interference with the cell cycle and mitotic apparatus. Importantly, AgNPs have also shown promise in overcoming drug resistance, a major challenge in cancer treatment. However, while the therapeutic potential of AgNPs is promising, their application in cancer treatment is not without challenges. The potential toxicity of AgNPs to healthy cells and the environment raises safety concerns. Therefore, future research should aim to optimize the design of AgNPs to maximize their therapeutic efficacy while minimizing their potential toxicity. Moreover, efficient and targeted delivery of AgNPs to tumor sites remains a significant challenge. The modification of the nanoparticle surface with various ligands can help improve their stability, prolong their circulation time, and enhance their selectivity towards cancer cells. Furthermore, the combination of AgNPs with other anticancer agents could enhance therapeutic outcomes and potentially allow for dose reduction, thus minimizing side effects. In conclusion, AgNPs hold promise for the future of breast cancer treatment. However, their successful clinical application requires further research to fully understand their biological behavior, optimize their therapeutic properties, and address potential toxicity concerns. The development and clinical application of AgNPs for cancer treatment represent an exciting area of ongoing and future research.

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