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REVIEW ARTICLE

Hydroxyapatite-Based Targeted Drug Delivery Systems for HER2-Positive Breast Cancer: The Role of Trastuzumab and Folic Acid Functionalization

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ABSTRACT

Worldwide, breast cancer continues to be one of the most frequently diagnosed malignancies and continues to pose a significant challenge in cancer therapy. Despite the significant improvement in patient survival that HER2-targeted therapies, such as trastuzumab, have achieved, their therapeutic efficacy remains restricted by challenges such as cardiotoxicity, acquired resistance, limited tumor accumulation, and off-target effects. Recent developments in drug delivery systems have concentrated on the enhancement of treatment outcomes by utilizing targeted delivery strategies and controlled drug release. Among various carrier materials, hydroxyapatite (HAp) has attracted considerable attention due to its excellent biocompatibility, bioactivity, porous structure, high drug-loading capacity, and ease of surface functionalization. These characteristics make HAp a promising candidate for the development of advanced drug delivery platforms capable of enhancing therapeutic efficiency while minimizing systemic side effects. Furthermore, surface modification with targeting ligands such as folic acid offers additional opportunities for improving local cellular recognition and targeted drug delivery. This review summarizes current developments in hydroxyapatite-based drug delivery systems for breast cancer therapy, with particular emphasis on trastuzumab delivery, folic acid-mediated targeting approaches, and hydroxyapatite microsphere technologies. Recent studies investigating HAp-based carriers, targeted delivery systems, and multifunctional therapeutic platforms are discussed, together with their advantages, limitations, and clinical potential. In addition, the role of microsphere-based delivery systems in achieving sustained drug release and enhanced therapeutic performance is highlighted. Current literature demonstrates the potential of hydroxyapatite carriers for the delivery of various therapeutic agents; however, studies integrating trastuzumab delivery, folic acid-mediated targeting, and hydroxyapatite microspheres within a single platform remain limited. This gap highlights the need for multifunctional carrier systems capable of combining controlled release with selective cellular recognition and targeted delivery. The available literature suggests that hydroxyapatite microspheres may provide a versatile platform for HER2-targeted therapy, with trastuzumab potentially incorporated as a therapeutic cargo or used as a surface-bound targeting ligand. These approaches differ in their intended functions and may enable controlled drug release and HER2-mediated targeting, respectively.

Keywords: *Hydroxyapatite; microspheres; drug delivery system; HER2-positive breast cancer*

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1. Introduction

Breast cancer is still one of the most common malignancies and a leading cause of cancer-related mortality among women worldwide. According to global cancer statistics, breast cancer accounted for approximately 2.3 million new cases in 2022, representing one of the most frequently diagnosed cancers among women. Breast cancer remains associated with substantial morbidity and mortality, despite significant progress in early diagnosis and therapeutic strategies (Bray et al., 2024). Breast cancer is a heterogeneous disease consisting of multiple molecular subtypes with biological characteristics and clinical outcomes. Among these subtypes, human epidermal growth factor receptor 2 (HER2)-positive breast cancer is characterized by amplification and/or overexpression of HER2. Breast cancers with HER2 overexpression and/or amplification account for approximately 15–20% of cases and constitute a highly aggressive subtype (Pupa et al., 2021).

Current treatment approaches for breast cancer include surgery, radiotherapy, chemotherapy, endocrine therapy, immunotherapy, and targeted therapies. The selection of treatment strategies depends on tumor subtype, disease stage, receptor expression profile, and patient-specific factors (Waks & Winer, 2019). Although these therapeutic modalities have significantly improved patient survival, many conventional treatments may be associated with off-target exposure and treatment-related toxicity. For example, trastuzumab treatment is associated with cardiac toxicity, highlighting the need to consider treatment-related adverse effects in HER2-targeted therapy (Early Breast Cancer Trialists' Collaborative Group, 2021).

As a result of the development of HER2-targeted therapies, clinical outcomes for HER2-positive breast cancer have improved significantly. Nowadays, the most successful of these targeted therapies is trastuzumab, a monoclonal antibody that specifically targets HER2 receptors. However, trastuzumab treatment may be associated with cardiotoxicity, which remains an important challenge in its clinical use (Early Breast Cancer Trialists' Collaborative Group, 2021; Gu et al., 2024). These challenges have led to research into developing new drug delivery systems aimed at minimizing systemic side effects and enhancing therapeutic effects.

Among the various carrier systems investigated for cancer therapy, hydroxyapatite (HAp)-based microspheres have attracted considerable interest because of their biocompatibility, bioactivity, porous structure, and potential for drug loading and surface functionalization (Hou et al., 2022; H. Huang et al., 2020). In parallel, active targeting strategies using ligands such as folic acid (FA) have been explored in cancer drug delivery systems to enhance receptor-mediated cellular recognition and targeting selectivity (Sudimack & Lee, 2000). This review summarizes current literature on hydroxyapatite-based drug delivery systems for breast cancer therapy, with particular emphasis on microsphere formulations, folic acid-mediated targeting, and the feasibility of trastuzumab incorporation or surface conjugation for HER2-directed treatment. Current evidence, technological limitations, and key translational challenges are also discussed to identify priorities for future research.

1.1 Scope and Literature Search Strategy

A literature search was conducted to identify relevant studies published between 2000 and 2025 on hydroxyapatite-based drug delivery systems, hydroxyapatite microspheres, breast cancer, HER2-targeted therapy, trastuzumab, folic acid-mediated targeting, and related therapeutic

strategies. An extensive literature search was conducted in PubMed, Scopus, and Google Scholar databases to evaluate the current developments in hydroxyapatite-based drug delivery systems for HER2-positive breast cancer. The literature review was conducted with a particular focus on specific research areas, using the primary keywords "hydroxyapatite," "hydroxyapatite microspheres," "breast cancer," "HER2," "trastuzumab," "folic acid," "targeted drug delivery," and "controlled drug release." Inclusion criteria excluded pill carriers and decontextualized conference abstracts among these publications, as well as studies unrelated to drug delivery or targeted therapy. In addition to these reviews, about 200 or more records were scanned to define the boundaries, and 70 representative and peer-reviewed studies were selected.

2. HER2-Positive Breast Cancer and Targeted Therapies

HER2 belongs to the ErbB family of receptor tyrosine kinases and plays a crucial role in regulating cell proliferation, differentiation, migration, and survival. Amplification of the HER2 gene results in excessive receptor expression, leading to continuous activation of downstream signaling pathways such as PI3K/Akt and MAPK, ultimately promoting tumor progression and metastasis. The emergence of HER2-targeted therapeutics has completely transformed the management of this disease subtype. Trastuzumab is a humanized monoclonal antibody that selectively binds to the extracellular domain of HER2 receptors. Through receptor blockade, trastuzumab inhibits HER2-mediated signaling pathways, induces antibody-dependent cellular cytotoxicity (ADCC), and suppresses tumor growth (Nami et al., 2018). When trastuzumab is immobilized on the surface of a carrier like HAp, it's important to maintain the antibody's orientation and biological activity. Random adsorption or non-specific covalent binding can cause antibodies to have heterogeneous orientations, which can reduce accessibility of the Fab regions that recognize the antigen and affect HER2 recognition. Also, trastuzumab-mediated ADCC involves the interaction of the antibody's Fc region with Fcγ receptors on immune effector cells, especially natural killer cells, so the immobilization strategy needs to preserve the Fc region's accessibility and functional integrity (Collins et al., 2012; Wong & Lee, 2012). Directed immobilization strategies may help reduce steric hindrance and keep the antibody's binding sites accessible. In this context, Fc-directed conjugation, affinity-based immobilization, or linker-mediated attachment can be considered potential approaches for controlling the orientation of antibodies on the carrier surface (Nieto et al., 2020; Selepe et al., 2024). Additionally, it has been experimentally shown that proteins can be immobilized in a directed manner on HAp surfaces using bifunctional bisphosphonate linkers, which supports the feasibility of controlled presentation of proteins on HAp surfaces (Yewle et al., 2012). Nevertheless, whether surface-immobilized trastuzumab retains its HER2-binding and Fc-mediated effector functions after conjugation to HAp requires experimental validation.

In addition to trastuzumab, several HER2-targeted agents have been developed, including pertuzumab, lapatinib, trastuzumab deruxtecan, and ado-trastuzumab emtansine (T-DM1). These therapies have significantly improved progression-free survival and overall survival in HER2-positive breast cancer patients (Gu et al., 2024; Zheng et al., 2023). Although antibody-drug conjugates (ADCs) such as trastuzumab deruxtecan and T-DM1 have successfully achieved drug delivery targeting HER2 in clinical practice, HAp-based systems can offer different and potentially complementary delivery approach. Unlike conventional ADCs, HAp

carriers provide a porous matrix that allows the transport of therapeutic agents via encapsulation or surface loading, while also enabling controlled release and additional surface functionalization (De Lama-Odría et al., 2022; Dong et al., 2023). This modular structure may allow the integration of multiple functional components within a single carrier and provide greater flexibility in regulating drug loading and release characteristics. Therefore, HAp-based platforms should be considered as an alternative and potentially complementary strategy to established HER2-targeted therapies rather than as a replacement for clinically approved ADCs.

Despite these advances, several limitations remain. One of the major concerns associated with trastuzumab therapy is cardiotoxicity, which may lead to left ventricular dysfunction and heart failure in susceptible patients. Importantly, trastuzumab-associated cardiotoxicity is considered an on-target effect because HER2/ErB2 signaling also plays a crucial role in cardiomyocytes (Lin et al., 2022). Furthermore, systemic administration often results in suboptimal drug accumulation at tumor sites and may contribute to the emergence of acquired therapeutic resistance (Moo et al., 2018). To address these challenges, considerable efforts have focused on the development of advanced delivery platforms capable of enhancing trastuzumab stability, controlling drug release, improving local drug availability, and reducing systemic toxicity. Nanoparticles, liposomes, polymeric carriers, hydrogels, and microsphere-based systems have all demonstrated promising potential for improving the therapeutic performance of HER2-targeted agents (Truffi et al., 2018; Vanni et al., 2025). Recent studies have shown that surface-functionalized delivery systems decorated with antibodies or targeting ligands can improve cellular uptake and enhance therapeutic efficacy. Such approaches may provide more effective treatment strategies for HER2-positive breast cancer while minimizing adverse effects associated with conventional systemic administration (Sakhi et al., 2022; Truffi et al., 2018).

3. Drug Delivery Systems in Breast Cancer

Drug delivery systems (DDSs) have emerged as an essential strategy to improve the therapeutic efficacy and safety of anticancer agents. DDSs can improve therapeutic efficacy while reducing systemic toxicity by enabling controlled and sustained drug release, enhancing drug stability, and, depending on the carrier and administration strategy, improving drug distribution and local drug availability. Conventional chemotherapy, in contrast, often suffers from nonspecific biodistribution, rapid systemic clearance, dose-limiting adverse effects, and the development of multidrug resistance. Therefore, extensive research has been performed on the design of advanced delivery platforms to improve the pharmacokinetic and pharmacodynamic profiles of anticancer therapeutics (Peiris et al., 2014; Yao et al., 2020).

The drug delivery strategies can be classified as passive and active targeting approaches in a broad sense. Passive targeting mainly utilizes the enhanced permeability and retention (EPR) effect, while active targeting utilizes ligand-receptor interactions to increase selective cellular recognition and uptake. However, the EPR effect should not be considered a uniform or universally effective mechanism for tumor targeting. The extent of the EPR effect can vary depending on the tumor type and the heterogeneous tumor microenvironment. In addition, the presence and extent of the EPR effect are still debated, and the differences between preclinical models and human tumors may hinder the translation of passive targeting strategies based on the EPR effect into clinical practice (Shi et al., 2020). Among the different targeting ligands

explored, folic acid (FA) has been of particular interest since folate receptors are expressed in various tumors and can serve as attractive targets for tumor-specific drug delivery (Sudimack & Lee, 2000). Some of the carrier platforms investigated for breast cancer treatment include liposomes, polymeric nanoparticles, micelles, hydrogels, inorganic nanoparticles and microspheres (Zhai et al., 2024). Among them, microsphere-based systems have attracted increasing attention due to their high drug loading capacity, protection of encapsulated therapeutics, prolonged drug release and the possibility of surface functionalization (X. Li et al., 2024).

For drug delivery applications in breast cancer, polymeric microspheres made from biodegradable materials such as poly(lactic-co-glycolic acid) (PLGA), poly(lactic acid) (PLA), and their copolymers have been extensively studied. Natural polymers, including chitosan, gelatin, alginate, hyaluronic acid, and silk fibroin have also attracted considerable interest because of their favorable biocompatibility and tunable physicochemical properties (Cai et al., 2024; Zhai et al., 2024). These materials provide versatile delivery platforms because their degradation behavior, mechanical properties, and surface chemistry can be readily tailored to specific therapeutic requirements. However, their widespread clinical translation is still impeded by a variety of obstacles, such as the limited stability of certain therapeutic agents, the complexity of manufacturing, and the difficulties associated with large-scale production (X. Li et al., 2024; Wu et al., 2024). Most recently, hydroxyapatite (HAp)-based microspheres have attracted increasing attention as a promising alternative owing to their biomimetic composition, porous structure, excellent biocompatibility and versatile surface chemistry. These properties enable efficient drug loading while providing opportunities for controlled drug release and further functionalization with targeting ligands, while their potential for targeted drug delivery remains under investigation (Mondal et al., 2018; Qian et al., 2023).

4. Hydroxyapatite as a Drug Carrier

HAp, with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, is a calcium phosphate ceramic and is a principal inorganic component of human dental and bone tissue (Fiume et al., 2021). HAp has been extensively investigated for numerous biomedical applications, including bone regeneration, tissue engineering and drug delivery owing to its chemical similarity to native mineralized tissue. In addition to its established role as a bone substitute, there has been a growing interest in its potential as a multifunctional drug carrier due to its versatile surface chemistry and tunable physicochemical properties (Fiume et al., 2021; Hou et al., 2022).

HAp has some unique advantages as a drug carrier because of its porous structure, high specific surface area, and chemically active surface. These properties permit the efficient loading of various therapeutic agents such as small molecule drugs, peptides, proteins, and nucleic acids. In addition, the presence of calcium and phosphate groups offers active sites for drug adsorption and surface functionalization, allowing a wide variety of drug loading strategies (S.-M. Huang et al., 2022; Lara-Ochoa et al., 2021).

The release profile of HAp-based carriers depends on several factors like particle size, porosity, crystallinity, surface chemistry, and drug–carrier interactions (Mondal et al., 2018). The release of therapeutic agents may occur through diffusion, desorption from the surface of the carrier, gradual dissolution of the mineral matrix, or a combination of these mechanisms, depending on

the loading strategy (Mondal et al., 2018; N. Wang et al., 2019). This tunable release behavior can offer sustained therapeutic exposure and potentially reduce the frequency of administration while maintaining effective drug concentrations. These properties are especially advantageous in the setting of anticancer therapy, where sustained drug availability and reduced systemic exposure could enhance therapeutic efficacy and reduce adverse effects (Qian et al., 2023).

In recent years, HAp microspheres have emerged as particularly attractive delivery platforms due to their spherical morphology, porous structure, and favorable biological interactions. Compared with many conventional polymeric carriers, HAp microspheres combine favorable biocompatibility with intrinsic bioactivity and a mineral composition that closely resembles native hard tissues. These properties make HAp-based microspheres promising candidates for drug delivery applications (S. Li et al., 2023).

Several studies have demonstrated the successful incorporation of antimicrobial peptides, and chemotherapeutic agents within HAp microspheres, highlighting their versatility of HAp as carriers for localized and controlled drug delivery (Hong et al., 2021; H. Huang et al., 2020; Wu et al., 2022). These findings highlight the potential of HAp microspheres as multifunctional platforms capable of combining controlled drug release with further surface functionalization (Zhang et al., 2021). However, the effectiveness of such functionalizationfor active targeting in microscale HAp systems requires further investigation. Consequently, HAp-based delivery systems have become increasingly important in the development of next-generation cancer therapeutics (Qian et al., 2023).

Another potential advantage of HAp-based carriers is their pH-dependent dissolution behavior. HAp may exhibit higher solubility under acidic conditions; this can contribute to pH-dependent drug release and provide an advantage in acidic biological environments such as tumor microenvironment (Feng et al., 2013). In addition to its role as drug carrier, HAp, particularly in nanoparticle form, has also been reported to exhibit intrinsic antitumor activity. It has been shown that HAp nanoparticles cause intracellular Ca^{2+} loading in cancer cells, trigger calpain activation, and consequently activate mitochondrial apoptotic pathways (Dong et al., 2023). These findings suggest that HAp may offer an additional therapeutic potential beyond its conventional role as a drug carrier.

5. Folic Acid-Mediated Targeting Strategies

Active targeting is considered one of the most promising approaches for improving the efficacy and specificity of cancer treatment. Unlike passive targeting, which mainly relies on the EPR effect, active targeting employs specific ligands capable of recognizing molecular markers overexpressed on cancer cells (Bi et al., 2016). Among these ligands, folic acid (FA) has attracted considerable attention because as a targeting ligand, and its incorporation onto HAp surfaces has been investigated for developing FA-functionalized HAp systems (Cipreste et al., 2016).

Folate receptors are membrane-bound glycoproteins involved in cellular folate uptake. While their expression is generally low in most healthy tissues, significantly elevated expression levels have been reported in many cancer cells. This differential expression pattern provides an attractive opportunity for selective drug delivery (Sudimack & Lee, 2000; Tagde et al., 2020). FA is a small, inexpensive, non-immunogenic molecule that can be readily conjugated onto

carrier surfaces without significantly altering their physicochemical properties (Sudimack & Lee, 2000).

FA-functionalized drug delivery systems promote receptor-mediated cellular uptake following binding to folate receptors on cancer cells. As a result, therapeutic agents can be delivered preferentially into cancer cells, enhancing intracellular drug delivery and therapeutic efficacy. For instance, folate-functionalized liposomes have demonstrated FR-mediated internalization, enhanced cellular uptake, and improved anticancer activity in FR-overexpressing breast cancer cells (Dinakar et al., 2023).

The incorporation of FA onto HAp carriers represents an especially attractive strategy because it combines the favorable drug-loading characteristics of hydroxyapatite with thepotential for folate receptor-mediated cellular recognition and uptake (Cipreste et al., 2016; Verma et al., 2020). However, the therapeutic benefit of FA-mediated targeting is expected to depend on factors such as folate receptor expression and the physicochemical characteristics of the carrier system. Surface functionalization can be achieved through surface chemical modification using linker-mediated strategies like PEG-based functionalization (Qian et al., 2023). Following successful conjugation, FA-modified HAp carriers can enhance interaction with folate receptor-positive tumor cells (Verma et al., 2020).

Recent studies on folate-functionalized drug delivery systems and targeted microspheres suggest that folate-mediated targeting has promising potential for enhancing selective drug delivery and therapeutic performance (Yao et al., 2020; Zha et al., 2022). Consequently, the integration of FA-functionalized HAp microspheres with HER2-targeted therapeutics such as trastuzumab represents a potential design strategy that warrants further experimental and translational investigation.

6. Current Studies on Hydroxyapatite Microspheres for Cancer Therapy

6.1. Hydroxyapatite Microspheres in Drug Delivery Applications

Hydroxyapatite (HAp) microspheres have been increasingly investigated as versatile platforms for therapeutic drug delivery (Mondal et al., 2018). Their porous structure and tunable physicochemical properties enable efficient drug loading, while their pore structure can influence the kinetics of controlled and sustained drug release (Lai et al., 2016). Over the past decade, HAp microspheres have been investigated for the delivery of proteins and antimicrobial agents, demonstrating their versatility for the controlled delivery of biological molecules (Fu et al., 2011; Soriano-Souza et al., 2015). Furthermore, porous hollow HAp microspheres have demonstrated high drug-loading capacity and pH-responsive sustained-controlled release, highlighting their potential for anticancer drug delivery (Lai et al., 2016).

Previous studies have shown that HAp microspheres can encapsulate both small-molecule therapeutics and biomacromolecules and can maintain controlled release profiles (Fu et al., 2011; S. Wang et al., 2010). Apart from drug delivery, surface functionalization strategies have extended their applications to targeted and other specialized biomedical applications (Zhang et al., 2021).

Because of their micrometer-scale size, hydroxyapatite microspheres are more suitable for implantation for local or regional drug release purposes than systemic intravenous tumor

targeting (Fu et al., 2011; Long et al., 2013). In this approach, drug-loaded microspheres can be placed directly at the resection site after surgical removal of the tumor, ensuring local and long-term drug release. Indeed, local drug release, tumor regrowth, and metastasis development can be monitored following this process. This type of locoregional implantation approach allows the therapeutic agent to be delivered directly to the target site and controlled/sustainable release without requiring the microspheres to be extravasated into the tumor tissue by being delivered into the systemic circulation (Nwazojie et al., 2023). Table 1 presents representative studies in HAp microsphere-based systems for drug delivery.

Table 1 Representative studies investigating hydroxyapatite microsphere-based drug delivery systems

Therapeutic agent	Carrier	Size	Loading strategy	Model	Major Findings	Administration Route	Ref.
Tamoxifen	Porous HAp Microspheres	0.8-2 µm	Covalent attachment	The breast cancer cell line MCF-7	Sustained tamoxifen release with enhanced cytotoxicity against MCF-7 cells	Not reported	(Kırbıyık et al., 2022)
Doxorubicin	Porous HAp/Gelatin Composite Microspheres	45 µm	Chitosan-assisted bonding (Hydrogen bonding)	Human osteosarcoma-derived G-292 cells	99% drug entrapment efficiency with sustained doxorubicin release (45% release over 6 months) while maintaining anticancer activity.	Not reported	(Wu et al., 2022)
Paclitaxel (PTX)	PLGA-PCL microspheres	1.35–5.79 µm (SEM); 6.2–9.2 µm (DLS, DPBS)	Drug encapsulation in PLGA-PCL microspheres	MDA-MB-231 TNBC xenograft in athymic nude mice	Localized and sustained drug release after tumor resection; targeted formulations reduced/prevented local tumor regrowth and showed no detectable lung metastasis in the targeted groups	Locoregional / local implantation at the surgical resection site	(Nwazojie et al., 2023)
Ciprofloxacin	Mesoporous hierarchical HAp Microspheres	5 µm	Surface adsorption and pore loading	Not reported	61% drug loading with sustained ciprofloxacin release for 12 days; 79% of ciprofloxacin was released over 300 hours.	Not reported	(Daryan et al., 2025)
Resveratrol (Res)	n-HA/CS composite microspheres	30.75-110.87 µm (depends on formulation parameters)	Encapsulation during microsphere fabrication by emulsion chemical cross-linking	OVX-induced osteoporotic SD rats; femoral bone defect model	Enhanced osteogenic differentiation, reduced inflammation, accelerated entochondrostosis and bone regeneration; improved healing of osteoporotic bone defects	Local implantation into femoral bone defect	(L. Li et al., 2021)
HHC36 Peptide	Hierarchical HAp Microspheres	Not reported	Surface adsorption via electrostatic interactions	Antibacterial activity tested with E. coli and S. aureus	Highest peptide loading efficiency and sustained release over 9 days with prolonged antibacterial activity.	Not reported	(Hong et al., 2021)
Gentamicin	Mesopororus carbonated HAp microspheres	~5 µm	Adsorption/soaking	In vitro testing via S. epidermidis and human bone marrow stromal cells (hBMSCs)	70–75% drug-loading efficiency; sustained gentamicin release for up to 6 days; reduced bacterial adhesion and biofilm formation; good biocompatibility	Local drug delivery	(Guo et al., 2013)

In anticancer studies, HAp microspheres have been increasingly studied as carriers for therapeutic agents. Their porous structure, tunable physicochemical properties, and ability to sustain drug release make them attractive candidates for anticancer drug delivery. These features have led to an increasing interest in the exploration of HAp microspheres for the delivery of chemotherapeutic agents in different cancer models (Saber-Samandari et al., 2016).

The main goal has been to improve drug loading to prolong drug release while maintaining the therapeutic activity of the loaded agents. Examples of interest include the encapsulation of doxorubicin and tamoxifen, which showed sustained drug release and cytotoxic activity in cancer cell models (Kırbıyık et al., 2022; Wu et al., 2022). Representative studies have reported promising results in drug loading and efficiency. Wu et al. developed porous hydroxyapatite-gelatin composite microspheres loaded with doxorubicin and reported sustained release behavior together with enhanced drug loading efficiency. The incorporation of chitosan further improved release control and carrier stability (Wu et al., 2022).

Similarly, Kırbıyık et al. investigated tamoxifen-loaded hydroxyapatite microspheres for breast cancer treatment. Their findings demonstrated that HAp microspheres could effectively deliver tamoxifen to MCF-7 breast cancer cells while providing controlled release characteristics. These results highlighted the potential of HAp-based systems as drug delivery platforms for breast cancer treatment (Kırbıyık et al., 2022).

In addition to hydroxyapatite-based formulations, polymeric microsphere systems have also been investigated for targeted drug delivery in breast cancer. In particular, folate-mediated albumin microspheres have been developed to enhance targeting toward folate receptor-expressing tumor cells (Zha et al., 2022). This example shows the potential of ligand-mediated targeting in microsphere-based systems, although such targeting approaches should be distinguished from systemic tumor targeting by intravenously administered HAp microspheres.

6.2. Targeted Delivery Systems for Breast Cancer

One of the main advantages of microsphere-based delivery systems is the controlled release of drugs, however, selective accumulation at tumor sites still remains a major challenge. Sustained drug release alone cannot ensure optimal therapeutic efficacy unless sufficient amounts of the therapeutic agent reach the tumor site and are efficiently internalized by cancer cells. Hence, considerable efforts have focused on incorporating targeting ligands to improve cellular recognition and receptor-mediated uptake thereby improving therapeutic selectivity and reducing off-target effects (Yao et al., 2020).

Among these approaches, folic acid-mediated targeting has received considerable attention. Zha et al. developed folate-modified albumin microspheres and reported enhanced targeting efficiency toward folate receptor-positive cancer cells. Their findings demonstrated that folic acid modification significantly improved cellular uptake and therapeutic effectiveness compared with non-targeted formulations (Zha et al., 2022). These results demonstrate the potential of ligand-mediated targeting to complement controlled drug release through improved cellular internalization.

However, because folate receptor alpha (FRα) expression is not consistently high in this disease subtype, the justification for folic acid-mediated targeting in HER2-positive breast cancer needs to be carefully considered. Cheung et al. reported FRα expression in 15.6% of HER2-positive

tumors, compared with 36.8% of triple-negative breast cancers (TNBC). According to PAM50 molecular classification, FRα expression was observed in 10.7% of HER2-positive tumors and 33.3% of basal-like tumors (Cheung et al., 2018). These findings suggest that folate receptor-mediated targeting may be relevant to a subset of HER2-positive tumors with sufficient FRα expression rather than representing a universal targeting strategy for this subtype. O'Shannessy et al. similarly reported an association between FRα receptors and TNBC, further supporting the heterogeneous expression of this receptor across breast cancer subtypes (O'Shannessy et al., 2012). As a result, folic acid and trastuzumab combination shouldn't be seen as inherently better than HER2 targeting alone. As an alternative, in tumors that co-express HER2 and FRα, a dual-ligand approach might offer enhanced receptor engagement, which would enhance cellular recognition and uptake. Preclinical studies using gold nanoclusters functionalized with both folic acid and trastuzumab have reported enhanced cellular accumulation and therapeutic effects compared with non-targeted or single-targeted systems (Samani et al., 2020). However, whether this potential cooperative effect provides a meaningful advantage over single-ligand systems in HAp-based microspheres remains to be experimentally determined.

To better visualize these design principles and related molecular interactions with two illustrative figures, Figure 1 below presents a schematic representation of the hydroxyapatite microsphere detailing its porous surface and drug loadings. Furthermore, to address the biological spatial requirements discussed here, Figure 2 schematically compares the HER2 interaction with the spatial geometry of each ligand system, plotted to scale.

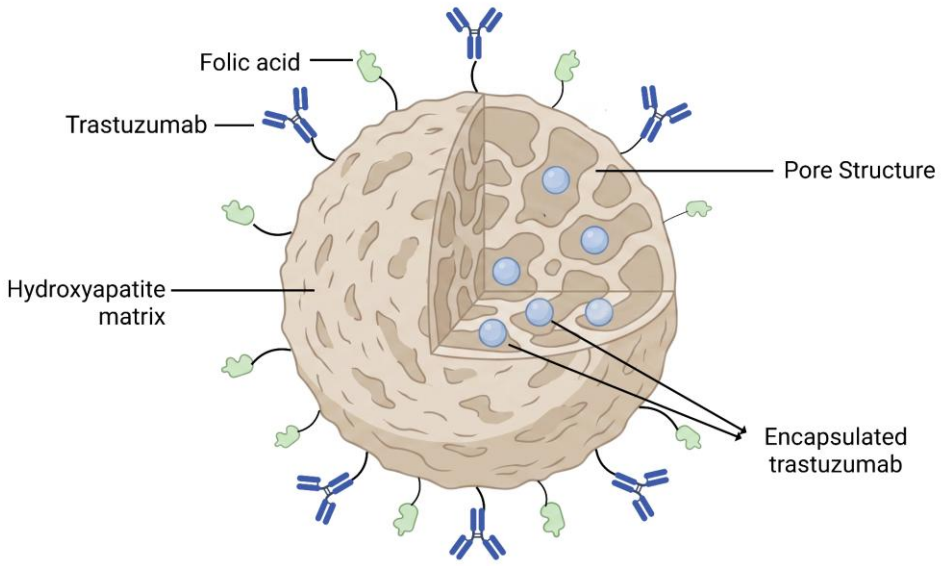

Figure 1 Schematic representation of the dual-functionalized hydroxyapatite (HAp) microsphere.

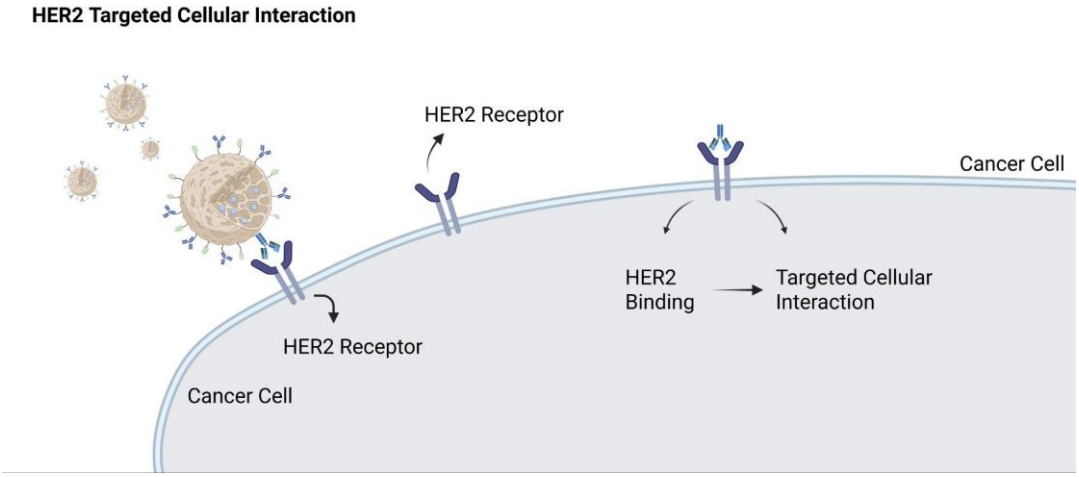

Figure 2 Mechanism of HER2 receptor-mediated interactions

For the literature examples of folic acid- and antibody-mediated targeting strategies investigated in different carrier systems are summarized in Table 2, including their targeting approaches, conjugation strategies, cellular models, and reported uptake outcomes.

Table 2 Representative folic acid- and antibody-functionalized drug delivery systems

Carrier	Targeting strategy	Ligand density / Modification degree	Conjugation chemistry	Cell line	Fold-change in uptake vs. non-targeted control	Major findings	Ref
Albumin microspheres	Folic acid	28.1% modification rate	EDC/Sulfo-NHS-mediated conjugation	MCF-7	Not reported	Folate modification enhanced cellular uptake and increased the inhibitory effect of nintendanib on MCF-7 cells.	(Zha et al., 2022)
HSA nanoparticles	Trastuzumab	14.92 ± 1.34 μg/mg nanoparticle	NHS-PEG5000-Mal-mediated covalent coupling	SK-BR-3 and MCF-7	Not reported	Trastuzumab-functionalized nanoparticles showed specific binding and intracellular uptake in HER2-overexpressing SK-BR-3 cells, whereas binding was marginal in HER2-low MCF-7 cells.	(Anhorn et al., 2008)
Polymeric nanoparticles	Folic acid + trastuzumab	Not reported	DCC-NHS mediated conjugation of folic acid and EDC-NHS-mediated conjugation for trastuzumab	MCF-7 and BT-474	Not reported	Dual-targeted nanoparticles exhibited greater cellular uptake than non-targeted and single-targeted formulations.	(Kumar et al., 2017)
Gold nannoclusters	Folic acid + trastuzumab	Not reported	EDC-NHS-mediated conjugation	SK-BR-3 and L929	3-fold for dual-targeted vs. non-targeted AuNCs	Dual-targeted AuNCs showed approximately threefold higher cellular uptake in SK-BR-3 cells and produced greater radiosensitization than non-targeted or single-targeted systems.	(Samani et al., 2020)

Carrier	Targeting strategy	Ligand density / Modification degree	Conjugation chemistry	Cell line	Fold-change in uptake vs. non-targeted control	Major findings	Ref
Polymersomes	Folic acid + trastuzumab	63.8 µg/mg trastuzumab for PLA-Dox-Her and 58.4 µg/mg for PLA-Dox-FA-Her	EDC/NHS-mediated conjugation	MCF-7 and BT-474	~9-fold vs. non-targeted polymersomes in BT-474	Dual-functionalized polymersomes showed enhanced cellular uptake and antitumor activity compared with non-targeted and single-targeted formulations.	(Lale et al., 2015)
Trastuzumab-conjugated iron oxide nanoparticles	Trastuzumab	Half-chain trastuzumab	Covalent conjugation to nanoparticle surface	SK-BR-3	Not reported	Trastuzumab conjugated nanoparticles showed high HER2-specific binding and internalization in SK-BR-3 cells, demonstrating the potential of antibody-mediated targeting.	(Truffi et al., 2018)

The studies summarized in Table 2 demonstrate that folic acid- and antibody-mediated targeting strategies have been investigated across a range of carrier platforms, with several studies reporting enhanced cellular uptake compared with non-targeted formulations. Among these approaches, antibody-mediated targeting has become an important strategy to increase the selectivity of breast cancer drug delivery. Trastuzumab is of particular interest due to its specific recognition of HER2 and its ability to bind HER2 and serve as a targeting ligand for HER2-positive breast cancer cells (Samani et al., 2020). However, the role of trastuzumab within a carrier system can vary depending on the targeted delivery strategy. These different roles and their effects on HAp-based delivery systems are considered in the following discussion.

Despite these promising advances, the bulk of targeted drug delivery research has been focused on polymeric nanoparticles rather than hydroxyapatite-based microspheres. However, the potential of HAp microspheres as carriers for receptor-targeted therapeutics is not exploited sufficiently. For example, the combination of controlled drug release and ligand-mediated targeting represents a developing research area with significant promise for the development of next-generation breast cancer therapies.

Although these advances are promising, there are still several important challenges before active targeting strategies can be effectively translated to hydroxyapatite-based microsphere systems. HAp microspheres have been mainly used for the delivery of small-molecule therapeutics, such as doxorubicin, tamoxifen, vancomycin, and ciprofloxacin (Daryan et al., 2025; Kırbıyık et al., 2022; Wu et al., 2022, 2024), and the application of HAp microspheres for delivery of biomocromolecules, especially monoclonal antibodies, remains limited. Similarly, although systems functionalized with folic acid and HER2-targeted delivery platforms have shown promising potential in cancer therapy, FA-functioalized HAp systems have also been investigated for targeted drug delivery, whereas the integration of folic acid and HER2-targeted strategies into a single HAp-based carrier represents a comparatively unexplored approach (Liu et al., 2019; Verma et al., 2020).

Overall, available reports suggest that active targeting combined with drug delivery represents a promising strategy for HAp-based microsphere systems in HER2-positive breast cancer. However, the use of trastuzumab —as a therapeutic cargo, a surface-bound targeting ligand, or potentially a component of a combined architecture— should be clearly defined according to the intended delivery strategy. Further studies are required to preserve the biological activity of

the antibody, determine the optimal architecture, and validate therapeutic efficacy through extensive preclinical and clinical investigations.

6.2.1. Distinct Roles of Trastuzumab in HAp-based Delivery Systems

Trastuzumab can be incorporated into HAp-based delivery systems through two distinct design strategies: as a therapeutic cargo or as a surface-bound targeting ligand. These approaches differ in their primary objectives and should be considered separately.

In the first strategy, trastuzumab is incorporated into the HAp carrier as a therapeutic agent and is intended to be released in a controlled manner. The feasibility of using HAp as a carrier for proteins has been demonstrated in several studies. Matsumoto et al. reported that protein adsorbed onto HAp particles could be released in a controlled manner, with the release behavior influenced by HAp solubility and the amount of adsorbed protein (Matsumoto et al., 2004) Similarly, HAp-containing microsphere systems have been investigated for protein delivery using double-emulsion approaches. Ho et al. demonstrated that HAp-containing PLGA microspheres could efficiently entrap BSA and provide sustained protein release, supporting the potential of HAp-containing microspheres for the delivery of therapeutic proteins (Ho et al., 2008). Therefore, the primary objective of using trastuzumab as a therapeutic cargo is to ensure the preservation of the antibody during its incorporation into the carrier and subsequently achieve its controlled release while maintaining its biological activity.

In the second strategy, the aim is to immobilize trastuzumab on the surface of the HAp carrier and enable it to function primarily as a HER2-targeting ligand rather than as a releasable therapeutic cargo. Trastuzumab functionalized carriers have been widely investigated for HER2-targeted drug delivery, with surface conjugation strategies designed to promote preferential interaction with HER2-overexpressing cancer cells (Nieto et al., 2020). In this strategy, the therapeutic payload is located within the carrier, whereas surface-bound trastuzumab facilitates receptor recognition and cellular interaction. Surface functionalization of HAp with biomolecules is also feasible through interactions between HAp surface ions and functional groups present in biological molecules, providing opportunities for the development of ligand-functionalized HAp systems (Kataoka et al., 2024).

The two strategies therefore serve different purposes. The cargo-based approach primarily aims to use HAp as a carrier for the controlled delivery of trastuzumab, whereas the surface - functionalized approach uses trastuzumab to provide HER2-mediated targeting for another therapeutic payload. A combined structure in which trastuzumab contributes to both therapeutic activity and targeting may also be conceptually possible; however, such a design would require careful consideration of antibody orientation, structural stability, and preservation of biological activity.

7. Challenges and Limitations

One of the major limitations involves achieving consistent drug loading efficiency while maintaining controlled release characteristics. Variations in microsphere morphology, pore structure, and particle size may significantly influence drug encapsulation and release kinetics (Sable et al., 2024).

Another important challenge is related to the delivery of biomacromolecules such as monoclonal antibodies. Unlike conventional small-molecule drugs, therapeutic antibodies possess complex three-dimensional structures that are more susceptible to structural instability during carrier fabrication and encapsulation. Processing conditions must therefore be carefully controlled to preserve protein stability and biological activity (Iafisco et al., 2012; Kim & Pack, 2006).

Targeting efficiency also remains a critical concern. Although folic acid-mediated targeting has demonstrated improved cellular uptake in experimental studies, variations in folate receptor expression among different tumor types may affect therapeutic outcomes (Sudimack & Lee, 2000; Tagde et al., 2020). Similarly, HER2-targeted therapies such as trastuzumab may encounter challenges related to heterogeneous treatment responses and therapeutic resistance, which can affect long-term treatment outcomes (Gu et al., 2024).

Beyond the biological challenges, reproducibility in manufacturing and large scale production remain critical obstacles to clinical translation of HAp-based systems. Maintaining consistent particle size distribution, porosity, drug-loading efficiency, and release characteristics across production batches can be challenging, especially during the scale-up of experimental HAp-based formulations (Veiga et al., 2023).

Furthermore, many studies investigating HAp-based delivery systems remain at the preclinical stage. Comprehensive in vivo evaluations, long-term toxicity assessments, pharmacokinetic investigations, and clinical trials are still lacking. Consequently, the clinical translation of promising laboratory-scale formulations remains an important challenge (Sable et al., 2024; Santos et al., 2017).

Another significant limitation is the limited number of studies integrating multiple targeting mechanisms within a single hydroxyapatite-based carrier system. Although HAp-based systems incorporating active targeting ligands and controlled drug release have been investigated (Liu et al., 2019; Verma et al., 2020), the integration of multiple receptor-mediated targeting mechanisms within a single HAp-based carrier remains comparatively underexplored.

8. Future Perspectives

The combination of advanced biomaterials, targeted delivery approaches, and biologically active therapeutics may contribute to the future development of HAp-based drug delivery systems. In particular, the development of stimuli-responsive HAp carriers responsive to tumor-associated triggers such as pH, enzymatic activity, or redox conditions may enable more precise control of drug release and potentially reduce systemic exposure (Mondal et al., 2018; Qian et al., 2023).

Another promising research direction is to expand the application of HAp microspheres from the delivery of conventional small-molecule drugs to biologics such as peptides, proteins, nucleic acids, and monoclonal antibodies. The immobilization of biomacromolecules into HAp-based delivery systems may require further optimization of surface functionalization and loading strategies to preserve biomolecular stability and biological activity (Iafisco et al., 2012; Kim & Pack, 2006).

For HER2-positive breast cancer, combining active targeting strategies with multifunctional HAp carriers represents a promising avenue for future research. In particular, the combination of folic acid-mediated targeting with HER2-directed therapeutics such as trastuzumab may provide complementary approaches to enhance tumor selectivity and controlled drug delivery (Nieto et al., 2020; Sudimack & Lee, 2000). Further advances in biomaterials engineering and targeted drug delivery may facilitate the development of multifunctional HAp-based therapeutic platforms for precision breast cancer treatment.

9. Conclusion

Hydroxyapatite microspheres represent a versatile platform because of their porous structure, tunable surface properties, and capacity to accommodate therapeutic agents while enabling controlled release. Recent studies in surface functionalization and targeted delivery have expanded their potential for multifunctional therapeutic systems.

Although folic acid-mediated targeting and HER2-targeted therapies such as trastuzumab offer an attractive framework for the treatment of HER2-positive breast cancer, standard production, maintenance of antibody stability, and rigorous in vivo verification are required to bridge the gap between laboratory synthesis and clinical application.

Declarations

Ethics Statement

This study did not involve human participants, human-derived data or samples, or live animals; therefore, ethics committee approval was not required. The study was conducted in accordance with generally accepted research and publication ethics.

Conflict of Interest

The authors declare there are no competing interests.

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References

Anhorn, M. G., Wagner, S., Kreuter, J., Langer, K., & von Briesen, H. (2008). Specific Targeting of HER2 Overexpressing Breast Cancer Cells with Doxorubicin-Loaded Trastuzumab-Modified Human Serum Albumin Nanoparticles. *Bioconjugate Chemistry*, *19*(12), 2321–2331. https://doi.org/10.1021/bc8002452

Bi, Y., Hao, F., Yan, G., Teng, L., Lee, R. J., & Xie, J. (2016). Actively Targeted Nanoparticles for Drug Delivery to Tumor. *Current Drug Metabolism*, *17*(8), 763–782. https://doi.org/10.2174/1389200217666160619191853

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *74*(3), 229–263. https://doi.org/10.3322/caac.21834

Cai, H., Li, A., Qi, F., Liu, R., Tang, X., Li, D., Gu, Y., & Liu, J. (2024). Application of biodegradable microsphere injections: an anticancer perspective. *Materials Advances*, *5*(8), 3094–3112. https://doi.org/10.1039/D3MA01150J

Cheung, A., Opzoomer, J., Ilieva, K. M., Gazinska, P., Hoffmann, R. M., Mirza, H., Marlow, R., Francesch-Domenech, E., Fittall, M., Dominguez Rodriguez, D., Clifford, A., Badder, L., Patel, N., Mele, S., Pellizzari, G., Bax, H. J., Crescioli, S., Petranyi, G., Larcombe-Young, D., … Karagiannis, S. N. (2018). Anti-Folate Receptor Alpha–Directed Antibody Therapies Restrict the Growth of Triple-negative Breast Cancer. *Clinical Cancer Research*, *24*(20), 5098–5111. https://doi.org/10.1158/1078-0432.CCR-18-0652

Cipreste, M. F., Gonzalez, I., Maria da Mata Martins, T., Goes, A. M., Augusto de Almeida Macedo, W., & Barros de Sousa, E. M. (2016). Attaching folic acid on hydroxyapatite nanorod surfaces: an investigation of the HA–FA interaction. *RSC Advances*, *6*(80), 76390–76400. https://doi.org/10.1039/C6RA14068H

Collins, D. M., O'Donovan, N., McGowan, P. M., O'Sullivan, F., Duffy, M. J., & Crown, J. (2012). Trastuzumab induces antibody-dependent cell-mediated cytotoxicity (ADCC) in HER-2-non-amplified breast cancer cell lines. *Annals of Oncology*, *23*(7), 1788–1795. https://doi.org/10.1093/annonc/mdr484

Daryan, S. H., Javadpour, J., & Khavandi, A. (2025). Synthesis and drug release study of ciprofloxacin loaded hierarchical hydroxyapatite mesoporous microspheres. *Results in Chemistry*, *14*, 102123. https://doi.org/10.1016/j.rechem.2025.102123

De Lama-Odría, M. del C., del Valle, L. J., & Puiggalí, J. (2022). Hydroxyapatite Biobased Materials for Treatment and Diagnosis of Cancer. *International Journal of Molecular Sciences*, *23*(19), 11352. https://doi.org/10.3390/ijms231911352

Dinakar, Y. H., Karole, A., Parvez, S., Jain, V., & Mudavath, S. L. (2023). Folate receptor targeted NIR cleavable liposomal delivery system augment penetration and therapeutic efficacy in breast cancer. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1867*(9), 130396. https://doi.org/10.1016/j.bbagen.2023.130396

Dong, X., Zang, C., Sun, Y., Zhang, S., Liu, C., & Qian, J. (2023). Hydroxyapatite nanoparticles induced calcium overload-initiated cancer cell-specific apoptosis through inhibition of PMCA and activation of calpain. *Journal of Materials Chemistry B*, *11*(32), 7609–7622. https://doi.org/10.1039/D3TB00542A

Early Breast Cancer Trialists' Collaborative Group. (2021). Trastuzumab for early-stage, HER2-positive breast cancer: a meta-analysis of 13 864 women in seven randomised trials. *The Lancet Oncology*, *22*(8), 1139–1150. https://doi.org/10.1016/S1470-2045(21)00288-6

Feng, D., Shi, J., Wang, X., Zhang, L., & Cao, S. (2013). Hollow hybrid hydroxyapatite microparticles with sustained and pH-responsive drug delivery properties. *RSC Advances*, *3*(47), 24975. https://doi.org/10.1039/c3ra44609c

Fiume, E., Magnaterra, G., Rahdar, A., Verné, E., & Baino, F. (2021). Hydroxyapatite for Biomedical Applications: A Short Overview. *Ceramics*, *4*(4), 542–563. https://doi.org/10.3390/ceramics4040039

Fu, H., Rahaman, M. N., Day, D. E., & Brown, R. F. (2011). Hollow hydroxyapatite microspheres as a device for controlled delivery of proteins. *Journal of Materials Science: Materials in Medicine*, *22*(3), 579–591. https://doi.org/10.1007/s10856-011-4250-6

Gu, S., Liu, Y., Huang, Y., Lin, W., & Li, K. (2024). Comparative efficacy and safety of targeted therapy and immunotherapy for HER2-positive breast cancer: a systematic review and network meta-analyses. *Frontiers in Oncology*, *14*. https://doi.org/10.3389/fonc.2024.1331055

Guo, Y.-J., Long, T., Chen, W., Ning, C.-Q., Zhu, Z.-A., & Guo, Y.-P. (2013). Bactericidal property and biocompatibility of gentamicin-loaded mesoporous carbonated hydroxyapatite microspheres. *Materials Science and Engineering: C*, *33*(7), 3583–3591. https://doi.org/10.1016/j.msec.2013.04.021

Ho, M. L., Fu, Y. C., Wang, G. J., Chen, H. T., Chang, J. K., Tsai, T. H., & Wang, C. K. (2008). Controlled release carrier of BSA made by W/O/W emulsion method containing PLGA and hydroxyapatite. *Journal of Controlled Release*, *128*(2), 142–148. https://doi.org/10.1016/j.jconrel.2008.02.012

Hong, D., Wu, J., Xiao, X., Li, X., Xu, D., & Du, C. (2021). Antimicrobial Peptides-Loaded Hydroxyapatite Microsphere With Different Hierarchical Structures for Enhanced Drug Loading, Sustained Release and Antibacterial Activity. *Frontiers in Chemistry*, *9*. https://doi.org/10.3389/fchem.2021.747665

Hou, X., Zhang, L., Zhou, Z., Luo, X., Wang, T., Zhao, X., Lu, B., Chen, F., & Zheng, L. (2022). Calcium Phosphate-Based Biomaterials for Bone Repair. *Journal of Functional Biomaterials*, *13*(4), 187. https://doi.org/10.3390/jfb13040187

Huang, H., Du, M., Chen, J., Zhong, S., & Wang, J. (2020). Preparation and characterization of abalone shells derived biological mesoporous hydroxyapatite microspheres for drug delivery. *Materials Science and Engineering: C*, *113*, 110969. https://doi.org/10.1016/j.msec.2020.110969

Huang, S.-M., Liu, S.-M., Ko, C.-L., & Chen, W.-C. (2022). Advances of Hydroxyapatite Hybrid Organic Composite Used as Drug or Protein Carriers for Biomedical Applications: A Review. *Polymers*, *14*(5), 976. https://doi.org/10.3390/polym14050976

Iafisco, M., Varoni, E., Di Foggia, M., Pietronave, S., Fini, M., Roveri, N., Rimondini, L., & Prat, M. (2012). Conjugation of hydroxyapatite nanocrystals with human immunoglobulin G for nanomedical applications. *Colloids and Surfaces B: Biointerfaces*, *90*, 1–7. https://doi.org/10.1016/j.colsurfb.2011.09.033

Kataoka, T., Liu, Z., Yamada, I., Galindo, T. G. P., & Tagaya, M. (2024). Surface functionalization of hydroxyapatite nanoparticles for biomedical applications. *Journal of Materials Chemistry B*, *12*(28), 6805–6826. https://doi.org/10.1039/D4TB00551A

Kim, K. K., & Pack, D. W. (2006). Microspheres for Drug Delivery. In *BioMEMS and Biomedical Nanotechnology* (pp. 19–50). Springer US. https://doi.org/10.1007/978-0-387-25842-3_2

Kırbıyık, B., Mazmancı, B., Yarlılar, Ş. G., Uğur, N., & Ocakoğlu, K. (2022). Tamoxifen Delivery to Breast Cancer Cells (MCF-7) Via Hydroxyapatite Microspheres. *Eurasian Journal of Biological and Chemical Sciences*, *5*((Ek sayı 1)), 111–118. https://doi.org/10.46239/ejbcs.1040161

Kumar, A., Lale, S. V., Aji Alex, M. R., Choudhary, V., & Koul, V. (2017). Folic acid and trastuzumab conjugated redox responsive random multiblock copolymeric nanocarriers for breast cancer therapy: In-vitro and in-vivo studies. *Colloids and Surfaces B: Biointerfaces*, *149*, 369–378. https://doi.org/10.1016/j.colsurfb.2016.10.044

Lai, W., Chen, C., Ren, X., Lee, I.-S., Jiang, G., & Kong, X. (2016). Hydrothermal fabrication of porous hollow hydroxyapatite microspheres for a drug delivery system. *Materials Science and Engineering: C*, *62*, 166–172. https://doi.org/10.1016/j.msec.2016.01.055

Lale, S. V., Kumar, A., Prasad, S., Bharti, A. C., & Koul, V. (2015). Folic Acid and Trastuzumab Functionalized Redox Responsive Polymersomes for Intracellular Doxorubicin Delivery in Breast Cancer. *Biomacromolecules*, *16*(6), 1736–1752. https://doi.org/10.1021/acs.biomac.5b00244

Lara-Ochoa, S., Ortega-Lara, W., & Guerrero-Beltrán, C. E. (2021). Hydroxyapatite Nanoparticles in Drug Delivery: Physicochemistry and Applications. *Pharmaceutics*, *13*(10), 1642. https://doi.org/10.3390/pharmaceutics13101642

Li, L., Yu, M., Li, Y., Li, Q., Yang, H., Zheng, M., Han, Y., Lu, D., Lu, S., & Gui, L. (2021). Synergistic anti-inflammatory and osteogenic n-HA/resveratrol/chitosan composite microspheres for osteoporotic bone regeneration. *Bioactive Materials*, *6*(5), 1255–1266. https://doi.org/10.1016/j.bioactmat.2020.10.018

Li, S., Guo, R., Chen, S., Zhang, J., Ikoma, T., Li, X., & Chen, W. (2023). Facile synthesis, in vitro biocompatibility, and antibacterial activity of porous hollow hydroxyapatite microspheres. *International Journal of Applied Ceramic Technology*, *20*(1), 341–349. https://doi.org/10.1111/ijac.14195

Li, X., Li, L., Wang, D., Zhang, J., Yi, K., Su, Y., Luo, J., Deng, X., & Deng, F. (2024). Fabrication of polymeric microspheres for biomedical applications. *Materials Horizons*, *11*(12), 2820–2855. https://doi.org/10.1039/D3MH01641B

Lin, M., Xiong, W., Wang, S., Li, Y., Hou, C., Li, C., & Li, G. (2022). The Research Progress of Trastuzumab-Induced Cardiotoxicity in HER-2-Positive Breast Cancer Treatment. *Frontiers in Cardiovascular Medicine*, *8*. https://doi.org/10.3389/fcvm.2021.821663

Liu, Q., Guo, W., Yang, M., Wang, K., Liu, W., & Wu, F. (2019). Release Behavior of Folic Acid Grafted Hollow Hydroxyapatite as Drug Carrier. *Advances in Polymer Technology*, *2019*, 1–9. https://doi.org/10.1155/2019/9562437

Long, T., Guo, Y.-P., Liu, Y.-Z., & Zhu, Z.-A. (2013). Hierarchically nanostructured mesoporous carbonated hydroxyapatite microspheres for drug delivery systems with high drug-loading capacity. *RSC Advances*, *3*(46), 24169. https://doi.org/10.1039/c3ra44497j

Matsumoto, T., Okazaki, M., Inoue, M., Yamaguchi, S., Kusunose, T., Toyonaga, T., Hamada, Y., & Takahashi, J. (2004). Hydroxyapatite particles as a controlled release carrier of protein. *Biomaterials*, *25*(17), 3807–3812. https://doi.org/10.1016/j.biomaterials.2003.10.081

Mondal, S., Dorozhkin, S. V., & Pal, U. (2018). Recent progress on fabrication and drug delivery applications of nanostructured hydroxyapatite. *WIREs Nanomedicine and Nanobiotechnology*, *10*(4). https://doi.org/10.1002/wnan.1504

Moo, T.-A., Sanford, R., Dang, C., & Morrow, M. (2018). Overview of Breast Cancer Therapy. *PET Clinics*, *13*(3), 339–354. https://doi.org/10.1016/j.cpet.2018.02.006

Nami, B., Maadi, H., & Wang, Z. (2018). Mechanisms Underlying the Action and Synergism of Trastuzumab and Pertuzumab in Targeting HER2-Positive Breast Cancer. *Cancers*, *10*(10), 342. https://doi.org/10.3390/cancers10100342

Nieto, C., Vega, M. A., & Martín del Valle, E. M. (2020). Trastuzumab: More than a Guide in HER2-Positive Cancer Nanomedicine. *Nanomaterials*, *10*(9), 1674. https://doi.org/10.3390/nano10091674

Nwazojie, C. C., Obayemi, J. D., Salifu, A. A., Borbor-Sawyer, S. M., Uzonwanne, V. O., Onyekanne, C. E., Akpan, U. M., Onwudiwe, K. C., Oparah, J. C., Odusanya, O. S., & Soboyejo, W. O. (2023). Targeted drug-loaded PLGA-PCL microspheres for specific and localized treatment of triple negative breast cancer. *Journal of Materials Science: Materials in Medicine*, *34*(8), 41. https://doi.org/10.1007/s10856-023-06738-y

O'Shannessy, D. J., Somers, E. B., Maltzman, J., Smale, R., & Fu, Y.-S. (2012). Folate receptor alpha (FRA) expression in breast cancer: identification of a new molecular subtype and association with triple negative disease. *SpringerPlus*, *1*(1), 22. https://doi.org/10.1186/2193-1801-1-22

Peiris, P. M., Toy, R., Abramowski, A., Vicente, P., Tucci, S., Bauer, L., Mayer, A., Tam, M., Doolittle, E., Pansky, J., Tran, E., Lin, D., Schiemann, W. P., Ghaghada, K. B., Griswold, M. A., & Karathanasis, E. (2014). Treatment of cancer micrometastasis using a multicomponent chain-like nanoparticle. *Journal of Controlled Release*, *173*, 51–58. https://doi.org/10.1016/j.jconrel.2013.10.031

Pupa, S. M., Ligorio, F., Cancila, V., Franceschini, A., Tripodo, C., Vernieri, C., & Castagnoli, L. (2021). HER2 Signaling and Breast Cancer Stem Cells: The Bridge behind HER2-Positive Breast Cancer Aggressiveness and Therapy Refractoriness. *Cancers*, *13*(19), 4778. https://doi.org/10.3390/cancers13194778

Qian, G., Xiong, L., & Ye, Q. (2023). Hydroxyapatite-based carriers for tumor targeting therapy. *RSC Advances*, *13*(24), 16512–16528. https://doi.org/10.1039/D3RA01476B

Saber-Samandari, S., Nezafati, N., & Saber-Samandari, S. (2016). The Effective Role of Hydroxyapatite Based Composites in Anticancer Drug Delivery Systems. *Critical Reviews in Therapeutic Drug Carrier Systems*, *33*(1), 41–75. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.v33.i1.30

Sable, P. R., Gawai, G. A., Rajput, J. V., Urkunde, A. S., & Atram, S. C. (2024). A Technical Note: on Microspheres. *Asian Journal of Pharmaceutical Research and Development*, *12*(1), 65–76. https://doi.org/10.22270/ajprd.v12i1.1350

Sakhi, M., Khan, A., Iqbal, Z., Khan, I., Raza, A., Ullah, A., Nasir, F., & Khan, S. A. (2022). Design and Characterization of Paclitaxel-Loaded Polymeric Nanoparticles Decorated With Trastuzumab for the Effective Treatment of Breast Cancer. *Frontiers in Pharmacology*, *13*. https://doi.org/10.3389/fphar.2022.855294

Samani, R. K., Tavakoli, M. B., Maghsoudinia, F., Motaghi, H., Hejazi, S. H., & Mehrgardi, M. A. (2020). Trastuzumab and folic acid functionalized gold nanoclusters as a dual-targeted radiosensitizer for megavoltage radiation therapy of human breast cancer. *European Journal of Pharmaceutical Sciences*, *153*, 105487. https://doi.org/10.1016/j.ejps.2020.105487

Santos, C., Gomes, P., Duarte, J. A., Almeida, M. M., Costa, M. E. V., & Fernandes, M. H. (2017). Development of hydroxyapatite nanoparticles loaded with folic acid to induce osteoblastic differentiation. *International Journal of Pharmaceutics*, *516*(1–2), 185–195. https://doi.org/10.1016/j.ijpharm.2016.11.035

Selepe, C. T., Dhlamini, K. S., Tshweu, L., Moralo, M., Kwezi, L., Ray, S. S., & Ramalapa, B. (2024). Trastuzumab-based nanomedicines for breast cancer therapy: Recent advances and future opportunities. *Nano Select*, *5*(5). https://doi.org/10.1002/nano.202300191

Shi, Y., van der Meel, R., Chen, X., & Lammers, T. (2020). The EPR effect and beyond: Strategies to improve tumor targeting and cancer nanomedicine treatment efficacy. *Theranostics*, *10*(17), 7921–7924. https://doi.org/10.7150/thno.49577

Soriano-Souza, C. A., Rossi, A. L., Mavropoulos, E., Hausen, M. A., Tanaka, M. N., Calasans-Maia, M. D., Granjeiro, J. M., Rocha-Leão, M. H. M., & Rossi, A. M. (2015). Chlorhexidine-loaded hydroxyapatite microspheres as an antimicrobial delivery system and its effect on in vivo osteo-conductive properties. *Journal of Materials Science: Materials in Medicine*, *26*(4), 166. https://doi.org/10.1007/s10856-015-5505-4

Sudimack, J., & Lee, R. J. (2000). Targeted drug delivery via the folate receptor. *Advanced Drug Delivery Reviews*, *41*(2), 147–162. https://doi.org/10.1016/S0169-409X(99)00062-9

Tagde, P., Kulkarni, G. T., Mishra, D. K., & Kesharwani, P. (2020). Recent advances in folic acid engineered nanocarriers for treatment of breast cancer. *Journal of Drug Delivery Science and Technology*, *56*, 101613. https://doi.org/10.1016/j.jddst.2020.101613

Truffi, M., Colombo, M., Sorrentino, L., Pandolfi, L., Mazzucchelli, S., Pappalardo, F., Pacini, C., Allevi, R., Bonizzi, A., Corsi, F., & Prosperi, D. (2018). Multivalent exposure of trastuzumab on iron oxide nanoparticles improves antitumor potential and reduces resistance in HER2-positive breast cancer cells. *Scientific Reports*, *8*(1), 6563. https://doi.org/10.1038/s41598-018-24968-x

Vanni, S., Caputo, T. M., Cusano, A. M., De Vita, A., Cusano, A., Cocchi, C., Mulè, C., Principe, S., Liverani, C., Celetti, G., Micco, A., Spadazzi, C., Miserocchi, G., Ibrahim, T., Mercatali, L., & Aliberti, A. (2025). Engineered anti-HER2 drug delivery nanosystems for the treatment of breast cancer. *Nanoscale*, *17*(15), 9436–9457. https://doi.org/10.1039/D4NR03907F

Veiga, A., Madureira, S., Costa, J. B., Castro, F., Rocha, F., & Oliveira, A. L. (2023). Tackling current production of HAp and HAp-driven biomaterials. *Materials Advances*, *4*(22), 5453–5478. https://doi.org/10.1039/D3MA00363A

Verma, G., Shetake, N. G., Pandrekar, S., Pandey, B. N., Hassan, P. A., & Priyadarsini, K. I. (2020). Development of surface functionalized hydroxyapatite nanoparticles for enhanced specificity towards tumor cells. *European Journal of Pharmaceutical Sciences*, *144*, 105206. https://doi.org/10.1016/j.ejps.2019.105206

Waks, A. G., & Winer, E. P. (2019). Breast Cancer Treatment. *JAMA*, *321*(3), 288. https://doi.org/10.1001/jama.2018.19323

Wang, N., Cheng, X., Li, N., Wang, H., & Chen, H. (2019). Nanocarriers and Their Loading Strategies. *Advanced Healthcare Materials*, *8*(6). https://doi.org/10.1002/adhm.201801002

Wang, S., Wang, X., Xu, H., Abe, H., Tan, Z., Zhao, Y., Guo, J., Naito, M., Ichikawa, H., & Fukumori, Y. (2010). Towards sustained delivery of small molecular drugs using hydroxyapatite microspheres as the vehicle. *Advanced Powder Technology*, *21*(3), 268–272. https://doi.org/10.1016/j.apt.2009.12.001

Wong, A. L. A., & Lee, S.-C. (2012). Mechanisms of Resistance to Trastuzumab and Novel Therapeutic Strategies in HER2-Positive Breast Cancer. *International Journal of Breast Cancer*, *2012*, 1–13. https://doi.org/10.1155/2012/415170

Wu, M.-Y., Kuo, Y.-T., Kao, I.-F., & Yen, S.-K. (2024). Porous Chitosan/Hydroxyapatite Composite Microspheres for Vancomycin Loading and Releasing. *Pharmaceutics*, *16*(6), 730. https://doi.org/10.3390/pharmaceutics16060730

Wu, M.-Y., Liang, Y.-H., & Yen, S.-K. (2022). Effects of Chitosan on Loading and Releasing for Doxorubicin Loaded Porous Hydroxyapatite–Gelatin Composite Microspheres. *Polymers*, *14*(20), 4276. https://doi.org/10.3390/polym14204276

Yao, Y., Zhou, Y., Liu, L., Xu, Y., Chen, Q., Wang, Y., Wu, S., Deng, Y., Zhang, J., & Shao, A. (2020). Nanoparticle-Based Drug Delivery in Cancer Therapy and Its Role in Overcoming Drug Resistance. *Frontiers in Molecular Biosciences*, *7*. https://doi.org/10.3389/fmolb.2020.00193

Yewle, J. N., Wei, Y., Puleo, D. A., Daunert, S., & Bachas, L. G. (2012). Oriented Immobilization of Proteins on Hydroxyapatite Surface Using Bifunctional Bisphosphonates as Linkers. *Biomacromolecules*, *13*(6), 1742–1749. https://doi.org/10.1021/bm201865r

Zha, Q., Zhang, L., Guo, Y., Bao, R., Shi, F., & Shi, Y. (2022). Preparation and Study of Folate Modified Albumin Targeting Microspheres. *Journal of Oncology*, *2022*, 1–9. https://doi.org/10.1155/2022/3968403

Zhai, M., Wu, P., Liao, Y., Wu, L., & Zhao, Y. (2024). Polymer Microspheres and Their Application in Cancer Diagnosis and Treatment. *International Journal of Molecular Sciences*, *25*(12), 6556. https://doi.org/10.3390/ijms25126556

Zhang, C., Shan, S., Hu, T., Wang, G., Zhi, Y., Su, H., Jiang, L., & Ni, Y. (2021). Recent progress on biomedical applications of functionalized hollow hydroxyapatite microspheres. *Ceramics International*, *47*(10), 13552–13571. https://doi.org/10.1016/j.ceramint.2021.01.214

Zheng, Y., Zou, J., Sun, C., Peng, F., & Peng, C. (2023). Ado-tratuzumab emtansine beyond breast cancer: therapeutic role of targeting other HER2-positive cancers. *Frontiers in Molecular Biosciences*, *10*. https://doi.org/10.3389/fmolb.2023.1165781