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# Transition Metal Layered Double Hydroxide Nanosheets for Breast Cancer Drug Delivery

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**ABSTRACT**

This review synthesizes research on transition metal layered double hydroxide nanosheets in breast cancerdrug encapsulation to address challenges in targeted delivery, drug resistance, and controlled release. Thereview aimed to evaluate synthesis and functionalization strategies, benchmark drug loading and releaseprofiles, elucidate metal doping effects on targeting and uptake, compare therapeutic outcomes, and identifyclinical translation barriers. Literature selection focused on experimental in vitro and in vivo studies publishedup to mid-2024, emphasizing physicochemical characterization, cellular internalization, and therapeuticefficacy. Findings reveal that transition metal doping and surface modifications enable ultrahigh drug loadingcapacities with pH-responsive, tumor-specific release, enhancing intracellular delivery via receptor-mediatedand endocytic pathways. Multifunctional nanosheets demonstrate synergistic anticancer effects throughcombined chemo-, photothermal, and immunotherapies, with favorable biocompatibility and low systemictoxicity in preclinical models. However, variability in synthesis reproducibility, incomplete mechanisticunderstanding of metal ion interactions, and limited long-term safety data impede clinical translation.Integrating these findings underscores the potential of transition metal-doped LDH nanosheets as versatileplatforms for breast cancer nanotherapy while highlighting the need for standardized evaluation andcomprehensive in vivo assessments. These insights inform future research directions toward optimizingnanosheet design and advancing clinical applications in precision oncology.

Keywords: Layered Double Hydroxide; nanosheets; transition metals; drug delivery

## I. INTRODUCTION

Research on transition metal layered double hydroxide (LDH) nanosheets in breast cancer drug encapsulationhas emerged as a critical area of inquiry due to their unique physicochemical properties, biocompatibility, andpotential for targeted drug delivery(Yu et al., 2023)(Peng et al., 2023). Over the past two decades, LDHs haveevolved from simple anion exchangers to sophisticated nanocarriers capable of controlled drug release andmultifunctional therapeutic applications(Kim et al., 2014)(Zhang et al., 2022). The practical significance of thisresearch is underscored by breast cancer's status as the most common cancer among women worldwide, withhigh morbidity and mortality rates necessitating improved treatment modalities(Hosseini et al., n.d.)(Gupta etal., 2025). LDH nanosheets offer promising solutions

to overcome limitations of conventional chemotherapy,such as poor drug solubility, systemic toxicity, and multidrug resistance(Bao et al., 2023)(Hakeem et al., 2018).

Despite advances, challenges remain in optimizing LDH nanosheets for effective breast cancer therapy. Thespecific problem lies in achieving high drug loading, targeted delivery, and controlled release within the tumormicroenvironment while minimizing off-target effects(Zhang et al., 2022)(Mei et al., 2018). Current literaturereveals a knowledge gap regarding the integration of transition metal doping to enhance therapeutic efficacyand multifunctionality of LDH nanosheets(Zhang et al., 2022)(Yang et al., 2024)(Tang et al., 2024). Moreover,controversies exist about the optimal synthesis methods and surface modifications to balance stability, cellularuptake, and biodegradability(Yu et al., 2023)(Liang et al., 2023)(Xu et al., 2021). Failure to address these gapsmay limit clinical translation and therapeutic outcomes(Omonmhenle & Ifijen, 2023). Thus, a comprehensiveunderstanding of the interplay between LDH composition, nanosheet architecture, and drug encapsulationmechanisms is essential(Maity et al., 2024)(Chen et al., 2023).

The conceptual framework for this review is grounded in the definition of LDHs as two-dimensionalnanomaterials composed of divalent and trivalent metal hydroxide layers with intercalated anions(Yu et al.,2023)(Peng et al., 2023). Transition metal doping modifies the electronic and structural properties of LDHs,enhancing drug loading capacity, pH-responsive release, and synergistic therapeutic effects(Zhang et al., 2022)(Yang et al., 2024). The relationship between nanosheet physicochemical characteristics and biologicalinteractions underpins their function as drug carriers, linking material design to therapeutic efficacy(Mei et al.,2018)(Li et al., 2021). This framework guides the systematic evaluation of LDH nanosheets in breast cancerdrug encapsulation.

The purpose of this systematic review is to critically analyze recent advancements in transition metal LDHnanosheets for breast cancer drug encapsulation, focusing on synthesis strategies, drug loading mechanisms,and therapeutic outcomes(Bao et al., 2023)(Zhang et al., 2022). By addressing the identified knowledge gaps,this review aims to provide a consolidated understanding that informs the rational design of multifunctionalLDH-based nanocarriers(Gupta et al., 2025)(Omonmhenle & Ifijen, 2023). The value added lies in synthesizingdispersed findings into a coherent narrative that supports future research and clinical translation.

This review employs a comprehensive literature survey of peer-reviewed studies from 2014 to 2025,emphasizing experimental and theoretical investigations of LDH nanosheets in breast cancer therapy(Bao etal., 2023)(Gupta et al., 2025). Inclusion criteria focus on transition metal-doped LDHs, drug encapsulationefficiency, and in vitro/in vivo therapeutic efficacy, while excluding purely diagnostic or synthesis-onlystudies(Yu et al., 2023)(Ren et al., 2022). Analytical frameworks include physicochemical characterization,cellular uptake mechanisms, and therapeutic performance metrics. The findings are organized thematically toelucidate synthesis methods, drug delivery capabilities, and clinical potential.

The objective of this report is to examine the existing research on "Transition metal layered double hydroxidenanosheets in breast cancer drug encapsulation" in order to elucidate the current advancements, mechanisms,and therapeutic potentials of these nanomaterials in targeted drug delivery. This review is important as itaddresses the critical challenges in breast cancer treatment, including drug resistance, targeted delivery, andcontrolled release, by exploring how transition metal-doped LDH nanosheets enhance drug loading efficiency, biocompatibility, and tumor specificity. The report aims to synthesize knowledge on the physicochemicalproperties, functionalization strategies, and biomedical applications of these

nanosheets, thereby providing acomprehensive understanding that can guide future research and clinical translation in nanomedicine forbreast cancer therapy.

## II. METHODOLOGY OF LITERATURE SELECTION

### II.1 Transformation of Query

We take your original research question "Transition metal layered double hydroxide nanosheets inbreast cancer drug encapsulation" and expand it into multiple, more specific search statements. Bysystematically expanding a broad research question into several targeted queries, we ensure that yourliterature search is both comprehensive (you won't miss niche or jargon-specific studies) and manageable(each query returns a set of papers tightly aligned with a particular facet of your topic).

Below were the transformed queries we formed from the original query:

a) Transition metal layered double hydroxide nanosheets in breast cancer drug encapsulation
b) Applications and advancements of layered double hydroxides in drug delivery systems for various cancersand their multifunctional properties
c) Innovative mechanisms of layered double hydroxides in targeted drug delivery and controlled release forbreast cancer therapies
d) Innovative mechanisms and applications of layered double hydroxides in targeted drug delivery systemsfor breast cancer therapies
e) Investigation of pH-responsive layered double hydroxides for enhanced drug delivery in breast cancertherapies

### II.2 Screening Papers

We then run each of your transformed queries with the applied Inclusion & Exclusion Criteria to retrieve afocused set of candidate papers for our always expanding database of over 270 million research papers.

### II.3 Relevance scoring and sorting

We take our assembled pool of 357 candidate papers (333 from search queries + 24 from citation chaining)and impose a relevance ranking so that the most pertinent studies rise to the top of our final papers table. Wefound 346 papers that were relevant to the research query. Out of 346 papers, 50 were highly relevant.

## III. RESULTS

### III.1 Descriptive Summary of the Studies

This section maps the research landscape of the literature on Transition metal layered double hydroxidenanosheets in breast cancer drug encapsulation, encompassing a diverse range of studies focused onsynthesis, functionalization, drug loading, and therapeutic applications. The reviewed works predominantlyemploy experimental methodologies involving in vitro and in vivo breast cancer models, with a strongemphasis on physicochemical characterization, cellular uptake mechanisms, and therapeutic efficacy. Thecomparison addresses critical parameters such as drug loading capacity, controlled release, cellularinternalization, therapeutic outcomes, and biocompatibility, thereby providing a comprehensive understandingrelevant to overcoming challenges in targeted breast cancer nanotherapy.

**Table 1.** Comparison of Characteristics and Therapeutic Efficacy of Various Nanoparticle Formulations in Cancer Therapy

| Study | Drug Loading Capacity | Controlled Release Profile | Cellular Uptake Efficiency | Therapeutic Efficacy | Biocompatibility and Safety |
|---|---|---|---|---|---|
| (Bao et al., 2023) | High loading of DOX and siRNA via electrostatic adsorption | Strong controlled release with pH sensitivity | Enhanced internalization of DOX and siRNA in MCF-7/ADR cells | Significant tumor inhibition and reversal of drug resistance in vitro and in vivo | Demonstrated low systemic toxicity in animal models |
| (Guo et al., 2021) | DOX loaded with ferrous ion doping, moderate loading | pH-dependent degradation and release, enhanced by acidic tumor microenvironment | Efficient cellular uptake with MRI-guided targeting | Synergistic chemo/photothermal therapy with tumor growth inhibition | Good biocompatibility, minimal side effects reported |
| (Pang et al., 2024) | Simvastatin intercalated with iron-based LDH, effective loading | pH-responsive release promoting ferroptosis and apoptosis | High cellular uptake and intracellular Fe2+ accumulation | Superior anticancer effects with ferroptosis-apoptosis synergy | Low systemic toxicity, good biocompatibility in vivo |
| (Peralta et al., 2023) | Carbamazepine loading up to 51% in magnetic LDH nanoclays | pH-responsive release, faster at lysosomal pH | Enhanced uptake aided by magnetic targeting | Improved efficacy over free drug in MDA-MB-231 cells | Magnetic properties enable targeted delivery, safe profile |
| (Peralta et al., 2024) | Gallic acid loading ~30% in Fe/Mg/Al magnetic LDH | Fast release at lysosomal pH, minimal release at blood pH | Improved uptake with magnetic guidance | 77% reduction in breast cancer cell viability in vitro | Superparamagnetic behavior reduces residual magnetization |

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| (Zhang et al., 2023) | miR-30a loaded with high binding efficiency on LDH | pH-sensitive release, lysosomal escape demonstrated | Effective uptake and retention in SKBR3 cells | Reduced proliferation and tumor size in vivo | No significant organ toxicity observed |
| (Ranjbar & Namazi, 2023) | DOX encapsulation efficiency ~78%, drug loading ~7.8% | pH-sensitive controlled release, higher at acidic pH | Enhanced uptake in MCF-7 cells | Higher cytotoxicity than free DOX, effective apoptosis induction | Non-hemolytic, biocompatible nanocarrier |
| (Zhu et al., 2023) | Ultrahigh ICG loading via electrostatic interaction | pH-responsive self-destructive release | Active transport and mitochondrial targeting | Combined phototherapy and immunotherapy with strong tumor suppression | Excellent biocompatibility, immune activation observed |
| (Li et al., n.d.) | Cu2+-doped LDH co-delivering diclofenac and LOX, stable loading | Acidic TME-triggered release of Cu2+ and drugs | Efficient cellular uptake and metabolic regulation | Synergistic chemodynamic/immunotherapy with tumor growth inhibition | Immunomodulatory effects, low toxicity reported |
| (Abdelgalil et al., 2023) | Pemetrexed intercalated with sericin-tagged LDH, sustained loading | Sustained release profile, reduced premature drug release | High cellular uptake in MDA-MB-231 cells | Enhanced cytotoxicity compared to free drug | Biocompatible with imaging capability |
| (Xia et al., 2023) | 5-FU intercalated in CuAl-LDH with HA coating | pH-degradable, rapid release in tumor cells | Targeted uptake via CD44 receptor | Synergistic chemodynamic and chemotherapy with immune activation | Promoted antitumor immunity, safe in vivo |
| (Zhang et al., 2022) | Ultra-thin LDH nanosheets with exceptionally high DOX loading (734%) | pH-responsive sustained release in tumor microenvironment | Increased cellular uptake and prolonged circulation | Superior therapeutic effect and reduced toxicity | Excellent biocompatibility and stability |

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| (Zhang et al., 2024) | High protein loading (182%) of gelonin on ultrathin LDH nanosheets | Controlled intracellular release with endosome escape | Enhanced uptake and deep tumor penetration | Improved anticancer efficiency in 2D and 3D models | Favorable safety profile in vitro |
| (Zhang et al., 2022) | Fe-doped LDH loaded with EGCG, targeted loading | pH-triggered release of iron ions and drug | Targeted uptake via CD44 receptor | Cooperative chemo-chemodynamic therapy with apoptosis and ferroptosis | Superior biocompatibility, minimal side effects |
| (Alves et al., 2024) | Indomethacin intercalated LDH with optimized synthesis | Controlled release influenced by synthesis conditions | Effective cellular uptake in multiple cancer cell lines | Inhibited proliferation in breast cancer cells | Reduced toxicity compared to free drug |
| (Khorsandi et al., 2015) | Curcumin intercalated LDH with ~50% loading | Improved solubility and pH- responsive release | Enhanced uptake in MDA-MB-231 cells | More effective photodynamic therapy than free curcumin | Biocompatible nanohybrid system |
| (Yu et al., 2023) | Review of multifunctional LDHs with variable drug loading | Controlled release and imaging capabilities | Enhanced cellular permeability reported | Multifunctional therapeutic and diagnostic applications | Generally good biocompatibility emphasized |
| (Peng et al., 2023) | Review on 2D LDHs for biomedical applications | pH responsiveness and functionalization strategies | Improved cellular uptake via surface modifications | Broad therapeutic applications in cancer | Biocompatibility and biodegradability highlighted |

### III.2 Critical Analysis and Synthesis

The literature on transition metal layered double hydroxide (LDH) nanosheets for breast cancer drugencapsulation reveals significant advancements in synthesis, functionalization, and therapeutic applications.Studies demonstrate enhanced drug loading, targeted delivery, and synergistic therapeutic effects throughmetal doping and surface modifications. However, challenges remain in achieving consistent in vivo efficacy,understanding complex cellular uptake mechanisms, and addressing clinical translation barriers. The diversityof experimental

models and methodologies also complicates direct comparisons across studies, highlightingthe need for standardized evaluation protocols.

**Table 2.** Comparison of Strengths and Weaknesses in Synthesis, Drug Loading, Cellular Uptake, and Therapeutic Efficacy of Transition Metal-Doped LDH Nanosheets for Cancer Therapy

| Aspect | Strengths | Weaknesses |
|---|---|---|
| Synthesis and Functionalization Strategies | The reviewed studies showcase versatile and robust synthesis methods, including hydrothermal co- precipitation and exfoliation-reassembling, enabling precise doping of transition metals such as Ni, Fe, Cu, and Zn into LDH nanosheets. Functionalization with polymers, targeting ligands, and biomimetic coatings enhances colloidal stability, biocompatibility, and tumor specificity, as seen in NiFe-LDH for siRNA/DOX co- delivery and sericin-tagged LDHs for combined therapy and imaging(Bao et al., 2023)(Abdelgalil et al., 2023). These strategies allow for high drug loading capacities and controlled release profiles tailored to tumor microenvironment conditions(Zhang et al., 2022)(Liang et al., 2023). | Despite these advances, synthesis reproducibility and scalability remain concerns, with some methods requiring complex multi-step processes that may limit clinical translation. Additionally, the impact of doping concentration and distribution on nanosheet stability and biological interactions is not uniformly addressed, leading to variability in therapeutic outcomes(Maity et al., 2024)(Yang et al., 2024). The lack of standardized protocols for functionalization complicates cross-study comparisons and optimization. |
| Drug Loading Capacity and Release Kinetics | Transition metal doping and nanosheet exfoliation have enabled ultrahigh drug loading capacities, exemplified by doxorubicin loading ratios exceeding 700% in ultra- thin LDH nanosheets(Zhang et al., 2022). pH-responsive release mechanisms are widely reported, facilitating selective drug release in acidic tumor environments, improving therapeutic index and reducing systemic toxicity(Hakeem et al., 2018)(Cao et al., 2018). Magnetic doping further allows for externally guided delivery and controlled release, enhancing tumor accumulation(Peralta et al., 2023)(Peralta et al., 2024). | However, many studies rely on in vitro release assays that may not fully replicate the complex in vivo tumor microenvironment, potentially overestimating release control and efficacy(Wang et al., 2023). The stability of drug- nanosheet interactions under physiological conditions varies, and premature drug leakage remains a challenge in some formulations(Hakeem et al., 2018). Moreover, the influence of protein corona formation on release kinetics is underexplored. |

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| Cellular Uptake and Targeting Mechanisms | Functionalization with targeting moieties such as folic acid, hyaluronic acid, and cell membrane cloaking has demonstrated enhanced cellular uptake and tumor specificity(Zhang et al., 2023)(Xia et al., 2023)(Wang et al., 2021). Studies employing enzyme modification (e.g., hyaluronidase) show improved tumor penetration by degrading extracellular matrix components(Li et al., 2021). The elucidation of endocytic pathways, including macropinocytosis and clathrin-mediated uptake, supports the rational design of LDH nanosheets for efficient intracellular delivery(Hakeem et al., 2018). | Despite these insights, the heterogeneity of breast cancer cell lines and tumor models used limits generalizability. The detailed intracellular trafficking and fate of LDH nanosheets post- uptake are not consistently characterized, leaving gaps in understanding potential off- target effects and long-term biocompatibility(Zhang et al., 2024)(Xu et al., 2021). Additionally, the influence of tumor microenvironment factors on targeting efficacy requires further investigation. |
| Therapeutic Efficacy and Synergistic Modalities | Numerous studies report enhanced anticancer efficacy through combined chemo, photothermal, photodynamic, chemodynamic, and immunotherapies using transition metal-doped LDHs(Guo et al., 2021) (Zhu et al., 2023)(Zhang et al., 2022)(Tang et al., 2024). Co-delivery of drugs and genetic materials (e.g., siRNA, miRNA) addresses multidrug resistance and promotes apoptosis and ferroptosis synergistically(Bao et al., 2023)(Pang et al., 2024)(Chen et al., 2023). In vivo models demonstrate significant tumor growth inhibition with reduced systemic toxicity(Zhang et al., 2023)(Zhang et al., 2022). | However, many therapeutic evaluations are limited to small animal models with short-term follow-up, raising concerns about long-term safety and efficacy(Bao et al., 2023)(Zhang et al., 2019). The complexity of multifunctional systems may introduce challenges in reproducibility and regulatory approval. Furthermore, the potential immunogenicity and off-target effects of metal ions and nanosheets are not comprehensively assessed. |

### III.3 Thematic Review of Literature

The literature on transition metal layered double hydroxide (LDH) nanosheets in breast cancer treatment predominantly highlights their multifunctional role as drug delivery platforms exhibiting high drug loading capacity, controlled and pH-responsive drug release, and enhanced targeting efficacy. Many studies focus on the synthesis and functionalization of LDH nanosheets with transition metals to improve therapeutic outcomes through mechanisms such as ferroptosis, chemodynamic therapy, and photothermal therapy. There is a notable emphasis on overcoming multidrug resistance, improving cellular uptake, and combining therapeutic modalities to enhance antitumor efficacy and minimize side effects. Clinical translation challenges and biocompatibility are also recurrent themes, reflecting ongoing efforts to optimize LDH nanosheets for practical breast cancer nanotherapy applications.

### III.4 Chronological Review of Literature

The literature on transition metal layered double hydroxide (LDH) nanosheets for breast cancer drug encapsulation has evolved significantly from foundational studies on drug intercalation and biocompatibility to advanced multifunctional theranostic platforms. Early research focused on improving drug loading, stability, and pH-responsive release mechanisms, while subsequent studies explored targeting strategies, overcoming multidrug resistance, and combining therapies such as photothermal, chemodynamic, and immunotherapy. Recent advancements highlight metal doping to enhance reactive oxygen species generation, ferroptosis induction, and integration with imaging modalities, positioning LDHs as versatile nanocarriers with promising clinical translation potential.

**Table 3.** Chronological Overview of Research Directions and Developments in LDH Nanoplatforms for Cancer Therapy

| Year Range | Research Direction | Description |
|---|---|---|
| 2012–2015 | Foundational Development of LDH Drug Carriers | Initial efforts established LDHs as biocompatible nanocarriers for anticancer drugs, focusing on intercalation techniques, improving drug stability and solubility, and enhancing photodynamic therapy efficacy against breast cancer cells. These studies demonstrated controlled drug release and the potential for improved therapeutic outcomes compared to free drugs. |
| 2018–2019 | Advancement in pH-Responsive and Multifunctional LDH Systems | Research emphasized pH-sensitive LDH nanoplatforms enabling controlled drug release in tumor microenvironments, incorporation of polymers for enhanced stability and cellular uptake, and combination therapies leveraging LDHs for synergistic apoptosis and cathepsin-mediated effects. Multifunctional hybrid materials integrating imaging and therapeutic agents began to emerge. |
| 2020–2021 | Integration of Magnetic and Photothermal Properties for Targeted Therapy | Studies developed magnetic LDH composites for external field-guided targeting and hyperthermia-triggered drug release, as well as photothermal and photodynamic therapy combinations. Efforts focused on enhancing tumor specificity, controlled release kinetics, and introducing imaging guidance to monitor therapeutic response. |
| 2022–2023 | Metal Doping and Stimuli-Responsive Nanoplatforms for Enhanced Therapy | The focus shifted to doping LDHs with transition metals (Fe, Ni, Cu) to improve reactive oxygen species generation, ferroptosis induction, and synergistic chemo-chemodynamic therapies. Advanced surface modifications with biomolecules and polymers facilitated tumor targeting, immune modulation, and improved in vivo efficacy, addressing multidrug resistance and tumor microenvironment challenges. |
| 2024– | Multifunctional Theranostic LDH | Recent works have engineered LDHs for combined therapeutic modalities including sonodynamic, photothermal, ferroptosis, and immunotherapy, with integrated imaging capabilities for real-time tracking. Innovations include biomimetic coatings for immune evasion, nanoplatforms for embryo-safe |

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| 2025 | Nanosheets and Clinical Translation Efforts | chemotherapy, and next-generation multifunctional nanocarriers designed for enhanced bioavailability, safety, and targeted delivery, aiming at clinical applicability. |

### III.5 Gaps and Future Research Directions

**Table 4.** Research Gaps, Future Directions, and Priorities in the Development of LDH Nanoplatforms for Cancer Therapy

| **Gap Area** | **Description** | **Future Research Directions** | **Justification** | **Research Priority** |
|---|---|---|---|---|
| Standardization of Synthesis and Functionalization Protocols | Current synthesis methods for transition metal-doped LDH nanosheets vary widely, affecting reproducibility and scalability. | Develop standardized, scalable synthesis protocols with controlled doping concentrations and uniform functionalization to ensure consistent physicochemical and biological properties. | Variability in synthesis leads to inconsistent therapeutic outcomes and hinders clinical translation (Bao et al., 2023) (Maity et al., 2024) (Yang et al., 2024). | High |
| In Vivo Drug Release and Stability under Physiological Conditions | Most studies rely on in vitro pH-responsive release assays that may not fully replicate the complex tumor microenvironment. | Conduct comprehensive in vivo studies to evaluate drug release kinetics, nanosheet stability, and premature drug leakage in physiological and tumor microenvironments. | In vivo conditions such as protein corona formation and enzymatic activity can alter release profiles, impacting efficacy and safety (Wang et al., 2023) (Hakeem et al., 2018). | High |
| Detailed Mechanistic Understanding of Cellular Uptake and Intracellular Trafficking | Limited characterization of intracellular fate and trafficking pathways of LDH nanosheets post-uptake. | Employ advanced imaging and molecular tracking techniques to elucidate endocytic pathways, lysosomal escape, and intracellular drug release mechanisms across diverse breast cancer models. | Understanding intracellular dynamics is critical to optimize targeting, minimize off-target effects, and improve therapeutic efficacy (Zhang et al., 2023) (Zhang et al., 2024) (Xu et al., 2021). | High |

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|---|---|---|---|---|
| Long-Term In Vivo Safety and Toxicity Profiles | Existing studies provide limited data on long-term systemic toxicity, biodistribution, and immune interactions of metal-doped LDHs. | Perform extended in vivo toxicity, immunogenicity, and biodistribution studies, including effects of metal ion release and accumulation in non-target organs. | Safety concerns, especially related to metal ion toxicity and immune responses, are major barriers to clinical application (Ranjbar & Namazi, 2023) (Shi et al., 2015) (Tang et al., 2024). | High |
| Optimization of Multifunctional LDH Platforms for Synergistic Therapies | Multifunctional LDHs combining chemo-, photothermal, photodynamic, and immunotherapies show promise but face complexity and reproducibility challenges. | Design simplified, modular LDH nanosystems with tunable multifunctionality; systematically compare therapeutic combinations and dosing regimens in clinically relevant models. | Complexity may impede reproducibility and regulatory approval; optimized platforms can maximize synergistic effects (Guo et al., 2021) (Zhu et al., 2023) (Zhang et al., 2019). | Medium |
| Influence of Tumor Microenvironment Heterogeneity on Targeting Efficacy | Limited exploration of how tumor heterogeneity and microenvironment factors affect LDH nanosheet targeting and penetration. | Investigate LDH nanosheet performance in diverse breast cancer subtypes and tumor microenvironments, focusing on ECM composition, pH | Tumor heterogeneity impacts targeting ligand efficacy and drug delivery, affecting clinical | Medium |

## VI. CONCLUSION

The collective body of research on transition metal layered double hydroxide (LDH) nanosheets for breast cancer drug encapsulation underscores their remarkable potential as multifunctional nanocarriers that address critical therapeutic challenges. Transition metal doping—especially with iron, copper, nickel, and ferrous ions— profoundly modulates the physicochemical properties of LDHs, enhancing drug loading capacities, facilitating controlled and stimuli-responsive drug release, and enabling synergistic therapeutic mechanisms such as chemodynamic therapy, photothermal therapy, and ferroptosis induction. These doped LDHs demonstrate high drug encapsulation efficiencies, ranging from moderate to ultrahigh levels, with sophisticated pH sensitive and tumor microenvironment-triggered release profiles that reduce off-target toxicity and improve drug bioavailability. Functionalization strategies, including surface modification with targeting ligands like hyaluronic acid, folic acid, cell membrane cloaking, and polymer coatings, significantly augment cellular uptake efficiency and tumor specificity. Such modifications facilitate receptor-mediated endocytosis and improved tumor penetration, while magnetic and photothermal properties further enhance tumor accumulation and intracellular delivery. The literature highlights that cellular internalization mechanisms are diverse, often involving macropinocytosis and clathrin-mediated pathways, but a comprehensive understanding of intracellular trafficking and fate

remains incomplete. Therapeutically, LDH nanosheet platforms consistently outperform free drugs across various in vitro and in vivo breast cancer models, achieving enhanced cytotoxicity, tumor growth inhibition, and synergistic effects through combination therapies. Notably, transition metal doping enables the integration of multiple therapeutic modalities, including immunotherapy activation, apoptosis, ferroptosis, and chemodynamic effects, to overcome drug resistance and exploit tumor microenvironment vulnerabilities. Biocompatibility profiles are generally favorable, with surface modifications mitigating immune clearance and systemic toxicity; however, long-term safety and pharmacokinetic data are still nascent and require further exploration. Despite these promising advancements, challenges persist in reproducibility, scalability, and clinical translation. Variability in synthesis methods, limited standardized evaluation protocols, and insufficient mechanistic elucidation of metal ion interactions constrain direct comparison and optimization. Moreover, gaps remain in understanding the influence of the tumor microenvironment on targeting efficacy and the long-term biodistribution and toxicity of metal-doped LDHs. Future research should prioritize comprehensive in vivo safety assessments, standardized characterization methodologies, and the development of clinically scalable synthesis processes to harness the full therapeutic potential of transition metal-doped LDH nanosheets in breast cancer nanomedicine.

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