Section: Research Paper

LACTOFERRIN ANCHORED NANOMATERIALS: A NOVEL APPROACH OF TARGETING BRAIN TUMOR

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ABSTRACT:

Brain tumors pose a substantial challenge due to their location within the brain and the existence of the Blood-Brain Barrier (BBB), which confines the access to many therapeutic agents. This article provides an overview of brain tumors, their cataloguing, causes, and symptoms, highlighting the life-threatening nature of these tumors. The main focus of this article is the novel approach of active targeting in nanoformulation for brain tumors by means of lactoferrin (Lf) as an active targeting ligand. The BBB's impermeability hampers the delivery of therapeutic agents to brain tumors, making it essential to find efficient drug delivery systems. Nanoparticles fabricated from various polymers such as synthetic and biopolymers have shown promise in this regard due to their diverse nature and biocompatibility. This includes a method using lactoferrin, a protein with iron-binding properties, as an active targeting ligand to improve brain tumor drug delivery. with highlighting the increasing research interest in using lactoferrin-based nanoparticles in cancer therapy, and the potential application of lactoferrin as a drug nanocarrier in the field of nanomedicine.

Keywords: Brain tumor, glioblastoma, lactoferrin, ligand, nanoparticles, nanomedicine

INTRODUCTION

A brain tumor is an abnormal growth of cells within the brain or the central nervous system.

These tumors can be benign (non-cancerous) or malignant (cancerous), and they can originate from different types of cells within the brain, such as neurons, glial cells, and other supportive tissues.

Benign tumors are generally slower-growing and less likely to spread to other parts of the body. However, even benign brain tumors can cause serious problems by pressing on or displacing surrounding brain tissue, leading to symptoms such as headaches, seizures, changes in vision, cognitive difficulties, and more.

Malignant brain tumors, also known as brain cancer, are more aggressive and have the potential to invade nearby tissues and spread to other parts of the brain or spinal cord. The growth of malignant brain tumors can lead to increased intracranial pressure, which can cause symptoms like severe headaches, vomiting, altered consciousness, and neurological deficits [1].

Overview of brain tumor and their impact

A brain tumor is an abnormal growth of cells in the brain or the surrounding tissues. They can be either **benign** (non-cancerous) or **malignant** (cancerous). Brain tumors are classified based on their origin, location, and behavior. They can originate from brain cells (primary brain tumors) or spread to the brain from other parts of the body (secondary or metastatic brain tumors). Primary brain tumors are further classified based on the types of cells they arise from, such as gliomas (originating from glial cells), meningiomas (arising from the meninges), and more [2].

Impact: The impact of a brain tumor can be significant and vary depending on several factors, including the tumor's type, size, location, and rate of growth. Here are some ways brain tumors can impact individuals:

- **Neurological Symptoms:** Brain tumors can cause a wide range of neurological symptoms, such as headaches, seizures, vision problems, difficulty speaking or understanding language, weakness in the limbs, balance issues, and changes in mood or personality. The symptoms can vary greatly based on the tumor's location in the brain.

- **Cognitive Function:** Depending on the location of the tumor, cognitive functions such as memory, concentration, and problem-solving may be affected. This can impact a person's ability to perform daily tasks, work, or engage in social activities.

- **Motor Function:** Tumors located in areas that control movement can lead to muscle weakness, coordination problems, and difficulties with fine motor skills.

- **Emotional and Psychological Impact:** Dealing with a brain tumor diagnosis and its effects can lead to emotional distress, anxiety, and depression for both patients and their loved ones.

- **Treatment Side Effects:** Treatments such as surgery, radiation therapy, and chemotherapy can have their own set of side effects, including fatigue, hair loss, nausea, and cognitive changes.

- **Quality of Life:** Brain tumors and their treatment can significantly affect an individual's overall quality of life. Depending on the severity of symptoms and their impact on daily activities, individuals may experience limitations in work, relationships, and recreational activities.

- **Prognosis:** The prognosis for brain tumors varies widely depending on factors such as the tumor type, grade, location, and the individual's overall health. Some benign tumors can be successfully treated with surgery and have a good prognosis, while malignant tumors can be more challenging to treat and have a less favorable outlook.

The cerebrospinal fluid (CSF) that normally circulates around and through the brain might become blocked by some types of brain tumours. When the ventricles swell in response to a rise in intracranial pressure, the condition is known as hydrocephalus. Oedema is a common symptom of brain tumours. Many of the symptoms are caused by the "mass effect," which is a combination of size, pressure, and oedema.

Types of brain tumors

More than 120 distinct kinds of tumours can develop in the brain. Common types of brain tumours include:

1. **Gliomas**: These tumors originate from glial cells, which are supportive cells in the brain. Gliomas are the most common type of primary brain tumors. They are further categorized based on the type of glial cell they arise from:

    a. **Astrocytomas**: Arise from astrocytes, star-shaped glial cells. They can be low-

grade (slow-growing) or high-grade (fast-growing and more malignant), such as glioblastoma multiforme, which is one of the most aggressive brain tumors.

b. **Oligodendrogliomas**: Arise from oligodendrocytes, another type of glial cell. These tumors are typically slow-growing but can be malignant.
c. **Ependymomas**: Arise from the ependymal cells lining the ventricles and central canal of the spinal cord. They can occur in both children and adults.

2. **Meningiomas**: These tumors originate from the meninges, the protective layers surrounding the brain and spinal cord. Meningiomas are typically slow-growing and often benign, but they can cause symptoms due to their location.

3. Schwannomas (Acoustic Neuromas): These tumors arise from Schwann cells, which produce the protective myelin sheath around nerve fibers. They often occur on the vestibular nerve, affecting hearing and balance.

4. **Medulloblastomas**: These are malignant tumors that primarily affect children and arise in the cerebellum. They are a type of embryonal tumor.

5. **Pituitary Adenomas**: These tumors form in the pituitary gland, which is located at the base of the brain. Depending on their size and hormone-secreting activity, they can cause hormonal imbalances and various symptoms.

6. **Craniopharyngiomas**: These are rare tumors that develop near the pituitary gland and often affect children. They can cause endocrine and visual problems due to their location.

7. **Primary Central Nervous System Lymphomas**: These are non-Hodgkin lymphomas that originate in the brain, spinal cord, or the eyes. They typically affect individuals with weakened immune systems.

8. **Metastatic Brain Tumors**: These tumors are secondary tumors that have spread (metastasized) to the brain from cancers originating in other parts of the body, such as the lungs, breasts, or skin.

9. **Chordomas**: These tumors develop from remnants of the notochord, a structure present during early development. They often occur at the base of the skull or along the spine.

10. **Hemangioblastomas**: These rare tumors can occur in the brain or spinal cord and are often associated with von Hippel-Lindau (VHL) disease, a genetic disorder.

These are just a few examples of the many types of brain tumors that exist. Each type has its own characteristics, growth patterns, and treatment options. Diagnosing and treating brain tumors requires careful evaluation by medical professionals, often involving imaging tests, biopsies, and other diagnostic techniques to determine the tumor's type and grade. Treatment plans are tailored to the specific type of tumor, its location, and the overall health of the individual.

Causes of brain tumors

The exact causes of brain tumors are not always well understood, and in many cases, the development of a brain tumor is complex and can involve a combination of genetic, environmental, and lifestyle factors [3]. Here are some factors that have been studied in relation to the development of brain tumors:

1. **Genetic Factors**: Certain genetic mutations and inherited conditions can increase the risk of developing brain tumors. For example, individuals with certain hereditary conditions such as neurofibromatosis type 1 and type 2, von Hippel-Lindau (VHL) disease, and Li-Fraumeni syndrome have an elevated risk of developing specific types of brain tumors.

2. **Radiation Exposure**: Exposure to ionizing radiation, especially at a young age, has been linked to an increased risk of brain tumors. This can include exposure from medical treatments (such as radiation therapy) or environmental sources.

3. **Family History**: While most brain tumors are not directly hereditary, having a family history of certain genetic conditions or brain tumors can slightly increase the risk of developing a brain tumor.

4. **Age**: The risk of brain tumors generally increases with age, with some types of tumors being more common in specific age groups.

5. **Immune System Suppression**: Individuals with weakened immune systems, such as those undergoing organ transplantation or individuals with certain immune disorders, may have a higher risk of developing brain tumors.

6. **Cell phone use**: There has been ongoing research into whether long-term and heavy use of cell phones could be associated with an increased risk of brain tumors due to the electromagnetic radiation emitted by phones. However, most studies have not provided conclusive evidence of a strong link.

7. **Environmental Exposures**: Some studies have explored potential links between exposure to certain chemicals, such as pesticides or industrial chemicals, and the risk of brain tumors. However, the evidence is often inconclusive or conflicting.

8. **Hormones**: Hormonal factors, such as hormone replacement therapy or exposure to hormones during pregnancy, have been investigated as possible contributors to brain tumor development.

9. **Viruses**: Some research suggests that certain viruses, such as the Epstein-Barr virus, might play a role in the development of specific brain tumors, particularly primary central nervous system lymphomas.

It's important to note that for the majority of brain tumors, the exact cause remains unclear, and many cases do not have a clear identifiable risk factor. In many instances, brain tumors seem to arise spontaneously, and their development may be influenced by a combination of genetic predisposition and environmental factors [4].

Symptoms

The symptoms of a brain tumor can vary widely depending on factors such as the tumor's location, size, type, and rate of growth. It's important to note that many of these symptoms can also be caused by other medical conditions, so a proper medical evaluation is essential for accurate diagnosis. Here are some common symptoms associated with brain tumors:

1. **Headaches**: Persistent and worsening headaches, especially in the morning or after lying down, can be a common symptom. The headaches may be accompanied by nausea and vomiting.

2. **Seizures**: New-onset seizures, especially in individuals without a history of seizures, can be indicative of a brain tumor. Seizures might involve convulsions, jerking movements, or staring spells.

3. **Neurological Deficits**: These can include various symptoms related to impaired brain function and may vary based on the tumor's location. Examples include:
    a. Weakness or numbness in the limbs, often on one side of the body.
    b. Difficulty speaking or understanding language.
    c. Changes in vision, such as double vision, blurred vision, or loss of peripheral vision.
    d. Balance and coordination problems.
    e. Memory difficulties and changes in cognitive function.
4. **Personality or Behavioral Changes**: Brain tumors can sometimes cause changes in mood, personality, or behavior. These changes may include depression, irritability, mood swings, and social withdrawal.
5. **Cognitive Changes**: Difficulties with thinking, reasoning, problem-solving, and memory can be indicative of a brain tumor, especially if these changes are sudden or severe.
6. **Speech Problems**: Trouble speaking, slurred speech, or difficulty finding the right words can be a symptom.
7. **Changes in Sensation**: Tingling or numbness in certain parts of the body can occur due to the tumor's impact on sensory pathways.
8. **Visual Disturbances**: Visual symptoms can include blurred or double vision, loss of peripheral vision, or visual hallucinations
9. **Nausea and Vomiting:** If not caused by other factors like a stomach virus, persistent nausea and vomiting can be associated with a brain tumor.
10. **Fatigue and Weakness**: Feeling unusually tired or weak, even after adequate rest, can be a symptom.
11. **Difficulty Swallowing**: Problems with swallowing and changes in taste perception can occur if the tumor affects the areas responsible for these functions.

12. **Unexplained Weight Loss or Gain**: Changes in appetite and weight can sometimes be linked to brain tumors.

The occurrence of headaches that have a propensity to exacerbate during the early hours.

1. The manifestation of seizures.
2. The individual experiences impaired coordination, vertigo, and challenges with ambulation.
3. One common issue that individuals may experience is speech difficulties, which can manifest as challenges in word retrieval or finding the appropriate word to express their thoughts.
4. The presence of visual impairments and atypical ocular motions
5. One of the observed manifestations is hemiparesis, which refers to a weakness or decreased motor function on one side of the body.
6. An elevation in intracranial pressure leads to symptoms such as somnolence, cephalalgia, emesis, and delayed reactions.

Pathogenesis of brain tumor

The pathogenesis of brain tumors involves complex processes that lead to the development and growth of abnormal cells within the brain. While the exact mechanisms can vary depending on the type of brain tumor, there are common underlying factors that contribute to their formation [5-7]. Here's an overview of the general pathogenesis:

1. **Genetic Mutations**: Genetic alterations play a significant role in the development of brain tumors. Mutations can affect the normal regulation of cell growth, division, and death. These mutations can be inherited or acquired during a person's lifetime due to various environmental factors, such as radiation exposure or chemical exposure.
2. **Uncontrolled Cell Growth**: Brain tumors arise when certain cells within the brain start to divide and grow uncontrollably. This uncontrolled cell growth can lead to the formation of a mass or tumor.
3. **Cell Differentiation**: In normal brain tissue, cells have specific functions and

characteristics. In brain tumors, these cells often lose their normal differentiation and take on more primitive or immature characteristics.

4. **Angiogenesis**: Tumors require a blood supply to provide nutrients and oxygen for their growth. Angiogenesis is the process by which tumors stimulate the formation of new blood vessels to support their growth.

5. **Invasion and Metastasis**: Malignant brain tumors can invade nearby tissues and spread to other parts of the brain or even to other areas of the body. This process involves the ability of tumor cells to break away from the primary tumor, enter the bloodstream or lymphatic system, and establish new tumor growths at distant sites.

6. **Immune System Evasion**: Tumor cells can develop strategies to evade the immune system's surveillance and attack mechanisms, allowing them to continue growing despite the body's defense mechanisms.

7. **Tumor Microenvironment**: The microenvironment surrounding the tumor plays a crucial role in its growth and progression. Various cells, including immune cells and support cells, interact with the tumor and can influence its behavior.

8. **Cell Signaling Pathways**: Aberrant activation of signaling pathways that control cell growth and survival can contribute to the development of brain tumors. Mutations in genes involved in these pathways can lead to unregulated cell division.

9. **Epigenetic** Changes: Epigenetic modifications, which involve changes in gene expression without altering the underlying DNA sequence, can impact the development of brain tumors by influencing how genes are turned on or off.

10. **Tumor Subtypes**: Different types of brain tumors have distinct pathogenic mechanisms based on their cell of origin and genetic mutations. For example, glioblastomas have unique genetic alterations that drive their aggressive growth.

It's important to note that brain tumors are a diverse group of diseases, and the pathogenesis can vary significantly between different types and subtypes of tumors. Researchers continue to study these mechanisms to gain a deeper understanding of brain tumor development, which could lead to more targeted and effective treatments in the future.

Tumor Location

The location of a brain tumor refers to the specific area within the brain where the tumor is located. The location is a critical factor in determining the symptoms a person may experience and the potential impact on various bodily functions. Different areas of the brain are responsible for different functions, so a tumor's location can influence the type of symptoms it causes. Here are some common regions of the brain and the potential effects of tumors in those areas:

1. **Frontal Lobe**: The frontal lobe is involved in higher cognitive functions, personality, behavior, and motor control. Tumors in this area can cause changes in personality, mood swings, impaired judgment, difficulties with decision-making, and weakness or paralysis on one side of the body.

2. **Parietal Lobe**: The parietal lobe is responsible for sensory perception, spatial awareness, and integration of sensory information. Tumors here can lead to problems with spatial perception, sensory disturbances, and difficulty recognizing objects or body parts.

3. **Temporal Lobe**: The temporal lobe is associated with memory, language processing, and emotions. Tumors in this region can cause memory problems, language difficulties, changes in mood, and auditory hallucinations.

4. **Occipital Lobe**: The occipital lobe is responsible for processing visual information. Tumors in this area can result in visual disturbances, such as vision loss, visual hallucinations, and difficulty recognizing objects.

5. **Cerebellum**: The cerebellum controls coordination, balance, and fine motor skills. Tumors in this region can lead to difficulties with balance, coordination, and fine motor control.

6. **Brainstem**: The brainstem controls vital functions such as breathing, heart rate, and blood pressure. Tumors in the brainstem can affect these functions, leading to symptoms like difficulty breathing, swallowing problems, and changes in heart rate.

7. **Pituitary Gland**: The pituitary gland regulates hormone production and controls various bodily functions. Tumors in this area can disrupt hormone balance, leading to

hormonal imbalances and related symptoms.

8. **Cerebral Ventricles**: Tumors within the fluid-filled spaces (ventricles) of the brain can obstruct the flow of cerebrospinal fluid, leading to increased intracranial pressure and symptoms such as headaches, vomiting, and changes in consciousness.

9. **Skull Base**: Tumors at the base of the skull can affect cranial nerves and nearby structures, leading to various neurological and sensory symptoms.

Tumor Growth Rate

The growth rate of a brain tumor refers to how quickly the tumor increases in size over a specific period of time. The growth rate can vary widely depending on the type of tumor, its location, grade, and other factors. Here are some key points to understand about tumor growth rates:

1. **Slow-Growing Tumors**: Some brain tumors are considered slow-growing, which means they increase in size gradually over an extended period of time. These tumors often include certain types of benign tumors like meningiomas and low-grade gliomas. Slow-growing tumors might not cause noticeable symptoms for a long time, and they can remain stable or slowly progress over years.

2. **Fast-Growing Tumors**: Other brain tumors, particularly high-grade gliomas such as glioblastomas, are aggressive and grow rapidly. These tumors can double in size in a matter of weeks to months. Fast-growing tumors tend to cause more noticeable symptoms in a shorter period due to their rapid expansion and the pressure they exert on surrounding brain tissue.

3. **Steady Growth**: Some brain tumors exhibit a steady, intermediate growth rate. These tumors can fall between the extremes of slow and fast growth. The rate of growth can depend on various factors, including the tumor's genetic characteristics and the microenvironment around it.

Factors Affecting Growth Rate

1. **Tumor Type and Grade**: The histological type (cell type) and grade (degree of malignancy) of the tumor influence its growth rate. Higher-grade tumors tend to grow

more quickly.

2. **Vascularization**: The presence of blood vessels supplying the tumor can impact its growth rate. Tumors with more blood vessels may grow more rapidly due to enhanced nutrient supply.

3. **Location**: Tumors in certain brain regions might have limited space to expand, which can affect their growth rate and symptoms they cause.

4. **Cellular Proliferation**: The rate at which tumor cells divide and replicate plays a crucial role in growth. Some tumors have more aggressive cell division mechanisms.

Lactoferrin: Lactoferrin was discovered in 1939, is a multifunctional glycoprotein found in various bodily fluids, including milk, saliva, and tears [8]. It plays a vital role in iron binding and transportation, immune response modulation, and antimicrobial activity. Lactoferrin is present in mammals, including humans, and it has gained attention for its potential health benefits. Due to its biological properties, lactoferrin has been explored as a ligand in nanoparticle-based drug delivery systems and other biomedical applications [9, 10]. Here's an overview of using lactoferrin as a ligand in nanoparticles:

Nanoparticles in Drug Delivery: Nanoparticles are tiny particles with dimensions typically in the range of nanometers. They can be engineered to carry drugs, genes, or other therapeutic agents, enabling targeted and controlled delivery to specific cells or tissues. Nanoparticles offer advantages such as prolonged drug release, reduced side effects, and improved bioavailability of therapeutic agents [11].

Lactoferrin as a Ligand: Lactoferrin can be used as a ligand, which is a molecule that binds to a specific target molecule, to functionalize the surface of nanoparticles. By attaching lactoferrin to the nanoparticle surface, researchers aim to achieve several goals:

- **Targeting:** Lactoferrin can act as a targeting ligand by binding to specific receptors that are overexpressed on the surface of certain cells, such as cancer cells. This allows nanoparticles to selectively accumulate at the target site, enhancing the therapeutic effect and reducing off-target effects.

- **Enhanced Cellular Uptake:** Lactoferrin-mediated targeting can promote the uptake of nanoparticles by cells, especially those that have receptors for lactoferrin. This can improve the internalization of therapeutic cargo carried by the nanoparticles.

- **Enhanced Stability:** Lactoferrin can help stabilize nanoparticles by preventing aggregation and maintaining their structural integrity in biological fluids.

- **Biocompatibility:** Lactoferrin is a naturally occurring protein with low immunogenicity and biocompatibility, making it suitable for use in biomedical applications.

- **Additional Functionalities:** Apart from targeting, lactoferrin's other biological activities, such as antimicrobial properties and immunomodulation, can be leveraged to enhance the therapeutic potential of nanoparticle-based systems.

Applications

- **Cancer Therapy:** Lactoferrin-functionalized nanoparticles can be designed to target cancer cells specifically, delivering chemotherapeutic agents directly to the tumor site while minimizing damage to healthy tissues.

- **Brain Drug Delivery:** Lactoferrin can facilitate the transport of therapeutic agents across the blood-brain barrier, which is often a challenge in treating neurological disorders.

- **Antimicrobial Nanoparticles:** Lactoferrin-coated nanoparticles can be used for delivering antimicrobial agents to combat infections.

- **Inflammatory Diseases:** Lactoferrin-functionalized nanoparticles can potentially target sites of inflammation, such as those associated with autoimmune diseases.

Lactoferrin a novel approach as an active targeting ligand more than other targeting ligands

Lactoferrin has gained interest in the field of cancer research, including brain tumor targeting, due to its potential as a targeting ligand for nanoparticles and drug delivery systems. Using lactoferrin as a targeting moiety can enhance the specificity of drug delivery to brain tumor cells, potentially improving treatment outcomes while minimizing damage to healthy tissue

[12]. Here's how lactoferrin can be utilized for brain tumor targeting:

1. **Receptor Expression:** Lactoferrin receptors are overexpressed on the surface of various cancer cells, including certain types of brain tumor cells. This receptor overexpression makes lactoferrin a suitable ligand for targeted drug delivery.

2. **Blood-Brain Barrier Penetration:** One of the challenges in brain tumor treatment is the blood-brain barrier (BBB), a protective barrier that limits the passage of substances from the bloodstream into the brain. Lactoferrin has shown potential to enhance the transport of therapeutic agents across the BBB, making it valuable for brain tumor treatment.

3. **Enhanced Cellular Uptake:** Lactoferrin-functionalized nanoparticles can bind to lactoferrin receptors on the surface of tumor cells, leading to increased cellular internalization of the nanoparticles carrying therapeutic cargo.

4. **Reduction of Systemic Side Effects:** Targeted delivery using lactoferrin can potentially reduce systemic side effects associated with chemotherapy, as the therapeutic agent is more concentrated at the tumor site and less likely to affect healthy tissues.

5. **Synergistic Effects:** Lactoferrin itself has shown anticancer and anti-inflammatory properties that could have synergistic effects with other therapeutic agents, enhancing the overall efficacy of the treatment.

6. **Personalized Treatment:** Lactoferrin-functionalized nanoparticles can be designed to carry different types of therapeutic cargo, such as chemotherapeutic drugs, targeted therapies, or even imaging agents. This allows for personalized treatment approaches based on the specific characteristics of the tumor.

Need for effective drug delivery systems for brain tumor lactoferrin as an active targeting ligand

There is a significant need for effective drug delivery systems for brain tumors, and lactoferrin can serve as an active targeting ligand in these systems.

- **Blood-Brain Barrier (BBB) impermeability:** The key task in dealing with brain

tumors is presence of the blood-brain barrier, a defensive sheet that separates blood circulation from the brain tissue. The BBB restricts the entry of many therapeutic medications into the brain, rendering it difficult to deliver drugs effectively to brain tumor sites.

- **Specificity and Selectivity:** Lactoferrin is a glycoprotein that can act as a targeting ligand because it binds specifically to receptors that are overexpressed on exterior of brain tumor cells.

  By using lactoferrin as a ligand, drug delivery systems can achieve higher specificity and selectivity for brain tumors, minimizing off-target effects and reducing damage to healthy brain tissue.

- **Enhanced Drug Accumulation:** Lactoferrin-mediated drug delivery systems can improve drug accumulation at the tumor site. When drugs are conjugated or coated with lactoferrin, they can bind to the tumor cells and be internalized through receptor-mediated endocytosis, increasing drug delivery to the tumor area.

- **Overcoming Drug Resistance:** Brain tumors often exhibit drug resistance, leading to treatment failure. Lactoferrin-targeted drug delivery can help overcome this resistance by improving drug uptake and bypassing resistance mechanisms.

- **Reduced Systemic Toxicity:** By utilizing lactoferrin as a targeting ligand, the drug delivery system can minimize systemic toxicity and side effects. This is because drugs are more efficiently delivered to the tumor, and fewer drugs are distributed throughout the rest of the body. Non-Invasive Approach: Lactoferrin-based drug delivery systems can be administered non-invasively, such as through intravenous injections, allowing for easier and more patient-friendly treatment regimens.

Methodology of nanocarrier's surface functionalization by lactoferrin [13]

Synthesis of Nanocarriers: The first step is to synthesize the nanocarriers, such as liposomes, micelles, polymeric nanoparticles, or silica nanoparticles, depending on the specific application and drug delivery requirements.

Preparation of Lactoferrin Solution: Lactoferrin, a milk protein, can be obtained from natural sources (e.g., bovine milk or human milk) or produced through recombinant

technology. Lactoferrin is typically purified and then dissolved in an appropriate solvent to prepare a lactoferrin solution.

Activation of Nanocarrier Surface: The surface of the nanocarriers needs to be activated to introduce functional groups that can react with lactoferrin. Commonly used functional groups for activation include carboxyl groups (COOH) or amino groups (NH2. The lactoferrin solution is then mixed with the activated nanocarriers, and a conjugation reaction takes place between the functional groups on the nanocarrier surface and the specific binding sites on lactoferrin.

This reaction may involve covalent bonding or other chemical interactions, depending on the type of nanocarrier and lactoferrin [13]. After the conjugation reaction, the nanocarriers are purified to remove any unbound lactoferrin and other impurities. Techniques such as centrifugation, dialysis, or ultrafiltration are commonly used for purification. The functionalization of the nanocarrier surface with lactoferrin is confirmed through many depiction procedures, such as Fourier-Transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR).

Loading of Therapeutic Agents: Once the lactoferrin is successfully attached to the nanocarrier surface, the nanocarriers can be loaded with therapeutic agents, such as anticancer drugs or neuroprotective agents. The lactoferrin- functionalized nanocarriers are then evaluated in vitro using cell culture studies to assess their cellular uptake and targeting efficiency. Furthermore, *in vivo* studies are conducted using animal models to evaluate the nanocarrier's ability to cross BBB and target brain tumor cells [14,15.]

Carbodiimide coupling reaction: The carbodiimide coupling reaction is a widely used chemical reaction in bioconjugation and crosslinking processes. It involves the use of carbodiimide reagents to activate carboxylic acid groups for the formation of amide bonds with primary amines. This reaction is commonly employed in the field of biochemistry and bioconjugation to link proteins, peptides, or other biomolecules to various molecules or surfaces [16]. The carbodiimide coupling reaction is as follows:

Carboxylic acid + Carbodiimide → O-acylisourea intermediate → Amide bond + Byproduct

In this reaction, the carbodiimide molecule (usually N-ethyl-N'-(3- dimethylaminopropyl) carbodiimide or EDC) first reacts with the carboxylic acid group of one molecule, forming an

O-acylisourea intermediate. The O-acylisourea intermediate is highly reactive and reacts with a primary amine group (e.g., from a protein or peptide) to form an amide bond, covalently linking the two molecules together. The byproduct generated during the reaction is typically a soluble urea derivative. The carbodiimide coupling reaction is widely used in bioconjugation and protein immobilization applications, including the covalent attachment of proteins to solid surfaces, antibody conjugation, and the synthesis of peptide-protein conjugates. It is a versatile and efficient method for functionalizing biomolecules and creating complex bioconjugates with controlled orientations and stoichiometries.

Maleinimide–thiol coupling reaction: The maleimide-thiol coupling reaction is a common and versatile bioconjugation method used in chemistry and biochemistry to link molecules containing maleimide groups to molecules containing thiol groups. This reaction is highly specific and efficient and is widely used in various fields, including protein labeling, antibody-drug conjugates, and the synthesis of other bioconjugates. The reaction occurs between a maleimide group (a molecule containing a maleimide functional group) and a thiol group (a molecule containing a thiol functional group). The reaction proceeds through a Michael addition, where the thiol nucleophilically attacks the electron-deficient double bond of the maleimide, resulting in the formation of a stable thioether linkage [17]. The general reaction scheme is as follows:

Maleimide-containing molecule + Thiol-containing molecule → Thioether- linked bioconjugate

Here are the key steps of the reaction:

- **Formation of the thiolate:** In the presence of a mild base (such as amines or hydroxides), the thiol group is deprotonated to form a thiolate ion. This thiolate ion is a more reactive nucleophile than the neutral thiol.

- **Michael addition:** The thiolate ion attacks the electron-deficient carbon-carbon double bond of the maleimide, leading to the formation of a covalent bond between the maleimide and the thiolate.

- **Formation of the thioether:** Upon protonation, the thiolate ion is converted back to the thiol group, and the maleimide-thiol coupling reaction results in the formation of a stable thioether linkage between the two molecules.

This reaction is widely used in the conjugation of proteins or peptides to thiol- containing molecules, such as cysteine residues on proteins or thiolated DNA. The maleimide-thiol coupling reaction is mild and specific, making it particularly useful for the modification of biomolecules without significantly affecting their structure and function [19-21].

Covalent conjugation using sulfo-GMBS crosslinking agent: Sulfo-GMBS (N-γ-Maleimidobutyryloxy) sulfosuccinimide ester) is a heterobifunctional crosslinking reagent commonly used in bioconjugation and protein chemistry. It possesses two functional groups: a maleimide group and a sulfosuccinimide ester group.

The maleimide group reacts specifically with free thiol groups (cysteine residues) on proteins or peptides, while the sulfosuccinimide ester group reacts with primary amines (such as lysine residues) at physiological pH [78].

- **Activation:** Sulfo-GMBS is dissolved in an appropriate solvent (usually DMSO or DMF) to create a reactive solution. The sulfo-GMBS solution is added to the solution containing the protein or peptide with free thiol groups. The maleimide group reacts with these thiol groups to form steady thioether bonds. After the maleimide reaction, it is essential to quench any unreacted maleimide groups to prevent non-specific binding. This is typically achieved by adding a thiol- containing compound, such as DTT (dithiothreitol) or 2-mercaptoethanol.

- **Reaction with amine-containing molecule:** If there are any primary amine groups present in the reaction mixture (e.g., lysine residues on proteins), the sulfo-GMBS will also react with them. This can lead to the formation of amide bonds between the protein and amine-containing molecules. Using sulfo-GMBS, there can create covalent linkages between various biomolecules, such as attaching peptides or other proteins to antibodies, immobilizing enzymes, or forming conjugates for biomedical and biotechnological applications.

1. **Electrostatics complexation:** Electrostatics complexation refers to the process by which charged molecules or ions interact and form complexes based on their electrostatic charges. It occurs between oppositely charged species and is driven by attractive forces between the charges.

2. **Protein-DNA Interactions:** Positively charged amino acid residues in proteins can interact with the -vely charged phosphate backbone of DNA, forming stable complexes.

These interactions are essential for DNA binding and gene regulation.

3. **Protein-Ligand Binding:** Electrostatic interactions can influence the binding of small molecules (ligands) to proteins. Charged regions on the protein surface can attract oppositely charged ligands, affecting binding affinity. Polymers with charged functional groups, known as polyelectrolytes, can form complexes with each other through electrostatic interactions. These polyelectrolyte complexes have applications in drug delivery and materials science. Static connections play a substantial part in the formation of micelles and further self-assembled structures of ionic surfactants in solution.

4. **Nanoparticle interactions:** Nanoparticles carrying opposite charges can form stable complexes through electrostatic interactions, which can be exploited in drug delivery and nanotechnology. Electrostatic complexation is the basis for many ion exchange processes used in water treatment, chromatography, and purification techniques. Electrostatic complexation is highly subtle to the ionic strength and pH of the environment, as these factors affect the strength of the electrostatic interactions. Understanding and controlling electrostatic complexation are crucial in various scientific and technological applications, ranging from drug design to materials engineering [22,23].

5. **Surface activation method:** The surface activation method is a process used to modify the surface properties of materials to improve their adhesion or reactivity with other substances. This technique is particularly important in areas like coatings, adhesives, and surface functionalization. There are several ways to activate a surface, and the choice depends on the material and desired outcome. Some common surface activation methods include:

6. **Plasma treatment:** Plasma is an ionized gas that can be used to introduce reactive species on a material's surface, creating functional groups that enhance adhesion or reactivity.

7. **Chemical treatment:** Using chemical agents, such as acids or bases, to modify the surface by etching, functionalization, or creating anchor sites for subsequent reactions.

8. **UV/ozone treatment:** Exposing a material's surface to ultraviolet (UV) light and ozone

can lead to the formation of reactive sites, making the surface more amenable to bonding or coating. The surface activation method is essential for a wide range of applications, including improving the adhesion between materials, promoting the deposition of thin films or coatings, and enabling specific chemical reactions on surface [24-26].

9. **Phase separation method:** Phase separation can occur in various systems, such as polymer blends, liquid mixtures, and thin films. It involves creating a homogeneous mixture of two or more components and then inducing conditions that cause them to separate into distinct phases. These phases may have different compositions, structures, and properties, providing a way to control and tailor the material's characteristics [27]. There are several techniques to induce phase separation, and some common methods like:

    a. **Temperature-induced phase separation**: Altering the temperature of the material can lead to phase separation, especially in mixtures with components that have different solubilities at different temperatures.

    b. **Solvent-induced phase separation**: Changing the solvent composition or the solvent evaporation rate in a solution can trigger phase separation in polymer blends and other systems.

    c. **Non-solvent-induced phase separation**: Introducing a nonsolvent into a solution can cause phase separation and precipitation of one component. There is another method which is spinodal decomposition**:** This method involves creating conditions where the system becomes thermodynamically unstable, leading to spontaneous phase separation. In thin-film fabrication, phase separation can occur during film deposition and subsequent processing steps, resulting in nanostructured surfaces and interfaces. The phase separation method is widely used in the development of various materials, including membranes, coatings, polymer composites, and biomaterials [28].

Various nanocarriers coated by lactoferrin

Various nanocarriers can be coated with lactoferrin to enhance their properties for drug delivery, imaging, and therapeutic applications. Lactoferrin is a naturally occurring

glycoprotein with multifunctional properties, including antimicrobial, anti-inflammatory, and immunomodulatory activities. It also exhibits strong affinity for specific receptors over expressed on cellular exteriors, making it an attractive ligand for targeted delivery to specific tissues or cells. When used as a coating material, lactoferrin imparts several advantages to the nanocarriers, such as improved biocompatibility, prolonged circulation in the bloodstream, enhanced cellular uptake, and reduced non-specific interactions. Some of the common nanocarriers coated with lactoferrin include:

- **Lactoferrin-coated liposomes:** Liposomes are lipid-based vesicles used as drug delivery systems. By coating liposomes with lactoferrin, their surface properties can be modified, enabling targeted delivery to specific cells or tissues, such as cancer cells that overexpress lactoferrin receptors. Lactoferrin-coated liposomes can improve drug delivery efficiency and reduce off-target effects [19].

- **Lactoferrin-coated nanoparticles:** Various kinds of nanoparticles, for instance polymeric nanoparticles or inorganic nanoparticles, can be coated with lactoferrin. This coating can enhance the nanoparticles' stability, biocompatibility, and cellular uptake. Lactoferrin-coated nanoparticles are explored for site-specific transport of medicines, genes, and imaging agents [29].

- **Lactoferrin-conjugated micelles:** Micelles are self-assembled structures formed by amphiphilic molecules. Lactoferrin can be conjugated to the micellar surface to improve their colloidal stability and targeting capabilities. Lactoferrin- conjugated micelles have shown promise in cancer therapy and other diseases [73].

- **Lactoferrin-coated nanogels:** Nanogels are three-dimensional crosslinked networks that can encapsulate therapeutic agents. Lactoferrin coating on nanogels can improve their cellular internalization and targeted delivery, enabling efficient release of drugs at the desired site [40].

- **Lactoferrin-coated magnetic nanoparticles:** Magnetic nanoparticles coated with lactoferrin can be guided to specific tissues using an external magnetic field. This approach is particularly useful for targeted drug delivery, hyperthermia-based therapies, and magnetic resonance imaging (MRI) [30-32].

- **Lactoferrin-modified dendrimers:** Dendrimers are highly branched macromolecules

that can be modified with lactoferrin to improve their cellular acceptance and explicit delivery of medications or diagnosing agents. By utilizing lactoferrin as a coating material, researchers aim to improve the therapeutic efficacy of nanocarriers while minimizing their potential toxicity and side effects. The targeted delivery enabled by lactoferrin-coated nanocarriers holds great promise for personalized medicine and precision therapeutic [56].

- **Inorganic nanoparticle coated by lactoferrin:** Inorganic nanoparticles coated by lactoferrin refer to nanoparticles made from non-carbon-based materials (inorganic) that have been modified or covered with a layer of lactoferrin, a glycoprotein found in various bodily fluids, including milk, tears, and saliva. Lactoferrin is known for its multifunctional properties, including antimicrobial, anti-inflammatory, and antioxidant activities, making it a promising candidate for various biomedical applications [34,35].

Synthesis of Inorganic Nanoparticles: The first step is to synthesize the inorganic nanoparticles. These nanoparticles can be made from a wide range of materials, such as metal oxides (e.g., iron oxide, zinc oxide), metal nanoparticles (e.g., gold, silver), or other inorganic materials (e.g., silica, quantum dots). The choice of material depends on the specific application and desired properties of the nanoparticles.

The process of coating inorganic nanoparticles with lactoferrin involves several steps:

1. **Surface modification:** Once the nanoparticles are synthesized, they are typically coated with a surfactant or other stabilizing agents to prevent agglomeration and ensure stability. This step creates a surface conducive for the attachment of lactoferrin molecules.

2. **Lactoferrin coating:** The lactoferrin molecules are then attached to the surface of the inorganic nanoparticles. This can be attained through different methods, such as physical adsorption, covalent bonding, or electrostatic interactions, depending on the nanoparticle material and lactoferrin properties.

The advantage of binding ligand to the nanoparticles can be summarized in the following points:

- **Biocompatibility:** Lactoferrin is a naturally occurring protein in the body and is generally well-tolerated, making the coated nanoparticles more biocompatible.

- **Enhanced Stability:** The lactoferrin coating can improve the stability of the nanoparticles, preventing aggregation and ensuring a longer shelf life.

- **Controlled Release:** Lactoferrin-coated nanoparticles can also facilitate controlled release of drugs or other active agents. The presence of lactoferrin on the surface can act as a gatekeeper and release the encapsulated substance in response to specific environmental triggers, such as changes in pH or the presence of certain enzymes.

- **Targeted Drug Delivery:** Lactoferrin-coated nanoparticles can potentially be used for targeted drug delivery to specific cells or tissues. Lactoferrin receptors are overexpressed in certain cancer cells, and by utilizing this targeting ability, drugs or therapeutic agents can be delivered more selectively to cancer cells.

Anti-Inflammatory and Antioxidant Effects: Lactoferrin's inherent properties, such as its anti-inflammatory and antioxidant effects, can complement the therapeutic action of the loaded drug or agent (28,29,30).

PAMAM dendrimer coated by lactoferrin: PAMAM (Poly(amidoamine) dendrimers are a type of nanoscale, highly branched polymers with unique properties, making them attractive for various biomedical applications. These dendrimers consist of a core, interior branches, and an exterior surface, allowing for controlled and precise functionalization. One potential application of PAMAM dendrimers is in drug delivery systems. Lactoferrin is a multifunctional glycoprotein found in milk and various other biological fluids. It plays a significant role in the body's immune response and has antimicrobial, anti-inflammatory, and iron-binding properties. Due to its biocompatibility and ability to target certain receptors overexpressed in cancer cells and other diseased tissues, lactoferrin has been investigated for use in drug delivery systems [33]. When PAMAM dendrimers are coated with lactoferrin, the resulting nanocomplex can offer several advantages:

- **Targeted drug delivery**: Lactoferrin can act as a targeting moiety, directing the dendrimer-drug complex to specific cells or tissues that overexpress lactoferrin receptors. This targeted delivery approach helps improve drug efficiency and reduce off-target effects.

- **Enhanced biocompatibility**: Lactoferrin-coated PAMAM dendrimers are likely to have improved biocompatibility compared to unmodified dendrimers. Lactoferrin's natural presence in the body reduces the risk of immunogenic responses and adverse reactions.

- **Controlled drug release**: The dendrimer structure allows for the encapsulation of drugs within its interior, and the lactoferrin coating can help control drug release kinetics. This controlled release can lead to sustained drug concentrations at the target site, enhancing therapeutic efficacy.

- **Reduced toxicity**: By encapsulating drugs within the dendrimer and adding a lactoferrin coating, it may be possible to reduce the systemic toxicity of the drugs, as they are shielded from direct interaction with healthy tissues.

- **Imaging applications**: Lactoferrin-coated PAMAM dendrimers can also be employed in imaging applications. By conjugating imaging agents to the dendrimer surface, they can be used for non-invasive imaging of specific tissues or disease sites.

Table 1: Various nanoparticles coated by Lactoferrin [36]

Coated nanocarrier	Medications	LF coupling method	Target organ	Consequences	References
LF-SPIONs		EDC/NHS covalent conjugation	Brain	*In vivo* higher uptake in brain glioma tissues.	[37]
LF-PEG-Fe_3O_4		EDC/NHS covalent conjugation	Brain	Enhanced *in vivo* MRI contrast by brain glioma tissues.	[38]
LF-MPNA		EDC/NHS covalent conjugation	Brain	*In vivo* higher uptake in brain glioma tissues.	[49]
LF-MDCs	DOX/CUR	EDC/NHS covalent conjugation	Brain	Enhanced antitumor effect in BALB/c female nude mice bearing RG2 cells.	[50]
LF-MIONs	PFH-PTX	EDC/NHS covalent conjugation	Brain	Improved *in vivo* antitumor effect in solid brain tumor.	[52]
LF-IONPs		EDC/NHS covalent	Brain	Higher cellular internalization into C6	[52]

		conjugation		cells.	
LF-PDNCs	CUR	EDC/NHS covalent conjugation	Brain	Improved *in vivo* anti-tumor efficiency in brain tumor-bearing rats.	[53]
LF-PAEEP-PLLA/OMA-MNPs		Maleimide-thiol coupling reaction	Brain	Enhanced *in vivo* MRI contrast in brain glioma tissues.	[54]
LF-GO@Fe3O4	DOX	EDC/NHS covalent conjugation	Brain	Increased anticancer activity against C6 glioma cells.	[55]
LF-FeGd-HN@Pt /RGD2	Cisplatin	EDC/NHS covalent conjugation	Brain	Augmented antitumor activity in mice bearing brain tumor.	[56]
LF-magnetite NPs		EDC/NHS covalent conjugation	-	Enhanced in vitro anticancer effect on 4T1 breast cancer cells.	[57]
LF-PAMAM-PGG	DNA	Maleimide-thiol coupling reaction	Brain	Higher *in vivo* brain accumulation and transfection activity.	[58]
LF-PAMAM-PEG	hGDNF	Maleimide-thiol coupling reaction	Brain	Enhanced *in vivo* neuroprotective effect in PD rat model.	[59]
Lf-conjugated Poly(propylene imine) (PPI) dendrimers	MTX	EDC/NHS covalent conjugation	Lung	Higher drug accumulation in the lung with improved anticancer activity.	[60]
LF-DAB	Dendriplex encoding TNFα	EDC/NHS covalent conjugation	Skin, Brain	Improved tumor repression in case of DAB-LF-dendriplexes compared to uncoated ones.	[61,62]
LF-DAB	Dendriplex encoding either TNFα TRAIL, or IL-12	Sulfo-GMBS as a cross-linking agent	Prostate	Enhanced anticancer effect in prostate tumors animal model.	[63]
LF-PAMAM	RIV	EDC/NHS covalent conjugation	Brain	Enhanced learning ability in AD animal model.	[64]
LF-PEG–PLA	UCN	Maleimide-thiol coupling reaction	Brain	Attenuated striatum lesions in AD bearing rats.	[66]

LF- PEG–PLA	PTX	Maleimide-thiol coupling reaction	Brain	Enhanced anti-glioma effect in nude mice.	[68]
LF-mPEG-PLA	α-asarone	Maleimide-thiol coupling reaction	Brain	Improved *in vivo* brain accumulation.	[69]
LF-PLGA	TMD	Epoxy-activated PLGA NPs conjugated with thiolated LF	Brain	Increased *in vivo* cellular uptake with prolonged analgesic effect.	[70]
LF-PLGA	BA	Electrostatic complexation	-	Stronger anti-leishmanial effect on cultured *L. donovani*-infected macrophages.	[71]
LF-PLGA	AMB	Ionic adsorption	Liver	Increased accumulation in the liver and spleen with improved anti-leishmanial activity in the infected hamsters.	[72]
LF-PLGA-TMC	HupA	Maleimide-thiol coupling reaction	Brain	Higher cellular uptake *in vitro* on 16HBE cell line and *in vivo* in the brain tissue.	[73]
LF/FA-PLGA	ETP	Covalent conjugation using EDC and NHS	-	Enhanced *in vitro* anticancer activity in U87MG glioblastoma cell line.	[74]
LF-PEG-PLGA	Rotigotine	Maleimide-thiol coupling reaction	Brain	Decreased nigrostriatal dopaminergic neurodegeneration in PD animal model.	[75]
LF-PEG-PLGA	SHK	Maleimide-thiol coupling reaction	Brain	Higher drug accumulation in the brain with improved pharmacokinetic profile.	[76]
LF-B- mPEG-PLGA	Dopamine	Maleimide-thiol coupling reaction	Brain	Improved dopamine accumulation the lesioned striatum in PD animal model.	[77]
LF-PEG-co-PCL	NAP	Maleimide-thiol coupling reaction	Brain	Enhanced brain accumulation with improved memory in	[78]

				AD-induced rats.	
LF-PAEEP-PLLA NBs	Perfluoro pentane	Maleimide-thiol coupling reaction	Brain	Increased accumulation in tumor-bearing BALB/c nude mice.	[79]
LF-PEG-S-S-PLA-PCL-OH	bacosides	EDC/NHS covalent conjugation	Brain	*In vivo* higher brain accumulation associated with a reversed amnesia in Swiss albino mice.	[80]
LF-PCL	DOX	Electrostatic complexation	Brain	Enhanced *in vivo* anti-glioma activity and reduced toxicity.	[81]
LF-PO-	DOX/TET	EDC/NHS covalent conjugation	Brain	Increased cellular uptake and *in vivo* drug accumulation at the tumor site.	[82]
LF-PEG PLS	DOX	EDC/NHS covalent conjugation	Liver	Improved *in vitro* and *in vivo* antitumor efficacy.	[83]
LF-LPS or OX26-LPS	Senktide (SNK)	Maleimide-thiol coupling reaction	Brain	Higher senktide accumulation in the brain with increased dopamine transmission.	[84]
BSA- LF-NLs		Electrostatic complexation	GIT	In vitro gastrointestinal digestion indicated higher stability for BSA- LF-NLs	[85]
LF-SLNs	RIF	EDC/NHS covalent conjugation	Lung	Improved *in vivo* cellular uptake with increased drug delivery.	[86]
LF-SLNs	PTX	EDC/NHS covalent conjugation	Lung	Enhanced *in vivo* antitumor efficacy with better pharmacokinetic profile	[87]
LF-NLC-	CUR	Electrostatic complexation	Brain	*In vivo* higher drug accumulation in the brain with regression in AD progression.	[88]

LF/TX-SLNs	BCNU-	EDC/NHS covalent conjugation	-	Improved *in vitro* anti-glioma effect on U87MG cells with higher BBB permeability.	[89]
LF-SLNs-	DTX	EDC/NHS covalent conjugation	Brain	Increased *in vitro* anticancer effect on U-87 MG cells with enhanced *in vivo* biodistribution.	[90]
LF/Hyaluronic acid-SLNs	BER/RAP	Electrostatic complexation	Brain	Reinforced *in vivo* antitumor effect in lung cancer-bearing mice.	[91]
LF-R-SLNs	VCR/ TMZ	Maleimide-thiol coupling reaction	Brain	Superior anti- tumor effect in tumor xenograft mouse model.	[92]
LF-CD	IR-775 chloride	Maleimide-thiol coupling reaction	Brain	Enhanced *in vivo* brain accumulation with reduced toxicity	[93]
LF-HA nanocrystals	-	Electrostatic complexation	-	Efficient osteogenic differentiation of MSCs.	[94]
LF-HA naocrystals	Cell free supernatant from probiotic *Lactobacillus paracasei*	Electrostatic complexation	GIT	Enhanced in vitro antibacterial effect.	[95]
LF- PEGylated BSA-NPs	DOX	Electrostatic complexation	Brain	Induced anticancer activity in C6 glioma bearing rats.	[96]
LF-Zein Core/shell NPs	Nile red Cy5.5	Phase separation conjugation	GIT	The prolonged retention time for labelled LF- zein nanocarriers in GIT.	[97]
LF- gelatinase-cleavable peptide and cell-penetrating peptide (CPP)-fused recombinant TCS toxin	TCS toxin	NHS-PEG3500-Maleimide bifunctional crosslinking	Brain	Enhanced tumor suppression in GL261 tumor xenografts and orthotropic animal models.	[98]

LF-hyaluronic acid / chitosan hydrochloride NPs	CUR	PEG20000 coupling reaction	Brain	Higher cellular uptake and internalization of CUR in brain tissue.	[100].
LF-Zein nanospheres	LUT/EXM	Electrostatic interaction	Breast	Improved anticancer activity in breast cancer-bearing female mice.	[101]
LF–quantum dots–chondroitin sulfate nanocapsules	CXB/HNK	EDC/NHS covalent conjugation	Breast	Increased *in vivo* tumor accumulation and antitumor efficacy.	[102]
LF–gliadin nanospheres	CXB/DSN	Electrostatic complexation	Liver	Superior anticancer activity in HCC animal model.	[103]
LF-fucoidan	fucoidan	Electrostatic complexation	-	Enhanced in vitro cytotoxic effect against PANC-1 pancreatic cancer cells.	[104]
LF/zein composite NPs	7,8-DHF	Anti-solvent precipitation method	Brain	Improved stability with high entrapment efficiency and good redispersibility.	[105]
LF/hyaluronic acid-lignosulfonate NPs	quinacrine	Electrostatic complexation	Pancreas	Increased *in vivo* anticancer effect associated with better survival.	[106]
LF-chondroitin sulfate NPs	DTX /EA	Electrostatic complexation	Lung	Increased lung deposition with enhanced *in vivo* anticancer activity.	[107]
LF/chondroitin sulfate liquid crystal NPs	PMT/RSV	Electrostatic complexation	Lung	Superior *in vivo* antitumor effect and reduction in cancerous lung foci.	[108]
LF-deferasirox	Deferasirox	EDC/NHS covalent conjugation	Brain	Enhanced neuroprotective effect in AD animal model.	[109]

Lipid-based nanocarriers coated by lactoferrin: Lipid-based nanocarriers coated with lactoferrin are a type of drug delivery system that combines the advantages of lipid-based nanoparticles with the functional properties of lactoferrin, a glycoprotein found in milk and

other biological fluids. These nanocarriers are designed to efficiently deliver therapeutic agents, such as drugs or nucleic acids (e.g., siRNA or DNA), to specific target cells or tissues.

Lipid-based Nanocarriers: Lipid-based nanoparticles are colloidal structures composed of lipids, such as phospholipids or triglycerides, which self-assemble to form a core-shell structure. These nanocarriers can encapsulate hydrophobic drugs within their lipid core and have hydrophilic shells that stabilize the nanoparticles and improve their biocompatibility.

Advantages of lipid-based nanocarriers:

- **Enhanced drug solubility**: Lipophilic drugs can be encapsulated in the lipid core, increasing their solubility and bioavailability.
- **Controlled drug release**: The lipid shell can be engineered to release the drug in a controlled manner, leading to prolonged therapeutic effects.
- **Protection of drugs**: The lipid shell provides protection to the encapsulated drug from degradation and clearance by the body's immune system.

Lactoferrin Coating: Lactoferrin is a multifunctional glycoprotein that plays essential roles in iron transport and modulating the immune response. It is also known to have specific receptors on the surface of certain cell types, making it an attractive ligand for targeted drug delivery.

Advantages of lactoferrin coating:

- **Targeted drug delivery**: Lactoferrin-coated nanocarriers can be engineered to bind specifically to receptors overexpressed on the target cells, improving drug delivery efficiency and reducing off-target effects.
- **Enhanced cellular uptake**: Lactoferrin facilitates receptor-mediated endocytosis, leading to increased internalization of the nanocarriers by target cells.
- **Immunomodulatory effects**: Lactoferrin's presence on the nanocarrier surface can also influence the immune response, potentially enhancing the therapeutic efficacy.

Therapeutic agents conjugate by lactoferrin: Therapeutic agent conjugates by lactoferrin refer to a type of drug delivery system in which therapeutic agents, such as drugs or

biomolecules, are chemically linked or physically associated with lactoferrin. Lactoferrin is a glycoprotein found in various bodily fluids, such as milk, tears, saliva, and mucosal secretions. It plays a crucial role in the innate immune system, acting as an iron-binding protein that helps regulate iron metabolism and exhibits antimicrobial and anti-inflammatory properties.

The conjugation of therapeutic agents with lactoferrin offers several advantages in drug delivery, particularly for targeting specific cells or tissues, enhancing drug stability, and improving therapeutic efficacy. Here are some key aspects and potential benefits of therapeutic agent conjugates by lactoferrin.

- **Targeted delivery**: Lactoferrin receptors are expressed on the surface of various cells, including cancer cells and cells of the immune system. By attaching therapeutic agents to lactoferrin, the drug delivery system can specifically target these cells, increasing drug accumulation at the desired site while minimizing off-target effects. Enhanced Cellular Uptake: Lactoferrin can facilitate the internalization of the drug into the target cells through receptor-mediated endocytosis. This mechanism can improve the cellular uptake of the therapeutic agent, leading to better treatment outcomes.

- **Improved drug stability**: Some therapeutic agents may be susceptible to degradation in the bloodstream or gastrointestinal tract. Conjugation with lactoferrin can protect the drug from enzymatic degradation, thereby increasing its stability and bioavailability. Lactoferrin is generally considered safe and biocompatible. By using lactoferrin as a carrier for therapeutic agents, the potential for toxicity and adverse effects may be reduced compared to other drug delivery methods.

- **Multifunctional Platform**: Lactoferrin can act as a multifunctional carrier, offering both drug delivery capabilities and inherent biological activities, such as antimicrobial and anti-inflammatory effects. This combination can be particularly beneficial for certain disease conditions.

Applications of therapeutic agent conjugate by lactoferrin include drug delivery for cancer treatment, anti-inflammatory therapies, treatment of infectious diseases, and targeted delivery of bioactive compounds to specific organs or tissues. It's essential to note that while lactoferrin-based drug delivery systems hold promise, their development and application require careful consideration of factors such as stability, immunogenicity, and specific targeting requirements

for different diseases (34)

Conclusion and future perspective: The study of lactoferrin-anchored nanomaterials as a novel approach for active targeting in nanoformulation for brain tumors shows prodigious potential for refining the effectiveness and specificity of brain tumor treatment. In conclusion, lactoferrin-anchored nanomaterials represent a promising and innovative approach for active targeting in nanoformulation for brain tumors. The combination of active targeting, enhanced drug delivery, and potential for combination therapy makes these nanomaterials a compelling avenue for advancing brain tumor treatment and improving patient outcomes in the future. and also Future studies could focus on optimizing the lactoferrin anchoring strategy to further enhance brain tumor targeting. This could involve exploring different types of nanomaterials and understanding the impact of lactoferrin density on targeting efficiency.

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