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Recent advances in nanocarrier-based vaccines for enhanced immunotherapy

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Abstract

Nanocarrier vaccines represent a groundbreaking advancement in cancer immunotherapy, leveraging nanotechnology to enhance vaccine efficacy and specificity. This review examines the latest advancements in nanocarrier-based cancer vaccine formulations, focusing on the types of nanocarriers utilized and the critical role of their physicochemical properties in influencing immune responses. Key nanocarriers include liposomes, polymeric nanoparticles, lipid nanoparticles, self-assembled protein nanoparticles, inorganic nanoparticles, and virus-like particles. These nanocarriers improve antigen stability, protect against degradation, enable controlled release, and enhance uptake by antigen- presenting cells, resulting in stronger and more durable immune responses. The physicochemical characteristics of nanocarriers, including dimensions, form, surface charge, hydrophobicity, and degradability significantly influence vaccine efficacy by affecting cellular uptake, lymphatic trafficking, antigen presentation, and immune activation. Recent advancements optimize nanocarrier formulations to enhance antigen retention, immune interactions, and tumour modulation. Integrating immune-stimulatory agents like toll-like receptor agonists and cytokines, boosts immunogenicity, overcoming immune tolerance and improving outcomes. The emergence of new patents in nanocarrier- based cancer vaccines highlights innovative approaches in antigen stabilization, adjuvant selection, and targeted delivery. These patented technologies are driving the next generation of cancer immunotherapies, offering promising strategies for achieving precise, effective, and personalized cancer treatment. By synthesizing the latest findings, this review acts as a crucial reference for researchers and clinicians committed to progressing cancer nano vaccine innovation and understanding the emergence of new patents.

Keywords: Adjuvants; Cancer immunotherapy; Interactions; Nanocarriers; Patents; Physiochemical properties; Vaccines

1. Introduction

In recent years, vaccines have emerged as a vital tool in the fight against infectious diseases. Their exceptional therapeutic and prophylactic efficacy has a significant attention; particularly in the face of emerging intractable diseases.[1] The present review article discusses the latest developments on cancer nano vaccines as follows:

The three types of vaccines are categorized as follows:

- Live attenuated vaccine, which includes bacteria or viruses but is less pathogenic than the naturally occurring pathogen;
- Inactivated vaccine, which offers pathogens that have been made inactive by heat or chemical treatment; and
- Subunit vaccine that is made from the pathogen's components and regarded as safer than both live attenuated and inactivated vaccines. Subunit vaccination does, however, have many drawbacks, including low effectiveness and insufficient immune response. In contrast, live attenuated vaccines are constrained by the

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possibility of increased post-immunization morbidity and virulence recovery due to insufficient inactivation of bacteria or viruses. Even though a lot of novel vaccinations have been created recently.[2]

Vaccines activate the immune system's adaptive response, enabling it to recognize and eliminate foreign substances. The vaccine's potency is directly tied to the intensity of this immune response. By boosting the adaptive immune system, vaccines help the body remember and defend against specific pathogens.[3]

The adaptive immune response is initiated in lymph nodes by lymphocytes, including CD4+ helper T cells, CD8+ T cells, and B cells, which reside alongside antigen-presenting cells (APCs) such as macrophages, dendritic cells (DCs), and follicular dendritic cells. APCs trigger the immune response through specialized antigen uptake mechanisms, allowing T and B lymphocytes to recognize and respond to vaccinations and foreign antigens. Follicular dendritic cells maintain long-lasting immunity by capturing circulating antigen-antibody complexes and retaining them within lymph nodes.[4]

Vaccines work by triggering the immune system to fight foreign pathogens. For vaccines to be effective, they must be delivered efficiently to immune-related organs, ensuring optimal availability and retention. This requires advanced delivery strategies using specialized platforms. These platforms enable targeted delivery to the immune system and controlled release of vaccine agents, minimizing immune-related side effects like hypersensitivity.[5]

However, conventional free vaccine administration often suffers from low targeting efficiency and systemic side effects. To overcome this challenge, various innovative vaccine delivery systems have been developed, including liposomes, polymers, cells, inorganic materials, DNA, peptides/proteins, and virus-based systems. These advanced systems significantly enhance antigen delivery to lymph nodes, improving targeting accuracy, delivery efficiency, and biosafety. Furthermore, the administration routes for these systems can be tailored to specific diseases, antigens, and delivery platforms, offering greater flexibility and optimization of vaccine efficacy.[6, 7]

2. Cancer immunotherapy

Cancer is a category of diseases distinguished by uncontrolled cell proliferation as well as the invasion and spread of cells from their point of origin, or primary site, to other parts of the body.[8] In general, there are four distinct immunotherapeutic techniques. These include immune checkpoint inhibition, cytokine treatment, cellular therapy, and therapeutic vaccinations.[9] Tumour cells use immune-regulatory mechanisms to prevent immune responses and suppress them within the tumour microenvironment.[10] Several immune-related cells contribute to the formation of an immunosuppressive microenvironment, including regulatory T cells, dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs), and regulatory B cells. Cancer cells and immune cells in the tumour's microenvironment produce inhibitory cytokines and checkpoint inhibitors, reducing the effectiveness of anti-tumour T cells.[11]

Immune Checkpoint Inhibitors (ICIs) perform by disrupting immunological checkpoints, which regulate self-tolerance and prevent excessive immune responses.[9] The most frequently targeted immune cell checkpoints for cancer immunotherapy include cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), programmed cell death protein-1 (PD-1), T-cell immunoglobulin and ITIM domain (TIGIT), T-cell immunoglobulin-3 (TIM-3), and lymphocyte activation gene 3 (LAG-3). Six medications, encompassing one CTLA-4 blocker (ipilimumab), two PD-1 blockers (nivolumab and pembrolizumab), and three PD-L1 blockers (atezolizumab, avelumab, and durvalumab) have been approved for the treatment of various cancer types, including hematological tumours like classic Hodgkin's lymphoma as well as solid tumours like melanoma, lung cancer, head and neck cancer, bladder cancer, and Merkel cell cancer.[12] Inhibitors of CTLA-4, including ipilimumab, act by binding directly to the corresponding checkpoint proteins and preventing them from interacting with their ligands on cancerous cells. A disruption in signaling allows T cells to identify and eliminate cancer cells with more specificity, thus "releasing the brakes" on the immune system.[12, 13]

Adoptive T cell transfer (ACT) is a new type of transfusion medicine in which lymphocytes are infused to produce anticancer, antiviral, or anti-inflammatory effects. From a promising form of immuno-oncology in preclinical models to the recent commercial licensure of chimeric antigen receptor (CAR) T cells for the treatment of lymphoma and leukemia, the area has moved quite quickly.[14]

Conversely, ACT involves isolating T cells from a patient and enhancing their anti-tumour activity by *ex vivo* modification. Recently, chimeric antigen receptors (CAR) have been used to improve T cell specificity by incorporating B and T cell receptor domains.[15]

In order to trigger a robust immune response against tumours, cancer vaccines, a significant area of immunotherapy, transfer tumour antigens to antigen-presenting cells (APCs). This could have both curative and preventive effects with

long-term anti-cancer advantages. However, challenges with antigen selection, immunogenicity, lymph node (LN) targeting ability, lysosomal escape capacity, immunological evasion, etc. may be the reason for the inadequate results of their clinical application. To overcome these obstacles, several preclinical and clinical studies show promising outcomes for cancer vaccines based on nanomaterials, with a notable increase in vaccine efficacy.[16]

For many years, the development of cancer vaccines has made use of nanotechnology including RNA and DNA vaccines, increase the delivery of tumour antigens, which leads to targeted immune responses. Cancer vaccines personalized approach represents the era of precision medicine, in addition to their benefits over earlier therapies. Advancements in vaccine technology, including RNA and DNA vaccines, increase the delivery of tumour antigens, which leads to targeted immune responses.[17]

Immunotherapy has been a focus of study in cancer treatment in recent years, with notable successes including CAR-T and ICB. Cancer vaccines are a significant area of immunotherapy that safely and effectively stimulate the immune system to produce an anticancer immune response. Moreover, adjuvant therapy with cancer vaccines is employed in conjunction with other immunotherapies to enhance the effectiveness of treatment.[16]

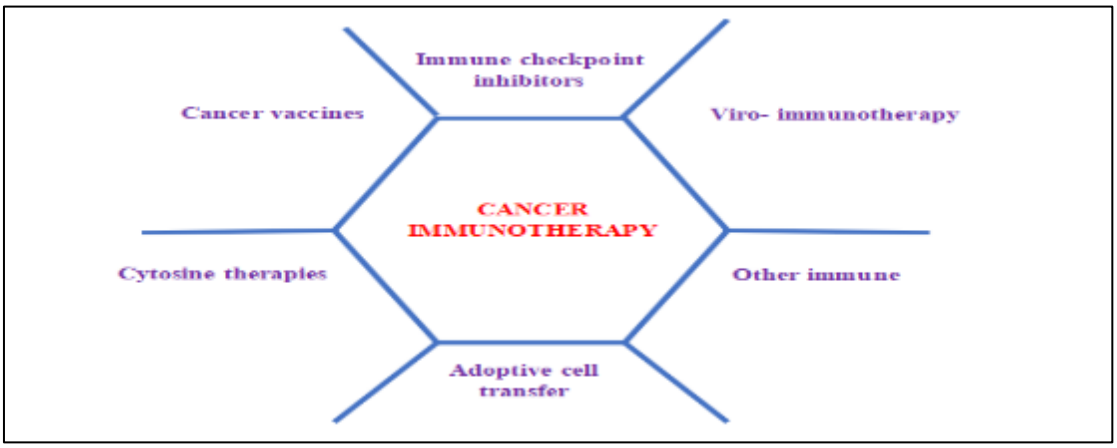


Figure 1 Cancer immunotherapy types include the use of immune-checkpoint inhibitors, cancer vaccines, cytokines, viruses, and adoptive cell transfer [101]

3. Nanocarriers

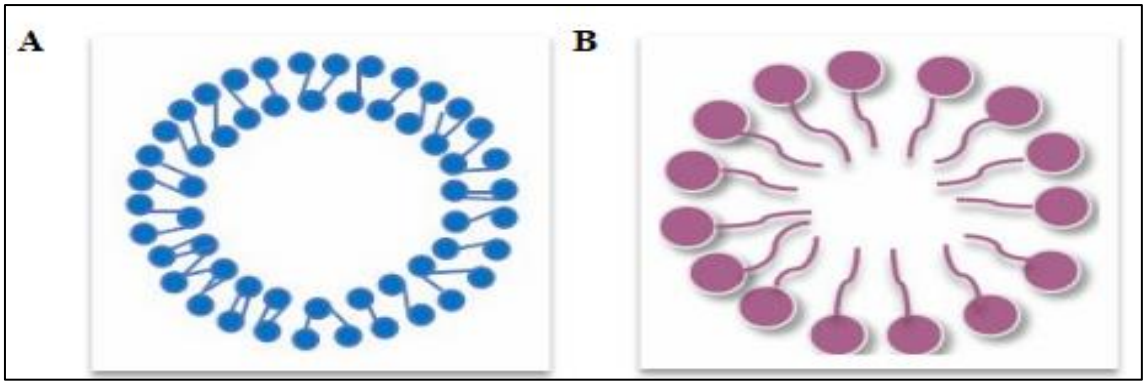
In order to get around the drawbacks of chemotherapy, scientists are creating novel drug delivery methods based on nanotechnology that will enable oncotherapy to advance significantly by delivering anticancer drugs to specific locations at higher concentrations. The use of nanocarriers as a novel cancer therapy tool has reduced many of the drawbacks associated with traditional drug delivery methods. Researchers have tested the potential of using nanotechnology-based drug carriers for cancer management, which has led to the possibility of using nano-drug carriers (10–100 nm) as unique cancer therapy treatments. Different nano-drug carriers have far greater potential applications and efficacies than conventional ones for anticancer drugs.[18, 19]

Compared to traditional drug delivery methods, nanocarriers have a number of advantages, including longer plasma half-lives, better biodistribution, and targeted drug delivery to tumour microenvironments via endothelial layers.[20]Researchers have investigated nanocarriers as a versatile tool for delivering drugs and bioactive molecules.[21]

Different types of nanostructures, including nanoparticles, nanocomposites, nanotubes, and nanofibers, are effective in the identification and management of a wide range of illnesses. These nanostructures are also used as transporting or carrier molecules for medications, vaccines, DNA, proteins, and enzymes.[22]

One of the distinct characteristics of nanocarriers is their improved pharmacokinetics and biodistribution.

- Increased stability,
- Increased solubility,
- A decrease in toxicity,
- Prolonged and Targeted delivery.[22]



A. LIPOSOMES **B. EMULSIONS**

Figure 2 Structure of nanocarriers for vaccine antigen delivery [102]

Recent trends in nanocarrier vaccines have shown promising potential in revolutionizing cancer immunotherapy. Nanoparticles have emerged as efficacious antigen carriers and immune cell activators, enhancing vaccine efficacy through targeted delivery and modulation of immune responses. Advances in modifying physicochemical properties enable nanoparticles to selectively target specific cells, optimizing anti-cancer activity. Furthermore, innovative nano-based vaccine platforms are being explored, offering alternative administration routes such as oral, nasal, and transdermal delivery. These cutting-edge developments hold significant promise for improving vaccine efficacy, overcoming traditional delivery limitations, and advancing personalized cancer therapy.[23] The present review article discusses the latest developments on cancer nano vaccines as follows:

4. Liposomes

Traditional liposomes, which range in diameter from 20 nm to a few micrometer's, have demonstrated exceptional promise in the delivery of vaccines because of their capacity to load hydrophilic and lipophilic components with accessibility and their biodegradability. Liposomes offer modified physicochemical features, controlled antigen release, and chemical changes for targeting. The physicochemical parameters of the antigen, such as its partition coefficient and polarity, as well as the liposome manufacturing techniques all affect the antigen loading efficiency. Conventional liposomes, however, have stability problems such as leakage, bilayer breakage, and early antigen release. Saturated lipids, freeze-drying, cryoprotectants, and sterically stabilized liposomes via polymer complexation or PEG grafting are among recent methods to get beyond these restrictions. Liposomes continue to be a widely used nanocarrier for vaccine delivery in spite of these difficulties.[24]

4.1. Formulations

The cationic lipoplex has been formulated by dissolving 4.46 μmol of DOTAP, cholesterol, and DSPE-PEG2000 in a chloroform: methanol (9:1, v/v) combination. A thin lipid film was the end product of nitrogen digestion and rotary evaporation used to eliminate the organic solvent. After 20 minutes at 60°C and 10 mM Tris-HCl buffer (pH 7.4), this film was rehydrated to produce a cationic liposome solution with a final concentration of 1.45 mg/mL. After that, the mRNA solution and the cationic liposome solution were combined at a weight ratio of 10:1:1 (liposome: protamine: mRNA), respectively, in the presence of protamine. For twenty minutes, this mixture was incubated at room temperature to allow a stable lipoplex complex to develop. The resultant lipoplex was made up of cationic liposomes that contained mRNA and were stabilized by PEGylation and protamine. It was appropriate for use in mRNA delivery applications.[25]

Using a Zetasizer ZS9 equipment (Malvern Paralytical Ltd.), transmission electron microscopy (TEM) and dynamic light scattering (DLS) were used to assess the physical properties of the LPC/mRNA vaccine and DOTAP liposome/mRNA complexes. Protamine sulphate was added to the mRNA solution in 10 mM Tris-HCl buffer at a weight-to-weight ratio of 1:1 in order to produce the complexes. This was followed by a 20-minute incubation period at room temperature. This process promoted mRNA condensation and stability. Subsequently, the LPC or DOTAP liposomes were combined with the protamine-mRNA complex, generating the respective lipoplex complexes. Then, using DLS, the particle size and zeta potential of these complexes were evaluated, offering information about their surface charge, potential interactions with biological membranes, and physical stability.[25]

4.2. Polymer based nano vaccines

Polymer-based particles are effective vaccination platforms and adjuvants due to their capacity to minimize antigen degradation and clearance, while also enhancing uptake by professional antigen-presenting cells (APCs). Polymer-based systems provide numerous benefits, including versatility and flexibility in design, the ability to incorporate immunomodulators/antigens, mimic infection in various ways, and act as a depot for adaptive immune responses. The major mechanisms are conjugation, encapsulation, adsorption, or simple mixing.[26]

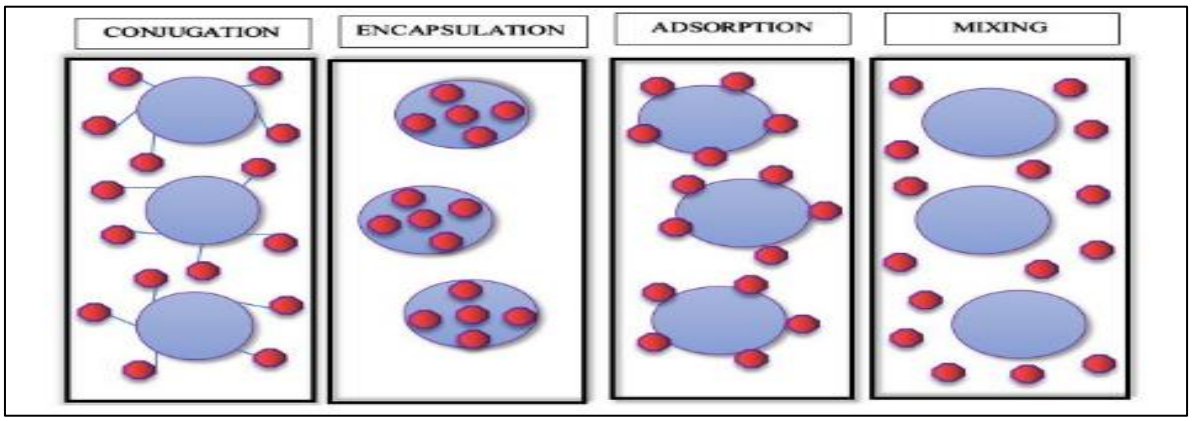


Figure 3 Interaction of nanoparticles with an antigen of interest. Formulation of nanoparticle and antigen of interest can be implemented through attachment (e.g., conjugation, encapsulation, or adsorption) or simple mixing).[103]

Stimulus-responsive nanomaterials enhance therapeutic effects and reduce drug-related cytotoxicity by selectively delivering encapsulated medicines to the target place. The encapsulated medications have been released from the nanomaterials using a variety of external energy sources, including temperature, light, magnetic fields, ultrasonic induction, etc. When delivering the medications to the site of injury, internal stimulus responsive carriers make use of the metabolic distinctions that naturally exist between healthy and malignant cells. Several enzymes have been utilized as triggers to release the contents from the proper carriers because they are over expressed in malignant cells.[27]

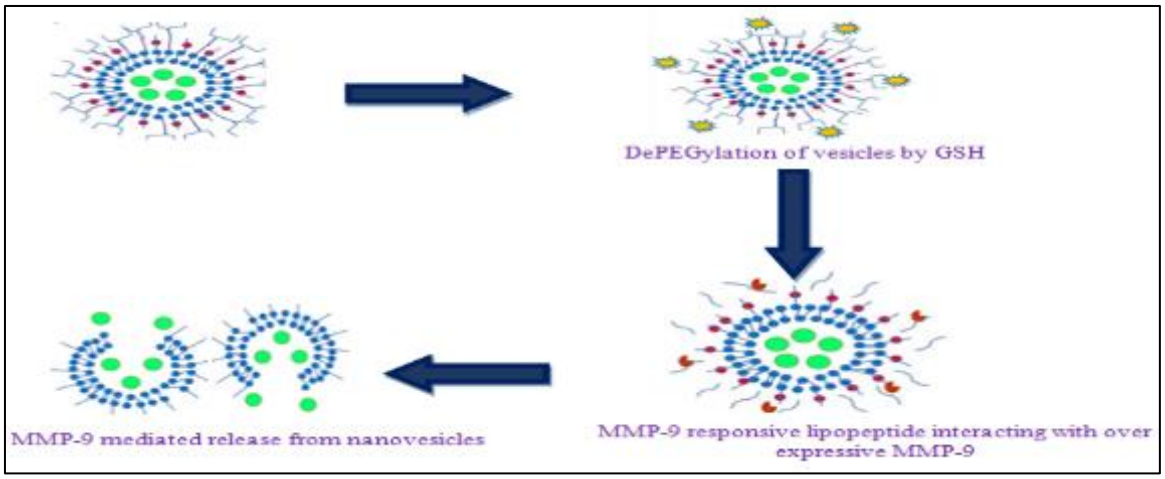


Figure 4 Schematic representation of nanovesicles incorporating MMP-9 substrate lipopeptides and reduction-sensitive POPE-SS-PEG which render the nanovesicles responsive to extracellular, elevated levels of MMP-9 & GSH.[104]

Matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, are overexpressed in various tumours and play a key role in cancer invasion and metastasis, making them valuable targets for enzyme-responsive drug delivery systems. Nanoparticles designed with enzyme-responsive peptides on their surfaces can exploit these enzymes for targeted drug release, but stability in the dynamic physiological environment is critical until they reach the tumour site. Coating nanoparticles with poly (ethylene glycol) (PEG), a process known as PEGylation, enhances their stability by reducing interactions with circulating proteins, lowering interfacial tension, and preventing protein adsorption. This PEG layer facilitates nanoparticle accumulation at the tumour site via the enhanced permeation and retention (EPR) effect.

However, for effective drug release and therapeutic action at the tumour site, the PEG coating must be removed to activate the carrier's desired functions.[28]

Matrix metalloproteinase (MMP) levels, particularly MMP-9, are often elevated in the extracellular matrix of various cancers, including pancreatic cancer. In this study, we synthesized an MMP-9-cleavable collagen-mimetic lipopeptide that forms nanosized vesicles when combined with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), cholesteryl-hemi succinate, and a reduction-sensitive PEGylated lipid, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE-SS-PEG5000). The PEG5000 in POPE-SS-PEG5000 provides long-circulating properties to the nanovesicles, while in the tumour's extracellular matrix, the high glutathione levels are expected to reduce the POPE-SS-PEG5000 polymer, shedding the PEG chains. This de-PEGylation exposes the MMP-9-responsive collagen-mimetic lipopeptides to enzymatic hydrolysis, destabilizing the nanovesicles and triggering the release of the encapsulated drugs at the tumour site.[29, 30]

4.3. Preparation of carboxyfluorescein encapsulated nanovesicles

The preparation of carboxyfluorescein-encapsulated nanovesicles, involved the molar ratios of 60:30:5:5 for POPC lipid, synthesized lipopeptide LP, POPE-SS-PEG5000, and cholesteryl hemi succinate. To create a thin lipid layer in a flask with a circular bottom, all of the lipids were dissolved in chloroform and then removed using a rotating evaporator. After vacuum-drying the film for a full night in a desiccator, it was hydrated for two hours at 60°C using a 100 mM carboxyfluorescein solution in HEPES buffer (pH 7.4). The resultant vesicles were extruded through 0.8 μm and 0.2 μm filters to obtain uniform size after being ultrasonicated for 45 minutes with an Aqua sonic bath sonicator (model 250D, power level 9). The vesicles were run down a Sephadex G50 size-exclusion column to remove unencapsulated dye, and the orange band of vesicles encapsulated in carboxyfluorescein was collected for subsequent release and imaging studies. The encapsulation % was not estimated because there was an excess of carboxyfluorescein utilized.

4.4. Preparation of gemcitabine-encapsulated nanovesicles

The pH gradient approach was used to create nanovesicles encapsulated with gemcitabine. Chloroform was used to dissolve the lipid-containing nanovesicles, which included POPC, LP, POPE-SSPEG, cholesteryl hemi succinate, and Lissa mine rhodamine lipid (in molar ratios of 59:30:5:5:1). After the chloroform was evaporated at lower pressure, the lipid film that was left over was vacuum-dried. This film was hydrated using a 20 mM citric acid buffer (pH 4), then it was extruded through a 0.2 μm filter and ultrasonically treated for 45 minutes at power level 9. Then, after going through a Sephadex G50 gel-filtration column, the nanovesicles were collected. During chromatography, lipid containing Lissa mine and rhodamine was added to provide colour for the vesicles to be seen. Using a 10:1 lipid-to-drug ratio, these eluted nanovesicles (pH 7.4) were incubated for two hours at 60°C with a 1 mg/mL gemcitabine solution. To extract unencapsulated gemcitabine, the drug-loaded nanovesicles were once more run down the Sephadex G50 column. The nanovesicles, whose entrapment efficiency was estimated to be 50%, were employed in cytotoxicity investigations.[31, 32]

The hydrodynamic diameters of the vesicles were measured using dynamic light scattering (DLS) (Malvern Zetasizer Nano-ZS90) at a 90° scattering angle in polystyrene cuvettes, with an equilibration time of 120 s and six readings averaged per sample. To study size changes, nanovesicles encapsulating gemcitabine were incubated with MMP-9 and GSH, and size variations were monitored over 24 hours using DLS. Morphological changes were analyzed using atomic force microscopy (AFM), where the vesicles were deposited on a mica sheet and imaged in tapping mode using a Multimode AFM with a Nano scope IIIa controller and J-type piezo scanner (Veeco Metrology Group) equipped with an antimony-doped silicon tip.[31]

4.5. Inorganic nanoparticles

Recently, cancer therapy research has increasingly focused on inorganic nanoparticles (INPs), drawing significant attention from scientists due to their unique physicochemical properties, which are influenced by their material composition and size.[33,34]

Inorganic nanoparticles (INPs) offer distinct advantages over organic counterparts, showcasing exceptional photosensitivity, superior conductivity, impressive optical properties, magnetic capabilities, and efficient thermal performance. Inorganic nanoparticles are derived from a range of materials, including metal oxides (e.g., iron, manganese, zinc), metals (e.g., gold, silver), carbons (e.g., carbon dots, carbon nanotubes), and semiconductors (e.g., quantum dots). These nanoparticles are widely studied as therapeutic tools for treating various cancers. Prominent examples include mesoporous silica nanoparticles (MSNs), cerium oxide nanoparticles (CeONPs), quantum dots (QDs), carbon nanotubes (CNTs), gold nanoparticles (AuNPs), iron oxide nanoparticles (Fe3O4NPs), silver nanoparticles (AgNPs), and zinc oxide nanoparticles (ZnONPs). These unique traits enable INPs to serve a dual purpose, acting as

effective carriers for drug delivery while simultaneously functioning as therapeutic agents to enhance cancer treatment outcomes.[35, 36]

Inorganic nanoparticles (INPs) stand out due to their simple synthesis, extensive surface area, and robust mechanical and chemical stability. Commonly derived from metals, metal oxides, and non-metallic materials like carbon and silica, these nanoparticles provide numerous benefits as drug carriers. Their advantages include enhanced quantum yield, superior drug-loading capacity, and the versatility to facilitate photothermal therapy (PTT) and photodynamic therapy (PDT), making them invaluable in cancer treatment. Inorganic nanoparticles (INPs) present remarkable benefits in cancer therapy, particularly through photothermal therapy (PTT) and photodynamic therapy (PDT). In PTT, INPs generate localized heat, while in PDT, they produce reactive oxygen species, enhancing treatment precision and minimizing harm to healthy tissues. Their high absorption coefficients, stability, and extended circulation time significantly boost therapeutic effectiveness. Moreover, surface modification enables INPs to function as efficient drug delivery systems, offering controlled drug release alongside multifunctional capabilities for bioimaging and therapy. These unique attributes position INPs as highly promising tools for advancing cancer treatments with improved efficacy and targeted action.[37]

4.6. Photothermal therapy (PTT)

Hyperthermia-based cancer therapies involve raising the temperature of targeted tissue to induce cancer cell death (thermal ablation, typically above 45°C) or to increase cancer cell sensitivity to other treatments (mild hyperthermia, with temperatures between 40 and 45°C).[38]

In photothermal therapy (PTT), NIR-II wavelengths (1000–1700 nm) offer better tissue penetration and improved safety compared to NIR-I (750–900 nm), due to lower scattering and absorption by biological tissues. As a result, NIR-II is more efficient for targeting deeper tumours while minimizing damage to surrounding healthy tissue.[39]

Conventional hyperthermia methods generally raise the temperature of the target tissue through external techniques, including regional hyperthermia, superficial hyperthermia, and whole-body hyperthermia, which use thermal baths, microwaves, or radiofrequency.[40]

However, this approach often creates a temperature gradient, with the highest temperatures occurring at the body surface and decreasing further from the external heat source. As a result, healthy tissues may also experience elevated temperatures, causing potential unwanted side effects.[41]

To address these limitations, researchers have focused on developing more efficient techniques, particularly those that can induce localized temperature increases at the tumour site. Nanoparticles (NPs) capable of generating heat in response to external stimuli have emerged as promising solutions, offering a targeted approach that overcomes the drawbacks of conventional hyperthermia methods.[42]

4.7. Photodynamic therapy (PDT)

Photodynamic therapy (PDT) is a non-invasive, painless treatment for various cancers and non-cancerous diseases, targeting cancer cells with high selectivity. It involves a photosensitizer (PS), light to activate the PS, and molecular oxygen from the tumour. When activated, the PS produces reactive oxygen species (ROS) that destroy tumour cells. The ideal wavelength for PDT is between 600–850 nm, known as the "phototherapeutic window. “First-generation (Hematoporphyrin derivative (HpD) and photofrin) PSs have harsh side effects, while second-generation (aminolaevulinic acid (ALA), esterified derivatives of ALA and phthalocyanine compounds) PSs offer reduced toxicity and improved ROS generation. Additionally, when second-generation photosensitizers (PSs) are conjugated with biological carriers, such as nanoparticles, they are classified as third-generation PSs. These "carrier" conjugates enable the PSs to selectively accumulate in cancer cells, enhancing their therapeutic effectiveness.[43]

4.8. Mechanism of PDT

Photodynamic therapy (PDT) involves two main mechanisms in tumour cells with molecular oxygen. Upon light irradiation, the photosensitizer (PS) transitions from a ground state to a single excited state, then to a triplet state. In the type I mechanism, the triplet PS interacts with biomolecules, generating reactive oxygen species (ROS) like hydrogen peroxide. In the type II mechanism, energy is transferred to oxygen, producing singlet oxygen. Both ROS and singlet oxygen cause tumour cell death through apoptosis, necrosis, or autophagy, depending on PS localization.[44, 45]

Among these, extra-large pore mesoporous silica nanoparticles (XL-MSNs) have shown significant potential as a prophylactic cancer vaccine. These nanoparticles are engineered to deliver both cancer antigens and danger signals directly to host DCs within the draining lymph nodes, thereby enhancing their ability to elicit robust immune responses. The use of XL-MSNs represents a transformative strategy in cancer immunotherapy, addressing the limitations of traditional DC-based vaccines and offering a scalable, efficient, and minimally invasive method for cancer prevention and treatment.

4.9. Synthesis and characterization of XL-MSNS:

Extra-large pore mesoporous silica nanoparticles (XL-MSNs) were prepared by mixing 500 µL of Fe_3O4 nanocrystals (6 mg/mL, 6 nm diameter), which were created by heating an iron-oleate complex, into 10 mL of an aqueous solution containing 0.055 M CTAB. The mixture was then vigorously stirred for 30 minutes. The mixture was mixed with a solution comprising 95 mL of DI water, 5 mL of methanol, 3 mL of ammonium hydroxide, and 20 mL of ethyl acetate after being heated to 60°C for 15 minutes. The mixture was then agitated for an additional 500 µL of TEOS and left overnight. The resultant MSNs were placed in 40 mL of ethanol and cleaned three times using ethanol. For three hours at 60°C, the MSNs were agitated in acidic ethanol containing HCl in order to extract the CTAB template and remove the Fe3O4 nanocrystal core. The MSNs were then preserved in 30 mL of ethanol for use in upcoming studies after being cleaned three more times with ethanol. The BET technique was used to analyze the MSNs' pore size and volume.

4.10. Amine modification and ritc conjugation of MSN

After adding APTMS to the MSN solution at a TEOS: APTMS molar ratio of 10:1, the reaction was allowed to continue for the entire night. The particles were then washed three times with ethanol. The amine-modified MSNs that resulted were then utilized to manufacture MSN vaccines. RITC-labeled MSNs were created by reacting RITC and APTMS at a 1:10 molar ratio in 750 µL of anhydrous ethanol (99.9%) for a full day in the dark. This resulted in the formation of RITC-APTMS, which allowed MSNs to be tracked *in vivo*. Then, TEOS and this RITC-APTMS were combined to create RITC-labeled MSNs (RITC-MSN).

5. Vaccine formulations

A solution of OVA (at a final concentration of 5 mg OVA/mL) was combined with 1 mg of amine-modified MSN in PBS to create the MSN vaccine. The mixture was then spun for two hours and PBS rinsed three times. In order to determine the OVA loading using UV-vis absorbance at 280 nm, the PBS supernatant was collected. After 30 minutes of mixing a CpG-ODN solution in 500 µL of PBS with the OVA-loaded MSNs, three washings were performed. In order to calculate loading, the UV-vis absorbance at 263 nm was measured using the CpG-ODN supernatant. To prepare the MSR vaccine, 5 mg of MSRs were gently shaken at room temperature for 4 hours with 1 µg of GM-CSF, 100 µg of OVA, and 10 µg of CpG-ODNs. Lyophilization and storage at -20°C were then performed. Prior to vaccination, a 17G needle was used to subcutaneously inject the MSR vaccine into the mouse's flank, resuspended in 200 µL of PBS. The MSR-MSN vaccine was created by combining 5 mg of MSRs with 1 µg of GM-CSF for 4 hours, lyophilizing the mixture, and storing it at -20°C. Prior to vaccination, the GM-CSF-loaded MSRs and 200 µL of the MSN vaccine were mixed to create the MSR-MSN vaccine, which was injected subcutaneously into the mouse flank using a 17G needle.

The use of mesoporous silica nanoparticles with extra-large pores (XL-MSNs) has demonstrated remarkable potential in cancer vaccine development, particularly in fostering long-term immunity against tumor recurrence. One of the key findings from recent studies is the significant inhibition of tumor growth in vaccinated, tumor-free mice that were rechallenged with tumors. This robust preventive effect was strongly associated with an elevated presence of memory T cells, which play a critical role in the immune system's ability to recognize and respond swiftly to recurring tumor antigens. The ability of XL-MSNs to stimulate and sustain such durable immune responses highlights their effectiveness as a cancer vaccine platform. These nanoparticles, designed to deliver cancer antigens and danger signals to dendritic cells in the draining lymph nodes, not only initiate strong antitumor immune responses but also establish long-lasting immunological memory. Consequently, XL-MSNs represent an innovative and promising approach for cancer immunotherapy, offering a scalable and efficient strategy to prevent tumor recurrence and improve patient outcomes.[46]

6. Self-assembled protein nanoparticles

In the past decade, we have developed a technology that enables precise control over the ability of peptides and proteins to self-assemble into nanoparticles with well-defined sizes and shapes. This breakthrough approach allows us to design nanoparticles that are both mechanically and chemically stable. By leveraging expertise in structural biology, biophysics, and computational protein design, we have created a novel method to design epitope strings that self-

assemble into self-assembling protein nanoparticles (SAPNs). This innovation opens new possibilities for applications in drug delivery, vaccine development, and cancer therapy, where the controlled assembly of protein nanoparticles is crucial for optimizing therapeutic efficacy and targeting. First presented in Raman *et al.,* this technology involves a protein chain composed of two coiled coils connected by a short linker region. The interaction between the coiled coils forces the monomers to self-assemble into spherical nanoparticles. These peptide nanoparticles resemble virus capsids, combining the strong immunogenicity of live attenuated vaccines with the purity and specificity of peptide-based vaccines. SAPNs offer several advantages, including no infection risk compared to live vaccines, and greater versatility and flexibility in design than virus-like particles. Additionally, their ease of expression, purification, and self-assembly significantly reduces production costs and time, making them ideal for large-scale vaccine development.[47]

Peptide-derived self-assembled molecules hold significant potential as drug nanocarriers, with their diverse structures offering unique advantages in various nanomedicine applications. Several self-assembled proteins and peptides, including albumin, ferritin, and virus-like particles (VLPs), have demonstrated promising roles in cancer therapy. These nanocarriers can be tailored to enhance drug delivery, targeting, and controlled release, making them valuable tools for improving cancer treatment outcomes. Their versatility in design enables optimization for specific therapeutic needs, offering a range of possibilities for advancing cancer therapies.

6.1. Albumin

Albumin is the most abundant protein in plasma, with various forms such as ovalbumin (OVA), human serum albumin (HSA), bovine serum albumin (BSA), and rat serum albumin (RSA) isolated for use. Due to its biodegradability, non-toxicity, and immunogenicity, along with the ability to conjugate drugs to its amino acid residues, albumin is a promising nanocarrier for drug delivery. Several albumin-based therapies have been FDA-approved. Additionally, albumin receptors (e.g., Gp18, Gp30, Gp60, and SPARC) are overexpressed on cancer cells, allowing albumin carriers to target tumours. Albumin-based hydrogels (CABH) are also being developed for controlled drug release in acidic environments.[48, 49]

6.2. Ferritin

Ferritin is composed of 24 subunits of heavy and light chains that self-assemble into a symmetric nanocage, measuring 12 nm in size with an internal diameter of 8 nm. The ratio of heavy to light chains can vary, being species and tissue-specific. Ferritin binds to transferrin receptor 1 (TFR1) via the heavy chain, facilitating cellular uptake. TFR1 is low in normal cells but overexpressed in cancers like breast and lung cancer, making ferritin vehicles tumour-selective without additional targeting ligands. Additionally, ferritin's properties, including thermal stability, pH resilience, monodispersed, and biodegradability, make it an ideal drug nanocarrier. Engineered ferritin can also be modified to improve targeting, enhancing safety and precision in cancer therapy.[50, 51,52]

6.3. Virus like particles

Virus-like particles (VLPs) are intricate, self-assembling protein structures composed of multiple subunits that closely resemble the morphology and architecture of native viruses or bacteriophages. However, they are devoid of viral genetic material, making them noninfectious and incapable of replication. Essentially, VLPs are hollow protein shells that mimic the structure of a virus without posing an infection risk. They can be categorized as either enveloped or non-enveloped, depending on whether a lipid envelope is present.[53]

VLPs are nanoparticles formed through the spontaneous self-assembly of viral structural proteins. Lacking the genetic material required for viral replication, VLPs provide a safe platform, eliminating the risk of unintended viral gene delivery. They serve as versatile tools for presenting various classes of epitopes on their surface, making them particularly valuable for vaccine development. Their capacity to engage dendritic cells (DCs) and stimulate robust B cell responses, along with specific CD4 and CD8 T-cell responses, significantly enhances the effectiveness of vaccines.[54]

The production of virus-like particles (VLPs) involves several critical steps: choosing a suitable expression system, genetically engineering the host cell or organism, expressing the target proteins, and then purifying and assembling the VLPs.[55]

Common expression systems for virus-like particle (VLP) production include bacterial systems (e.g., Escherichia coli), yeast systems (e.g., Pichia pastoris), insect cells (e.g., Drosophila melanogaster S2 cells), mammalian cells (e.g., human embryonic kidney [HEK] cells), and plant cells. Each of these systems exhibits unique characteristics that influence both the production yield a critical consideration given the substantial quantities required for vaccine development and the immunogenic properties of the resulting VLPs.[56]

Bacterial and yeast expression systems are commonly preferred due to their rapid growth rates and high production yields. Additionally, these systems are relatively straightforward to manipulate and are cost-effective, making them well-suited for large-scale VLP production.[57]

However, these systems, particularly bacterial expression systems, often lack the ability to correctly fold complex viral proteins or perform essential post-translational modifications (PTMs) such as glycosylation and phosphorylation. This limitation can affect the structural integrity and immunogenic properties of the resulting VLPs. Due to their limited capacity for post-translational modifications (PTMs), bacterial and yeast expression systems are typically used for the production of non-enveloped VLPs. In contrast, insect and mammalian cell systems are capable of producing VLPs with more accurate protein folding and comprehensive PTMs, resulting in particles that more closely mimic native viruses. These modifications play a critical role in enhancing the immunogenic potential of VLPs, thereby improving their ability to elicit robust immune responses.[58]

The production of VLPs begins with the genetic engineering of the host organism to express the viral structural proteins required for VLP formation. This process typically involves the construction of a recombinant plasmid or viral vector containing the gene of interest, which is then introduced into the host system. Following protein expression, the viral proteins are purified to isolate them from host proteins and other cellular components. Once purified, the viral proteins spontaneously self-assemble into VLPs, driven by the same molecular interactions that govern viral capsid formation during natural viral infections.[59]

Viruses are implicated in the development of certain cancers, with infectious agents estimated to contribute to approximately 20% of cancer cases worldwide. The most common infection-related cancers include cervical cancer, liver cancer, stomach cancer, and specific types of lymphoma. Among the primary oncogenic viruses are human papillomavirus (HPV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Currently, population-level cancer prevention has been successfully achieved through vaccination programs targeting cancer-associated viruses, particularly HBV and HPV.[60]

The WHO estimates 296 million HBV infections globally, with 1.5 million new cases annually, causing over 800,000 deaths. HBV vaccines, particularly VLP-based ones, are key to preventing HBV-related diseases. First-generation plasma-derived vaccines, while effective, raised concerns about blood-borne disease transmission. Second and third-generation recombinant vaccines, like Engerix-B and Heplisav-B, use self-assembled HBsAg VLPs and adjuvants to enhance immune responses. However, none achieve complete therapeutic remission, though novel VLP-based therapies like CR-T3 show promise in preclinical studies.[61, 62]

HPV, the most common sexually transmitted infection, comprises over 150 types, with high-risk strains like HPV16, 18, and others causing genital cancers. In 1991, Jian Zhou first described recombinant HPV VLP production. The FDA-approved HPV vaccines, such as Cervarix and Gardasil, are based on L1 VLPs and protect against high-risk genotypes. Gardasil 9, licensed in 2017, covers nine genotypes. L2-based vaccines are under preclinical investigation, offering potential cross-protection, though L2's inability to self-assemble poses challenges.[63, 64]

The success of VLP vaccines for HBV and HPV has spurred development of VLP vaccines for other oncogenic viruses like human herpesvirus 8 (HHV-8), associated with Kaposi sarcoma, and Epstein–Barr virus (EBV), linked to Burkitt's lymphoma. Preclinical studies on HHV-8 vaccines target glycoproteins such as gpK8.1, GB, and gH/gL, inducing neutralizing antibody responses. For EBV, VLP vaccines are being developed using recombinant genomes expressing self-assembling features without oncogenes or viral glycoproteins essential for entry.[65, 66]

The selection of an optimal antigen is critical for the efficacy of therapeutic cancer vaccines. An ideal target antigen should be exclusively expressed on cancer cells, absent in healthy cells, essential for tumor cell survival, and capable of eliciting a robust immune response. While targeting highly expressed antigens offers promise, consideration must be given to the risk of autoimmune reactions against normal tissues that express these antigens at lower levels, such as HER-2 in cardiac cells.[67,68]

Neoantigens are novel proteins that arise from somatic genomic alterations, such as single-nucleotide variants (SNVs), insertions and deletions (INDELs), and gene fusions, occurring within the DNA of tumor cells. These alterations generate unique peptide sequences that are not present in normal cells, making neoantigens potential targets for cancer immunotherapy.[69]

Tumor mutational burden (TMB) plays a dual role in cancer biology, particularly in malignancies such as melanoma, lung adenocarcinoma, gastric adenocarcinoma, colorectal carcinoma, and sarcomas. While high TMB contributes to

acquired resistance to therapies, including immune checkpoint inhibitors, it simultaneously presents a significant opportunity for immunotherapy. Specifically, the formation of neoantigens resulting from high TMB can drive a tumor-specific T-cell response, reducing the risk of off-target effects. This phenomenon can be exploited in the development of personalized cancer immunotherapies, such as therapeutic vaccines. Among the most extensively studied neoantigens are clonal mutations in driver genes such as KRAS, BRAF, and PIK3CA. Additionally, numerous other neoantigens are under investigation as potential targets for therapeutic vaccines across various cancer types.[70, 71]

A variety of antigens hold promise for the development of cancer vaccines. These include neoantigens, overexpressed antigens, cancer-testis antigens such as MAGE and NY-ESO-1, and foreign "non-self" antigens derived from viral origins. These antigen categories offer diverse targets for therapeutic strategies in cancer immunotherapy.[72]

Tumor antigens have been categorized based on specific characteristics to facilitate the identification of effective therapeutic targets. For instance, neoantigens are a class of persistent tumor antigens that play a causal role in tumor progression while remaining susceptible to immune recognition, making them promising targets for cancer immunotherapy.[73]

Neoantigens are typically expressed at low levels in normal cells but become overexpressed andor amplified in tumor cells, making them attractive targets for cancer immunotherapy. Notable examples include EGFR, HER-2, the mucin MUC1, CD20, and the idiotypes of neoplastic clones of B and T cells.[74]

Tumor antigens are categorized to identify effective therapeutic targets, including neoantigens, which drive tumor progression and remain immune-recognizable. Examples include EGFR, HER-2, MUC1, CD20, and IGF1R, with co-targeting strategies, such as HER-2 and VEGFR, showing efficacy against invasive cancer cells. Neoantigens are classified into plasma membrane (class I), tumor microenvironment (class II), and intracellular (class III) types. Tissue-specific differentiation antigens (e.g., PAP, PSA) and cancer germline antigens (e.g., MAGE, NY-ESO-1) are also targeted in cancer immunotherapy. Additionally, EMT- and stemness-associated antigens, like OCT-4 and CD44, present novel targets to reduce tumor progression and relapse.[75, 76]

6.4. Preparation of OVA-HBC VLPS AND GP100-HBC VLPS

Glycine-rich linkers were used to combine OVA257−264 (SIINFEKL), a pattern antigen for vaccine effectiveness, and gp100 (KVPRNQDWL), a melanoma-specific antigen, and insert them into HBc-183's main immunological area (between residues 78 and 81). A 6His tag was appended to the protein's C-terminal to aid in detection. Shanghai Generay Biotech Co., Ltd. synthesized the plasmids encoding these constructs, pET43.1(a)-HBc-OVA-6His and pET43.1(a)-HBc-gp100-6His, and introduced them into E. coli BL21 (DE3) to induce protein expression. At OD600 = 0.6, 1 mM IPTG was used to stimulate the expression of HBc-OVA-6His and HBc-gp100-6His. This was followed by a 14-hour incubation period at 16°C. Proteins were precipitated by adding 33% saturated ammonium sulphate after cell lysis by sonification and centrifugation. Diethylaminomethyl (DEAE) ion-exchange chromatography was used to further purify the crude proteins, which were then kept at -20°C. Western blot analysis was used to confirm protein expression.

6.5. Preparation and characterization of CY5.5-HBC-GP100 VLPS AND BHQ-3-HBC-OVA VLPS

Using Fmoc solid-phase peptide synthesis, Top-peptide Co., Ltd. created Cy5.5-MMP-2 substrate-SulfoSMCC by conjugating Cy5.5-NHS to the MMP-2 substrate (SHCPLGLAG-NH2) and then reacting with the maleimides of Sulfo-SMCC. The preparation of HBc-gp100-6His and HBc-OVA-6His monomers included incubation in a solution containing a high concentration of urea. The NH2 groups of HBc-gp100-6His monomers were conjugated to Cy5.5-MMP-2 substrate-Sulfo-SMCC, whereas the NH2 groups of HBc-OVA-6His monomers were conjugated to BHQ-3-NHS. Mass spectrometry using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) was used to characterize the molecular weight alterations of these VLP monomers. To create hybrid VLPs, various ratios of the monomers were combined and dialyzed after the monomers were reassembled by dialysis at 4°C in an assembly buffer. Using transmission electron microscopy (TEM), the morphology of hybrid VLPs, BHQ-3-HBc-OVA VLPs, and Cy5.5-HBc-gp100 VLPs was examined. The hybrid VLPs were placed in PBS with 10% FBS at 37°C for 12 hours to test the stability of the nanoparticles, and TEM was used to evaluate their morphologies. Dynamic light scattering (DLS) was used to analyze the VLPs' size and polydispersity index (PDI).[77]

7. Characterization of VLP physicochemical properties

The first step in evaluating VLP-based nano vaccines is to characterize their physicochemical properties, including size, surface charge, and morphology. Techniques such as dynamic light scattering (DLS), transmission electron microscopy (TEM), and atomic force microscopy (AFM) can be used to measure the size distribution and morphology of the VLPs.[78]

7.1. Nanoparticles and immunity

Nanoparticles (NPs) are extensively utilized as drug delivery systems to enhance therapeutic effects and minimize side effects. However, their interaction with the immune system raises concerns about potential immune responses, such as inflammation and hypersensitivity.[79]

These interactions are influenced by the physicochemical properties of NPs, including size, shape, hydrophobicity, stiffness, and surface components. For instance, lipid-based NPs, like liposomes and niosomes, have shown significant success in drug delivery, with market formulations such as Doxil and AmBisome. However, despite their advantages, these NPs often interact with immune cells, leading to varied uptake mechanisms and immune responses. Studies have demonstrated size-dependent uptake by mechanisms like pinocytosis, macropinocytosis, and clathrin-mediated endocytosis, influencing the extent of immune activation. Surface modifications, such as polyethylene glycol (PEG) coating, can reduce rapid immune clearance but may eventually trigger the "accelerated blood clearance" (ABR) phenomenon after repeated administrations.

The immune responses elicited by NPs vary based on their composition and surface properties. Hydrophobic or positively charged NPs often show higher immune activation due to increased cellular uptake and cytokine production. For example, positively charged gold NPs (AuNPs) exhibit enhanced macrophage uptake and serum protein binding, while hydrophobic polymer-based NPs amplify pro-inflammatory cytokine expression. Similarly, surface modifications can influence immune recognition and cytokine production. Liposomes, primarily composed of bilayer phospholipids, undergo opsonization, leading to rapid clearance by the immune system. However, PEG-coated liposomes improve stability and target-specific delivery, as seen in cancer models, though prolonged use may result in anti-PEG antibody production and rapid clearance.

Different NP types, such as metal-based, silica, and carbon-based NPs, elicit distinct immune responses. For instance, carbon nanotubes can induce inflammation and fibrosis, while mesoporous silica nanoparticles exhibit minimal immune activation. Shape and size also impact immune reactions; spherical AuNPs produce higher antibody levels and inflammatory cytokines than rod-shaped ones. Furthermore, studies have shown that NP delivery systems can trigger immune responses specific to their application, such as magnetite cationic liposomes inducing lymphocyte infiltration in tumour tissues or TiO2 NPs causing skin irritation and mast cell activation. These findings underscore the critical role of NP design in modulating immune responses and highlight the need for comprehensive studies to balance therapeutic benefits with immune safety.[80]

8. Liposomes

Liposomes have been explored as a means to improve the localization and efficacy of entrapped immunosuppressant drugs. For instance, Hong *et al*. and others demonstrated that encapsulating IL-10 genes in cationic liposomes enhanced allograft survival following heart transplants by promoting local over expression of IL-10 and reducing lymphocyte reactivity. Similarly, in liver transplant studies, canines treated with liposomal tacrolimus showed significantly longer survival compared to those receiving tacrolimus intravenously, highlighting the potential of liposomal formulations to optimize immunosuppressive therapies.[81]

8.1. Polymer np interaction

In cancer immunotherapy, polymer nanoparticles (NPs) and macromolecules like dendrimers demonstrate various immunosuppressive effects, which can be leveraged to modulate immune responses. Fullerenes (C60), including cerium oxide nanoparticles, play a crucial role in reducing oxidative stress by lowering reactive oxygen species (ROS) levels. While cerium oxide NPs reduce ROS directly, fullerenes utilize their aromatic structure to scavenge reactive oxygen species, including hydroxyl and superoxide radicals. Their free radical-scavenging properties can be further enhanced through bioconjugation with water-soluble ligands, making them a promising tool in managing oxidative stress and fine-tuning immune responses in cancer therapy.[82]

8.2. Inorganic nanoparticles

The cellular uptake of gold nanoparticles (AuNPs) has been shown to depend significantly on their size, as demonstrated in the work by Oli *et al.* Their study revealed that 10 nm AuNPs are internalized at a much higher rate than larger 50 nm particles. This enhanced uptake of smaller particles occurs primarily through clathrin and caveolae-mediated endocytosis, key pathways for receptor-specific and non-specific cellular internalization. The size-dependent behavior highlights the critical role of nanoparticle dimensions in optimizing their cellular interaction, making it an essential

consideration in designing AuNP-based applications, such as targeted drug delivery, diagnostics, and therapeutic systems.[83]

9. Effect of physicochemical properties of nanoparticle-based cancer immunotherapies

Nanoparticle-based cancer immunotherapies have shown significant promise due to their ability to enhance the therapeutic efficacy of immunomodulatory agents.

The physicochemical properties of nanoparticles, including size, shape, surface charge, surface chemistry, functionalization, and material composition, play a pivotal role in determining their biological interactions and therapeutic outcomes.

9.1. Size

The size of nanoparticles is one of the most critical determinants of their *in vivo* behavior, influencing biodistribution, circulation time, cellular uptake, and tumour targeting. Nanoparticles sized between 10–200 nm are typically optimal for tumour accumulation through the enhanced permeability and retention (EPR) effect, as they can penetrate the leaky vasculature of tumours while avoiding rapid clearance by the mononuclear phagocyte system (MPS).[84] Smaller nanoparticles (<10 nm) are cleared quickly by renal filtration, reducing their efficacy. Conversely, larger nanoparticles (>200 nm) are more likely to accumulate in the liver and spleen due to uptake by Kupffer cells, limiting their tumour-targeting potential.[85]

Studies also suggest that nanoparticle size affects the penetration depth within tumour tissues. Smaller nanoparticles demonstrate superior penetration, although their limited drug-loading capacity can reduce therapeutic efficacy. For instance, nanoparticles sized at ~50 nm strike a balance between EPR-based accumulation and intratumoral distribution.[86]

9.2. Shape

Nanoparticle shape impacts cellular uptake, biodistribution, and clearance mechanisms. Spherical nanoparticles are internalized more efficiently by cells via clathrin-mediated endocytosis compared to rod-shaped or disc-shaped nanoparticles. However, elongated shapes (e.g., nanorods) exhibit prolonged circulation times due to their reduced surface area for protein adsorption and immune recognition.[87]

Rod-shaped nanoparticles have demonstrated improved alignment along blood vessels, enhancing tumour extravasation and retention.[88] In contrast, disc-shaped nanoparticles have shown preferential margination toward vascular walls, which can enhance interactions with endothelial cells and facilitate tumour penetration.[89]

9.3. Surface charge

Surface charge is a critical factor influencing nanoparticle stability, cellular interaction, and biodistribution. Positively charged nanoparticles exhibit enhanced cellular uptake due to electrostatic interactions with negatively charged cell membranes but are often associated with higher cytotoxicity and increased immune activation.[90] Neutral or slightly negative nanoparticles tend to evade protein adsorption and avoid rapid clearance by the immune system, leading to extended blood circulation.[91]

Moreover, charge-related properties significantly influence the delivery of immunotherapies. For example, cationic nanoparticles are particularly effective in delivering nucleic acids like siRNA or DNA vaccines to antigen-presenting cells (APCs), as they facilitate endosomal escape.[92]

9.4. Surface chemistry

The surface chemistry of nanoparticles governs their interactions with biomolecules, influencing stability, immune evasion, and targeting efficiency. Hydrophobic surfaces promote protein adsorption and immune recognition, leading to rapid clearance. In contrast, hydrophilic surfaces, such as those modified with polyethylene glycol (PEG), enhance colloidal stability and prolong systemic circulation.[93]

PEGylation is a widely adopted strategy to prevent opsonization and phagocytosis. However, long-term use of PEG-coated nanoparticles can trigger the "accelerated blood clearance" (ABC) phenomenon, reducing their effectiveness.[94]

Alternatives, such as zwitterionic coatings, are being explored to address this limitation while maintaining stealth properties.[95]

9.5. Functionalization

Functionalization of nanoparticles with targeting ligands, including antibodies, peptides, or aptamers, enables specific interactions with cancer cells or immune cells. This active targeting approach improves therapeutic efficacy while minimizing off-target effects. For instance, nanoparticles functionalized with PD-1/PD-L1 inhibitors enhance T-cell activation in the tumour microenvironment, boosting anti-cancer immune responses.[96]

Dual-functional nanoparticles, combining immune activation and tumor targeting, are particularly promising. For example, nanoparticles co-functionalized with Toll-like receptor (TLR) agonists and tumor-specific antibodies can simultaneously stimulate innate and adaptive immunity.[97]

9.6. Material composition

The choice of material significantly impacts nanoparticle biodegradability, drug release profiles, and immune compatibility. Organic nanoparticles, such as liposomes and polymeric micelles, offer advantages in biocompatibility and controlled drug release but may suffer from stability issues.[98] Inorganic nanoparticles, such as gold or silica-based systems, exhibit excellent stability and tunable optical properties but often require surface modifications to reduce toxicity and enhance biodegradability.[99]

Hybrid nanoparticles, which combine organic and inorganic components, are gaining attention for their ability to integrate the advantages of both material types. For example, lipid-coated gold nanoparticles exhibit enhanced stability and improved interaction with immune cells, making them ideal for cancer immunotherapy applications.[100]

The physicochemical properties of nanoparticles intricately influence their in vivo fate and therapeutic potential in cancer immunotherapy. Optimizing these properties, such as size for EPR effect, surface chemistry for immune evasion, and functionalization for targeted delivery, is crucial for enhancing their efficacy and minimizing side effects. Future research should focus on tailoring multifunctional nanoparticles that can simultaneously address delivery challenges, modulate the immune microenvironment, and achieve sustained therapeutic effects

10. Patents

Table 1 The following Patent Numbers are obtained through European Patent Office

S.no	Patent topic	Nanocarrier used	Patent number
1	Preparation and application of cell membrane tumour vaccine.	Immunologic adjuvant.	CN117959416A
2	Concatemeric peptide epitope RNAs.	Concatemeric peptide epitope mRNAs.	US12150980B2
3	Fluorinated glycopolypeptide nano vaccine as well as preparation method and application thereof.	Alpha-fluoroalkyl-omega-mannosylated polycysteine.	CN118146502A
4	mRNA vaccine preparation for treating melanoma and pulmonary metastasis of melanoma as well as preparation method and application of mRNA vaccine preparation.	Co-entrap a TLR7 agonist (gardimod) and mRNA.	CN117357639A
5	Polymersomes and methods of using and making the same.	Polymersomes 1.Cationic polymer: PEG-PLGA (polyethylene glycol-polylactic-co-glycolic acid) 2. Helper polymer: helper group-PEG-PLGA.	WO2024215257A1
6	Cancer immunotherapy using virus particles.	Virus-like particles (VLPs).	US12070478B2

7	Nanoparticle complexes for enhanced safety.	Inorganic particle.	WO2024148169A1
8	Vaccine composition comprising gold-nanoparticle-carrier having double-stranded DNA bound thereof.	Gold nanoparticles (AuNPs).	WO2024144193A1
9	Formulated and/or Co-Formulated Lipid Nanocarriers Compositions Containing Toll-Like Receptor ("TLR") Agonist Prodrugs Useful In The Treatment of Cancer and Methods Thereof.	Lipid-based nanocarriers.	US2024108732A1
10	Dendritic peptide conjugated polymers for efficient intracellular delivery of nucleic acids to immune cells.	Synthetic polymer-based nanocarrier composed of a PEG-b-PPS-linker-DP polymer.	US2024158814A1
11	Preparation method and application of lipid nano-carrier inhalable dry powder.	Lipid nano-carriers, including lipidosomes and lipid nanoparticles (LNPs).	CN118576558A
12	Membrane encapsulated nanoparticles and method of use.	Membrane-encapsulated nanoparticle.	US12097290B2
13	Development and application of macrophage membrane hybrid lipid nano RNA vaccine.	Macrophage membrane hybrid lipid nanoparticle.	CN118460466A
14	Sarcoma cancer vaccines and uses thereof.	Recombinant SS18: SSX and cancer-testis antigen epitopes.	WO2024233388A2
15	Preparation of recombinant protein from recombinant Escherichia coli for inhibiting growth of prostate cancer cells.	Recombinant MUC-1-based polypeptide.	CN118063590A
16	Nucleic acid molecule, fusion protein and mRNA vaccine for treating liver cancer	Nucleic acid-based HBX protein and T cell epitope vaccine.	CN118147179A
17	PEG-PPS nanocarrier delivery of the RAS/RAP1 specific endopeptidase.	PEG-b-PPS (Polyethylene Glycol-block-Polypropylene Sulfide) system.	WO2024158781A2
18	Preparation and application of solidified self-microemulsion vaccine.	Squalene-based nano-carrier.	CN118662475A
19	Formulated and/or Co-Formulated Liposome Compositions Containing PD-1 Antagonist Prodrugs Useful in the Treatment of Cancer and Methods Thereof.	Liposomes.	US2024366772A1
20	Compositions and methods for ribonucleic acid vaccines encoding NY-ESO-1.	RNA molecule encoding an NY-ESO-1 derived peptide.	CN116970614A
21	Formulated and/or co-formulated Liposome compositions containing Immunogenic Cell Death (ICD) inducing prodrugs useful in the Treatment of cancer and methods thereof.	Lipid Nanoparticles (LNPs) and Solid Lipid nanoparticles (SLNPs).	US2023226031A1
22	Cancer immunotherapy using virus particles.	Virus-like particles (VLPs).	WO2024215257A1
23	Encapsulated biomolecules for intracellular delivery.	Metal-Organic Frameworks (MOFs).	WO2023021241A1
24	MRNA vaccine for treating lung cancer and bone metastasis thereof as well as preparation method and application of mRNA vaccine.	TLR4 agonist monophosphoryl lipid A (mPLA) and mRNA.	CN115920019A

25	Tumour vaccine as well as preparation method and application thereof.	Immunologic adjuvant.	CN115645518A
26	Tetanus vaccine platform for embedding covid-19 vaccine.	Detoxified recombinant Tetanus Neurotoxin (DrTeNT).	US2023201332A1
27	Dendritic cell-mesenchymal stem cell vaccine as well as preparation method and application thereof.	1V209-Chol-Liposome.	CN116898958A
28	Nano-carrier for inhibiting tumour dryness as well as preparation method and application of nano-carrier.	Fused exosome.	CN115990270A
29	Polypeptide vaccine delivery carrier and preparation method thereof.	Phenylalanine-based polyester amide polymer.	CN116603068A
30	A drug nanocarrier system to deliver a combination of Toll-Like Receptors (TLRs) agonists and/or a Lipoxin plus immunogenic cell death inducing chemotherapeutic agents for cancer immunotherapy.	Silicasomes and Liposomes.	WO2023172300A1
31	Nanotechnology based intranasal vaccine for covid-19 comprising chitosan.	Chitosan.	WO2023159082A2
32	Nucleic acid nano vaccine derived from bacterial outer membrane vesicle and use thereof.	Bacterial Outer Membrane Vesicles (OMVs).	WO2023142999A1
33	Cancer immunotherapy using virus particles.	Virus-like particle.	US11260121B2
34	Formulated and/or co-formulated compositions containing A2aR antagonist prodrugs useful in the treatment of cancer and methods thereof.	LNPs (Lipid Nanoparticles) and SLNPs (Solid Lipid Nanoparticles).	US2022401451A1
35	Tumour neoantigen DNA nano vaccine capable of riding red blood cells as well as preparation method and application of tumour neoantigen DNA nano vaccine.	PLGA (poly (lactic-co-glycolic acid).	CN114558127A
36	Use of parasites and extracellular vesicles obtained from parasites in cancer treatment.	Extracellular vesicles (exosomes).	CN114072167A
37	Methods and compositions for treating cancers.	Xenogeneic embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) with Valproic acid (VPA).	US11458194B2
38	Bladder cancer targeted nano-drug and preparation method thereof.	Amino-modified mesoporous silicon carrier.	CN115089728A
39	Nano-enabled immunotherapy in cancer.	Lipid-bilayer (LB)-coated nanoparticle.	CA3157508A1
40	Tumour vaccine based on dendrimer coated copper sulphide nanoparticles and preparation and application thereof.	Dendrimer-coated copper sulphide nanoparticle.	CN115300638A
41	Lipid nano-carrier loaded with anti-cancer drug as well as preparation method and application of lipid nano-carrier.	Lipid nano-carrier, specifically a solid lipid nanoparticle (SLNP).	CN114848594A
42	Self-assembled nanoparticle containing gB protein of EB virus, and preparation method therefor and use thereof.	Self-assembled nanoparticle.	WO2022120908A1
43	Dual-scale porous silica particle-based composition for preventing or treating cancer.	Dual-scale porous Silica particle.	KR20210045910A
44	Cationic liposomes for cancer immunotherapy.	Cationic Liposomes.	US10881612B2

45	Formulated and/or co-formulated liposome compositions containing Indoleamine 2,3-dioxygenase (IDO) antagonist prodrugs useful in the treatment of cancer and methods thereof.	Liposomes	US2021163418A1
46	BCG (bacillus Calmette Guerin) vaccine complex combined with nano drug carrier and preparation method of BCG vaccine complex.	Nano particles coated with a polylactic acid-glycolic acid (PLGA) copolymer.	CN112451679A
47	Remote modulation of bicontinuous nanospheres for controlled delivery applications	Bicontinuous Nanospheres.	US2021308065A1
48	Preparation method of nano vaccine with pH and reduction dual sensitivity and obtained product	PEI-modified mesoporous silica nanospheres.	CN112315941A
49	Preparation method and application of novel nano quasi-cell personalized tumour vaccine	Hyaluronic acid-based carrier.	CN113413463A
50	Golgi apparatus and genetic engineering exosome hybrid membrane coated retinoic acid in-situ spray hydrogel vaccine, and preparation method and application thereof	Golgi-exosome hybrid membrane.	CN113058031A
51	Poly (ethylene glycol)-block-poly (propylene sulfide) nanocarrier platform for enhanced efficacy of immunosuppressive agents	Poly (ethylene glycol)-block-poly (propylene sulfide).	US2020383917A1
52	Tumour vaccine combining exosome with immune checkpoint blocker and preparation method thereof	Exosome.	CN111840528A
53	Novel double-targeted nano drug of customized T-cell epitope vaccine, and preparation method and application thereof.	Nano drug that incorporates pMHC polymers (peptide-major histocompatibility complex polymers) and pancreatic-specific antibodies.	CN111135310A

11. Conclusion

Nanocarriers represent a versatile and innovative approach to vaccine formulation, offering improved delivery, stability, and efficacy for a wide range of vaccines. This review underscores the potential of nanocarrier-based vaccines as a transformative tool in modern immunization strategies, paving the way for advancements in both preventive and therapeutic applications. By addressing current challenges and exploring emerging trends, nanocarrier vaccines hold promise as a groundbreaking platform to combat infectious diseases, cancer, and other global health concerns.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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