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BIO-MEMBRANE BASED NANOVACCINES: ENGINEERING THE IMMUNE SYSTEM TO FIGHT AGAINST CANCER

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Abstract

Nanotechnology has transformed cancer therapy by overcoming limitations associated with conventional treatments. Although nanoparticle-based anticancer drugs hold promise, their efficacy is restricted by physiological barriers that hinder drug penetration and immune activation. Self-assembled nanomaterials have been designed to address these issues with programmable delivery and immune modulation, enhancing cancer immune therapy.

Cancer vaccines stimulate the immune system against tumors, yet conventional vaccines suffer from low immunogenicity, short-lived responses, and poor antigen presentation. Nanovaccines, utilizing carriers like liposomes, virosomes, and dendrimers, improve antigen stability, immune stimulation, and circulation time, optimizing their therapeutic potential. Biomembrane-based nanovaccines have emerged as a superior alternative, leveraging natural or engineered biological membranes to enhance immune evasion, antigen stability, and biocompatibility. These vaccines, derived from tumor cell membranes, dendritic cell vesicles, and bacterial OMVs, effectively evade immune clearance, improve lymphoid organ targeting, and enhance antigen presentation. Their mechanism involves MHC-I and MHC-II pathways, activating CD8+ and CD4+ T cells for a robust and sustained immune response. The capability of these nanovaccines to combine with immune checkpoint inhibitors expands their therapeutic potential, presenting them as a promising frontier in cancer immunotherapy.

INTRODUCTION

Nanotechnology has transformed cancer therapy, introducing novel approaches to overcome the limits of traditional treatments (Guo et al., 2023). While nanoparticle-based anticancer medicines have shown great promise, their therapeutic efficacy is frequently limited by physiological barriers that prevent drug penetration and immune activation (Rampado et al., 2022). To address these challenges, self-assembled nanomaterials have been created, offering programmable delivery, stimuli responsiveness, and immune modulation, thus enhancing the effectiveness of cancer immunotherapy (Zhou et al., 2023).

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Cancer vaccines, among the different approaches to cancer immunotherapy, appear to be an effective method for stimulating the immune system against tumor cells. Conventional cancer vaccines, on the other hand, have drawbacks such as low immunogenicity, short-lived responses, and poor antigen presentation, which reduce their efficacy (de Pinho Favaro et al., 2022; Hillman, 2024). Nanovaccines, which use advanced nanocarriers such as liposomes, virosomes, dendrimers, and polymeric nanoparticles, provide more targeted antigen delivery, immune stimulation, and sustained circulation, making them an appealing choice in cancer treatment (de Pinho Favaro et al., 2022). These nanoformulations allow for precise engagement with antigen-presenting cells (APCs), boosting immune responses while reducing off-target effects (Ding et al., 2022).

Despite these improvements, antigenic instability, poor endocytosis, and ineffective immune cell absorption remain significant problems (D. Wang et al., 2022). Biomembrane-based nanovaccines have emerged as a new option to improve the efficacy of cancer vaccinations (Wu et al., 2023). These nanovaccines, which use natural or designed biological membranes, improve biocompatibility, immune evasion, and antigen stability, providing a fresh technique for improving therapeutic results (B. Wang et al., 2022).

Biomembrane-based nanovaccines use tumor cell membranes, dendritic cell-derived vesicles, bacterial outer membrane vesicles (OMVs), and hybrid biomembrane formulations to improve antigen presentation and immune activation (Gu & Wang, 2024; Zheng et al., 2021). These vaccines closely resemble natural cell membranes, allowing them to elude immune system clearance while effectively delivering antigens to lymphoid organs. Additionally, the structural integrity of biomembranes shields the antigen from enzymatic degradation, ensuring long-term immune responses (Das & Ali, 2021; Ding et al., 2022; Liao et al., 2024). The usage of OMVs, in particular, has intrinsic immunostimulatory qualities because they naturally contain pathogen-associated molecular patterns (PAMPs) that activate toll-like receptors (TLRs), hence increasing immune responses (Ma et al., 2025; Tang & Li, 2024).

The mode of action of biomembrane-based nanovaccines involves effective absorption by APCs, followed by antigen presentation via both MHC I and MHC II pathways, which activates cytotoxic CD8+ T cells and helper CD4+ T cells. This promotes a strong and long-lasting immune response, which is essential for cancer treatment (Ding et al., 2022; Tang & Li, 2024). Furthermore, these nanovaccines can be coupled with immune checkpoint inhibitors like anti-PD-1 and anti-CTLA-4 therapies to reverse tumor-induced immune suppression and boost T-cell-mediated tumor killing (Ma et al., 2025).

Recent advances in mRNA lipid nanoparticle vaccines, such as those used to treat COVID-19, have fueled interest in nanotechnology-driven immunotherapy platforms. These accomplishments demonstrate the ability of nanovaccine formulations to be quickly transferred into clinical applications (Nie et al., 2023). However, problems like scalability, regulatory licensing, and manufacturing repeatability must be overcome before biomembrane-based nanovaccines may be widely used in oncology (Ding et al., 2022). Future studies will concentrate on enhancing biomaterial engineering, targeted delivery techniques, and immune regulation to improve the efficacy of these nanovaccines (Nie et al., 2023).

With ongoing advances in nanotechnology and immunotherapy, biomembrane-based nanovaccines represent a promising future in cancer treatment, providing a safe, efficient, and long-term way to mobilize the immune system against tumors (Ma et al., 2025). As research continues, these vaccinations could become a cornerstone of next-generation cancer immunotherapy, improving patient outcomes and furthering the area of personalized medicine (Tang & Li, 2024).

2. Advancements in Biomembrane-Based Nanovaccines for Cancer Immunotherapy

Biomembrane-based nanovaccines have emerged as a viable method for cancer immunotherapy, improving antigen delivery, cross-presentation, and immune activation. Modifying nanoparticles with ligands for mannose receptors, Fc receptors, DEC-205, CD11c/CD18, or DC-SIGN, which facilitate receptor-mediated endocytosis, allows for targeted distribution to antigen-presenting cells (APCs) (Feng

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et al., 2019; Zhao et al., 2023). However, effective antigen escape from the endosomal pathway is required for MHC-I presentation and CTL activation. To address this, pH-sensitive liposomes were created, adding 3-methylglutarylated hyperbranched poly(glycidol) (MGlu-HPG) to destabilize under acidic circumstances, hence increasing antigen release and presentation (Cheng et al., 2020; Feng et al., 2023).

Furthermore, exosome-based nanovaccines made from dendritic cells (DCs) have been demonstrated to improve immune activation by expressing co-stimulatory molecules and MHC peptides (Wang et al., 2021; Zhao et al., 2023). Ex vivo antigen loading and genetic alteration are two engineering methods that improve the efficacy of exosomes. In bacterial-derived outer membrane vesicle (OMV)-based vaccinations, intrinsic pathogen-associated molecular patterns (PAMPs) operate as adjuvants, and genetically altered OMVs with PD-1 ectodomains can diminish PD-L1-mediated immune suppression (Sun et al., 2021; Wang et al., 2021). Furthermore, the Plug-and-Display OMV method, which employs the SpyTag/SpyCatcher and SnoopTag/SnoopCatcher systems, enables quick tumor antigen integration for individualized immunization. While these advancements greatly improve nanovaccine efficacy, issues remain in scalability, immune response variability, and clinical translation (Souto et al., 2024; Sun et al., 2021).

3. **Bio-membrane based nanovaccines, A superior alternative to conventional approach:**

Biomembrane-based nanovaccines offer significant advantages over traditional nanovaccines, particularly in cancer immunotherapy. Conventional nanovaccines usually suffer from rapid immune system clearance, necessitating multiple doses and the addition of stabilizers to maintain antigen integrity (Zhang et al., 2022). Furthermore, their low antigen presentation capacity limits their ability to elicit a strong and long-lasting immune response, necessitating the application of stronger adjuvants to improve immunogenicity (Wang et al., 2023). Biomembrane-based nanovaccines, on the other hand, use natural cell membranes, such as red blood cells (RBC) or tumor cell membranes, to allow immune evasion and prolonged systemic circulation, hence improving vaccine durability and bioavailability (Gao et al., 2021). By permitting MHC I and II cross-presentation, these biomimetic characteristics further enhance antigen presentation, leading to potent adaptive immune activation and tumor-specific cytotoxicity (Liu et al., 2019). Additionally, because traditional nanovaccines lack specific biodistribution, biomembrane coverings enable precise homotypic tumor targeting (Xie et al., 2021). Despite these benefits, the challenges of membrane sourcing, functionalization, and large-scale production make it difficult to translate biomembrane-based nanovaccines into clinical practice, requiring new bioprocessing techniques (Yu et al., 2020). The necessity for more advanced biomimetic approaches in the creation of cancer vaccines is highlighted by the fact that, although being easier to produce and scale, conventional nanovaccines' limited immune activation and quick clearance limit their therapeutic efficacy (Li et al., 2023).

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	Feature	Conventional Nanovaccines	Biomembrane-Based Nanovaccines	Clinical Readiness	Cost & Scalability	References
1	**Antigen Stability**	Prone to degradation, requiring stabilizers	Natural biomembrane enhances antigen protection	Moderate, needs stability improvements	More complex due to biomembrane sourcing	Zhang et al., 2022
2	**Circulation Time**	Short, rapid clearance	Prolonged due to immune evasion (e.g., RBC membrane with CD47)	High potential but needs further clinical validation	Higher cost due to functionalization processes	Gao et al., 2021
3	**Immune Activation**	Requires strong adjuvants	Intrinsic stimulation via biomembrane components (e.g., OMVs, TLR activation)	Promising, but needs large-scale trials	Moderate, dependent on source material	Wang et al., 2023
4	**APC Targeting**	Limited, non-specific uptake	Biomembrane coatings enhance APC recognition	High, enhances vaccine efficiency	Challenging to optimize for large-scale use	Chen et al., 2020
5	**Antigen Presentation**	Less efficient cross-presentation	Strong MHC-I & MHC-II presentation	Strong potential for personalized therapy	Requires sophisticated production methods	Liu et al., 2019
6	**Immune Evasion**	Low; quickly recognized and cleared	High; mimics natural cell membranes	Experimental trials show improved longevity	Higher cost due to precise engineering	Huang et al., 2022
7	**Tumor Homing Ability**	Poor; non-specific distribution	Excellent; homotypic targeting with tumor-cell membranes	Encouraging preclinical results	Moderate cost, depends on membrane source	Xie et al., 2021
8	**Therapeutic Efficacy**	Moderate, multiple doses required	High, sustained immune responses	High potential in cancer immunotherapy	Higher due to complex production	Li et al., 2023
9	**Clinical Translation Challenges**	Easier to manufacture & scale	Complex biomaterial sourcing, regulatory concerns	Still experimental; needs further safety data	Requires innovative large-scale production solutions	Yu et al., 2020
10	**Personalized Medicine Potential**	Limited customization	High potential for personalized cancer immunotherapy	Increasing interest in precision medicine	Expensive but could become cost-effective with advancements	Zhang et al., 2023

Table 1: A comparison of conventional and biomembrane-based nanovaccines.

4. Bio-membrane based nanovaccines platforms:

4.1 Cell membrane-coated nanoparticles:

Cell membrane-coated nanoparticles (CM-NPs) are a promising biomimetic method for increasing the efficacy of nanovaccines in cancer immunotherapy. These nanoparticles are covered with plasma membranes derived from living cells, which allows them to maintain functional membrane proteins that aid in immune evasion, tumor targeting, and sustained circulation (Zhang et al., 2023). The top-down membrane extraction approach, which involves freeze-thaw cycles or hypotonic treatment, is widely used to generate empty membrane vesicles that can then be fused with nanoparticles to produce a core-shell structure (El-Sayed & Kamel, 2020).

Several cell sources have been investigated for membrane coating, including red blood cells (RBCs), platelets, leukocytes, macrophages, cytotoxic T lymphocytes (CTLs), and natural killer (NK) cells, each with unique immunological and pharmacokinetic properties (Farhoudi et al., 2023; Li et al., 2021). RBC membranes, for example, increase circulation time by inhibiting immune clearance through CD47-mediated "self-recognition," whereas tumor cell membranes retain tumor-associated antigens (TAAs), allowing for homotypic targeting and improved antigen presentation for immune activation (Farhoudi et al., 2023).Furthermore, hybrid membrane coatings, which are formed by fusing distinct cell membranes (for example, leukocyte-tumor cell hybrids), have been developed to boost tumor-targeting efficiency (Fang et al., 2014). Beyond passive targeting, designed alterations like pH-sensitive coatings, photosensitiser ornamentation, and functional protein insertion provide regulated antigen release and increased immune activation (Huang et al., 2022). The use of liposome-based approaches has increased the functionalization potential of CM-NPs, but issues remain in maintaining membrane integrity, maximizing scalability, and assuring clinical translation (El Tekle & Garrett, 2023).

4.2 Red Blood Cell Membrane-Coated Nanoparticles

Red blood cell membrane-coated nanoparticles (RBCM-NPs) have distinct advantages in cancer immunotherapy due to their prolonged circulation period, immune evasion capabilities, and oxygen-carrying capacity. The inclusion of CD47 self-marker proteins on RBC membranes allows these nanoparticles to avoid clearance by the reticuloendothelial system, extending their half-life in the bloodstream (Levin et al., 2020; Ma et al., 2018). This trait is crucial in tumor

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microenvironments, where hypoxia leads to treatment resistance. To address this, PFC@PLGA RBCM nanoparticles were designed, which combine oxygen-carrying perfluorocarbon (PFC) cores with RBC membranes, successfully reducing tumor hypoxia and increasing radiation therapy efficacy (Barresi et al., 2022; Shalhout et al., 2023).

RBCM-coated nanoparticles have also been investigated for chemo-photodynamic therapy, where they improve biocompatibility and circulation time while allowing ROS-mediated drug release. RBCM-coated nanoparticles have been created for photoacoustic imaging by integrating graphene quantum dot nanozymes (GQDzymes) that respond to H_2O_2 in the tumor microenvironment (Ma et al., 2018). This allows for selective imaging. Folic acid functionalization improves tumor targeting by attaching to cancer cell folate receptors that are overexpressed (Zhang et al., 2021). These developments highlight RBCM-coated nanoparticles as a viable platform for tumor-targeted administration, imaging, and multi-modal cancer treatment (J. Wang et al., 2022).

4.3 Tumor cell membrane coated nanoparticles:

Tumor cell membrane-coated nanoparticles (TCM-NPs) have shown great promise in cancer immunotherapy due to their homotypic targeting capability and significance in cancer vaccine development (Xia et al., 2020). Nanoparticles coated with membranes from tumor cells have a higher affinity for their source malignancies due to common surface antigens. This has been used for high-precision tumor imaging, as demonstrated by glioma-targeting nanoparticles coated with C6 glioma cell membranes, which increase MRI and photoacoustic imaging resolution (Y. Yang et al., 2023). Similarly, PLGA-based TCM-NPs have been used to create tailored cancer nanovaccines by including tumor cell membranes that naturally carry tumor-associated antigens (TAAs)(Y. Yang et al., 2023).

These membrane-coated vaccines, when combined with immunologic adjuvants like MPLA and R837 (a TLR7 agonist), effectively induce antigen-presenting cells (APCs), resulting in potent anti-tumor immune responses (Xia et al., 2020). Furthermore, a mannose-modified tumor cell membrane coating has been developed to improve APC uptake and vaccine effectiveness. Recent research has also shown that TCM-based nanovaccines can improve immunological memory and decrease post-surgical tumor spread, indicating a promising technique for tailored cancer immunotherapy (Chen et al., 2021).

4.4 Immune Cell membrane coated nanoparticles:

Nanoparticles coated with immune cell membranes (T cells, NK cells, and macrophages) exhibit tumor-targeting and immune evasion features, making them intriguing candidates for cancer immunotherapy (Feng et al., 2022). T cell membrane-coated nanoparticles use T cell receptors (TCRs) to identify tumor-specific antigens. However, because of tumor heterogeneity, their targeted efficacy may be restricted (Zheng et al., 2018). To improve specificity, bio-orthogonal chemistry-based changes have been applied, allowing azide-labeled T cell membranes (N3-TINPs) to selectively bind tumor cells treated with BCN-modified unnatural sugars, resulting in dramatically increased targeting efficiency (Y. Yang et al., 2023).

NK cell membrane-coated nanoparticles can also target tumors because NK cell surface proteins, such as RANKL, stimulate M1 macrophage polarization, which enhances immune activation. Furthermore, NK membrane-coated nanoparticles with photosensitizers such as TCPP enable photodynamic treatment (PDT), which effectively kills tumor cells and stimulates immune responses (Chen et al., 2021). Macrophage membrane-coated nanoparticles use toll-like receptors (TLRs) to target tumors and are programmed to release paclitaxel (PTX) in response to acidic tumor microenvironments, ensuring regulated drug delivery (Feng et al., 2022). While immune cell membrane-coated nanoparticles allow for wide tumor targeting independent of tumor type, specific tumor affinity remains a hurdle, necessitating further modifications to increase therapeutic efficacy (Shalhout et al., 2023; Zhao et al., 2022).

4.5 Outer cell membrane-coated nanoparticles:

Apart from immune cell membranes, different forms of cell-derived coatings have been investigated for nanovaccine production. Platelet membrane-coated nanoparticles have a longer circulation time and are biocompatible due to their natural role in tumor cell aggregation (Levin et al., 2020). Platelet membrane-

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coated Fe_3O_4 magnetic nanoparticles (PLT-MNs) are used for MRI imaging and photothermal treatment (PTT). Bacterial membrane-coated nanoparticles, such as Salmonella outer membrane vesicles (OMVs), can safely trigger immune responses and transport chemotherapeutic medicines, increasing tumor suppression by cytokine activation (TNF-α, IFN-γ, IL-12p40)(Ma et al., 2018; Xia et al., 2020; Zhao et al., 2022).

Cancer-associated fibroblast (CAF) membrane-coated nanoparticles have been designed to target FAPα-expressing tumor fibroblasts, allowing for synergistic PTT and PDT cancer treatment. Other innovative techniques include myeloid-derived suppressor cell (MDSC) membrane-coated nanoparticles, which have higher tumor accumulation than RBC-coated nanoparticles (El Tekle & Garrett, 2023; Fang et al., 2014). Exosome-coated nanoparticles have also been used for homotypic tumor targeting, with doxorubicin (DOX)-loaded exosome-based porous silicon nanoparticles (DOX@E-PSiNPs) accumulating 2.3-2.5 times more tumors than free DOX, indicating their potential for personalized nanovaccine development (Zhang et al., 2021).

4.6 Hybrid cell membrane-coated nanoparticles:

Hybrid membrane-coated nanoparticles combine the positive attributes of many cell types by incorporating numerous capabilities into a single nanovaccine platform. For example, melanin@RBC-M nanoparticles combine red blood cell membranes (RBCM) and MCF7 tumor cell membranes, resulting in lengthy circulation durations and tumor-homing capabilities for photothermal therapy (PTT). The optimum RBC-to-tumor membrane ratio considerably improves treatment efficacy (Fang et al., 2014; Y. Yang et al., 2023).

Similarly, platelet-cancer stem cell hybrid membranes (CSC-P-coated nanoparticles) indicate immune evasion and tumor-specific homing, which improves photothermal tumor ablation (Ma et al., 2018). Hybrid membranes have also been created by combining RBC and platelet membranes, which improves circulation and biocompatibility (Sakimoto et al., 2016). Future research into multimembrane coatings may allow for even more precise targeting and flexible uses in cancer nanomedicine (El Tekle & Garrett, 2023).

5. Mechanisms of Biomembrane-Based Nanovaccines in Cancer Immunotherapy

5.1 Enhancing Antigen Presentation for Robust Immune Action:

Biomembrane-based nanovaccines provide a highly efficient approach to antigen presentation, which is an important step in activating adaptive immunity (El-Sayed & Kamel, 2020). Unlike conventional vaccinations, which require on external adjuvants, these nanovaccines naturally include tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) on their surfaces, ensuring effective delivery to antigen-presenting cells (APCs), notably dendritic cells (Huang et al., 2021).

Exosome-derived nanovaccines, particularly those made from tumor cells, have shown tremendous promise in tailored cancer immunotherapy. Tumor-derived extracellular vesicles (TEVs) encapsulated with nucleic acid-based adjuvants, such as CpG ODNs and p(I:C), have been demonstrated to improve dramatically both humoral and cellular immune responses (Feng et al., 2019). Similarly, bacterial outer membrane vesicles (OMVs) use pathogen-associated molecular patterns (PAMPs) to activate immunological signaling, allowing for effective antigen transport to lymph nodes and activating T-cell-mediated immunity (Sun et al., 2021).

The lipid bilayer structure of biomembrane-based nanovaccines ensures controlled antigen release, allowing for prolonged antigen exposure and cross-presentation via MHC class I and II molecules, thereby activating cytotoxic CD8+ and helper CD4+ T cells (Das & Ali, 2021). Further, surface modifications such as PEG coatings and receptor-ligand functionalization (e.g., DEC-205, Clec9A) enhance targeting specificity, ensuring antigen uptake by specialized DC subtypes while reducing nonspecific interactions (Guo et al., 2023).

In addition, biomimetic coatings, such as erythrocyte or cancer cell membranes, allow nanovaccines to escape premature clearance by the mononuclear phagocyte system (MPS) (Zhao et al., 2023). CD47-expressing biological membranes send "don't eat me" signals to macrophages, preventing early degradation and maximizing antigen uptake by DCs. Additionally, immune cell-derived exosomal nanocarriers generally

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contain costimulatory molecules, further enhancing antigen presentation and immune activation (Ma et al., 2025).

5.2 Enhancing Dendritic Cell Activation and T-Cell Response

By processing and presenting tumor antigens to naïve T lymphocytes, dendritic cells (DCs) play a crucial role in cancer immunotherapy by triggering adaptive immunity (Ding et al., 2022). Biomembrane-based nanovaccines are effective tools for developing cancer vaccines because they increase DC activation, boost T-cell responses, and improve antigen absorption (de Pinho Favaro et al., 2022).

By activating Toll-like receptors (TLRs) on DCs, these nanovaccines resemble pathogen-associated molecular patterns (PAMPs), which results in increased production of CD80, CD86, CD40, and MHC molecules (Rampado et al., 2022). By improving antigen cross-presentation through MHC-I and MHC-II, this procedure activates helper CD4+ T cells as well as cytotoxic CD8+ T cells. A strong T-cell-mediated immune response is triggered by exosome-derived nanovaccines that contain microRNAs (Let-7i, miR-155, and miR-142) that further enhance DC maturation and cytokine production (IL-12, IFN-γ)(Chen et al., 2021; Zheng et al., 2018).

Nanovaccines can be designed to target DC subtypes in order to optimize antigen presentation specifically. MHC-I cross-presentation and CTL activation are facilitated by CD8+ DCs, which express DEC-205, whereas CD8− DCs (DCIR2+) mainly stimulate CD4+ helper T cells. Stronger T-cell priming results from modifications using mannosylated ligands or anti-DEC-205 antibodies, which improve DC-targeted antigen absorption (Barresi et al., 2022; Zhao et al., 2022).

Hybrid nanovesicles (hNVs), which contain tumor cell membranes, M1 macrophage-derived vesicles (M1-NVs), and platelet-derived vesicles (P-NVs), are recent developments that enhance T-cell activation by altering the tumor microenvironment. Furthermore, fusion vesicles that combine the membranes of tumor cells and DCs, such as NP@FM, directly improve immune priming and antigen presentation (Li et al., 2021).

5.3 Overcoming Immune Suppression in Tumor Environment

The effectiveness of cancer immunotherapy is limited by the tumor microenvironment's (TME) suppression of immune responses via regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and immune checkpoint pathways (PD-1/PD-L1)(B. Wang et al., 2022). By reprogramming the TME to promote anti-tumor immunity, biomembrane-based nanovaccines reverse these inhibitory pathways (Ding et al., 2022).

Combating Immune Evasion in Tumors

Biomembrane nanovaccines enhance immune activation by:

1. To restore T-cell function, use checkpoint blockade agents (anti-PD-L1, anti-CTLA-4).
2. Agonists for toll-like receptors to boost innate immunity.
3. Nanoparticles loaded with cytokines (IL-12, IFN-γ) to cause inflammation in the TME (B. Wang et al., 2022; F. Yang et al., 2023)

Reprogramming the Microenvironment of Tumors

The TME is influenced by biomembrane nanovaccines through:

1. TAMs (tumor-associated macrophages) repolarize from the immunosuppressive M2 phenotype to the pro-inflammatory M1 phenotype.
2. Reducing Treg infiltration and avoiding cytotoxic T cell suppression.
3. Enhancing anti-tumor immunity by increasing effector T-cell infiltration (D. Wang et al., 2022; F. Yang et al., 2023).

Nanovaccine Hybrids for Immune Modification

The outer membranes of bacteria and tumors are combined in hybrid vesicles, which further increase immune activation by:

1. T-cell function is restored by blocking immunological checkpoints (PD-1/PD-L1, CD47-SIRPα).
2. Enhancing cytotoxic T-cell infiltration and tumor phagocytosis mediated by macrophages.
3. Bacterial vesicle-expressed angiogenic factors (e.g., BFGF) that stimulate B-cell responses, resulting in more efficient tumor suppression (Sakimoto et al., 2016; Zheng et al., 2021).

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6. Challenges and Solutions

6.1 Scalability and manufacturing Challenges:

Regulatory approval, quality control, and large-scale production are major obstacles to the clinical translation of biomembrane-based nanovaccines. High production costs, intricate fabrication techniques, and inconsistent product quality are major obstacles (Wu et al., 2023).

Key Manufacturing Challenges

1. **Complex Fabrication and Standardization:** Standardizing the extraction and functionalization of cellular membranes while conserving bioactivity is challenging due to batch-to-batch variability.
2. **High Production Cost and Low Yield:** Advanced bioprocessing techniques, such as ultracentrifugation and membrane fractionation, increase costs, whereas biological membrane sources give lower vaccine yields than synthetic platforms.
3. **Quality Control and Regulatory Barriers:** Biomembrane-based vaccines' unique composition complicates particle size management, antigen presentation consistency, and sterility assurance, posing issues for regulatory approval (Nie et al., 2023; Tang & Li, 2024).

6.2 Immune system Recongnition and Clearance:

One of the main concerns in biomembrane-based nanovaccine development is the immune system's ability to recognize and quickly clear nanoparticles before they reach their intended targets. Nanovaccines can be less successful since the mononuclear phagocyte system (MPS) is responsible for recognizing and removing foreign particles (Fang et al., 2014; Sakimoto et al., 2016).

To tackle this difficulty, numerous solutions have been investigated:

Surface Modification with Polyethylene Glycol (PEG):

Coating nanoparticles with PEG inhibits opsonization and identification by macrophages, extending circulation duration. PEGylation increases nanoparticle stability in serum and reduces aggregation (Sun et al., 2021).

Biomimetic Coatings:

Using natural cell membranes from erythrocytes, cancer cells, or immune cells, nanoparticles can avoid immunological detection. The inclusion of CD47, a "don't eat me" signal on erythrocyte-derived nanovaccines, can hinder immune clearance (Chen et al., 2022).

6.3 Potential Toxicity of Nanovaccines:

Toxicity remains a big concern; especially as nanoparticles accumulate in important organs and cause unwanted inflammatory reactions. Studies have demonstrated that various nanomaterial features, such as size and shape, can influence the toxicity levels (Rampado et al., 2022).

1. Size-Dependent Toxicity:

Nanoparticles smaller than 10 nm can be cleared by the kidneys, while larger ones can collect in the liver and spleen, potentially leading to long-term retention and toxicity (Cheng et al., 2020).

2. Material Composition and Coating:

The selection of biomaterials has a considerable impact on toxicity. Iron oxide nanoparticles can cause macrophages to produce IL-1β, resulting in inflammatory reactions. To reduce these effects, biocompatible coatings such as phospholipids or polymers have been used to improve safety (Zhang et al., 2023).

6.4 Stability and Storage Issues:

Due to their biological nature and risk to degradation, biomembrane-based nanovaccines are difficult to stabilize and store for lengthy periods of time. These nanovaccines are based on lipid bilayers and membrane proteins, which are susceptible to oxidation, aggregation, and structural instability over time (de Pinho Favaro et al., 2022; Sakimoto et al., 2016). Lipid oxidation, in particular, causes membrane breakdown, which influences antigen presentation and vaccine efficacy. Additionally, biomembrane-coated nanoparticles often demonstrate instability during storage, resulting in aggregation or fusion, which affects their structural integrity. Many biomembrane-based vaccinations require specific low-temperature storage conditions, making them difficult to get, especially in resource-

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limited areas (El Tekle & Garrett, 2023; Fang et al., 2014).

7. Future Directions:

7.1. Future directions and potential solutions to overcome scalability and manufacturing challenges: Researchers are exploring on a number of approaches to deal with these issues, such as:

1. Automated Bioprocessing:
Reproducibility and scalability can be enhanced by implementing automated technologies for membrane isolation and nanoparticle assembly.

2. Genetically Modifying Source Cells:
Creating cell lines that generate biomembranes with ideal characteristics can improve the uniformity and effectiveness of vaccines (Sakimoto et al., 2016).

3. Hybrid Nanoplatforms:
By combining synthetic nanocarriers with vesicles formed from biomembranes, a scalable method that maintains the advantages of biomembrane-based delivery may be possible.

4. Advanced Characterization Techniques:
Proteomics, lipidomics, and high-resolution imaging can all be used to improve batch uniformity and quality control (Tang & Li, 2024).

7.2. Strategies to Reduce Toxicity and Enhance Biocompatibility:

1. Controlled Antigen Release: Encapsulating antigens within lipid bilayers ensures progressive and sustained antigen release, lowering the risk of excessive immune activation. This controlled strategy reduces the likelihood of hyperinflammatory responses.

2. Engineering nanoparticles for safe degradation: Using biodegradable polymers like poly (lactic-co-glycolic acid) (PLGA) allows nanoparticles to breakdown into non-toxic byproducts, preventing their accumulating over time.

3. Immune system modulation: Nanovaccines that include immune-modulating compounds, such as Toll-like receptor agonists, can enhance beneficial immune activation without causing excessive inflammation (Peng et al., 2024; D. Wang et al., 2022; Zheng et al., 2021).

7.3. Potential Solutions for Stability and Storage issues:

1. Strategies for Lipid Stabilization: Adding cholesterol or antioxidants (such α-tocopherol) can improve membrane stability and lower oxidation.

2. Methods of Cryopreservation: Maintaining membrane integrity during storage can be achieved by lyophilization or freeze-drying with cryoprotectants (sucrose, trehalose).

3. Nanocarrier Modification: Cross-linked hydrogels or polyethylene glycol (PEG) coatings can increase vaccine stability and extend shelf life (Ma et al., 2025; F. Yang et al., 2023).

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	Challenges	Description	Proposed Solutions	Impact on Vaccine Efficacy	Current Research & Experimental Solutions	References
1	**Scalability & Manufacturing**	High costs, variability in production	Automated bioprocessing, hybrid nanocarriers	Delays clinical application	New bioengineering methods for mass production	Zhang et al., 2023
2	**Immune System Recognition & Clearance**	Rapid clearance by immune cells	PEGylation, CD47 functionalization	Reduces bioavailability	Synthetic membrane coatings to mimic immune-evasive properties	Gao et al., 2021
3	**Antigen Stability & Degradation**	Antigen loss due to enzymatic degradation	Lipid stabilization, cryopreservation	Weakens immune response	Hydrophobic nanocarriers for prolonged antigen stability	Wang et al., 2023
4	**Poor Endocytosis & APC Uptake**	Inefficient antigen presentation	Ligand modifications for targeted uptake	Limits T-cell activation	Mannose-coated nanoparticles to enhance dendritic cell uptake	Chen et al., 2020
5	**Weak Immune Activation**	Lack of strong immune signals	Use of bacterial OMVs, TLR agonists	Leads to lower vaccine efficacy	Self-adjuvanting biomembranes under study	Liu et al., 2019
6	**Tumor Microenvironment (TME) Suppression**	Immunosuppressive factors (Tregs, PD-L1)	Immune checkpoint inhibitors, cytokine therapy	Can block effective immune responses	Combination therapy trials with checkpoint blockade agents	Huang et al., 2022
7	**Potential Toxicity & Long-Term Safety**	Nanoparticle accumulation in major organs	Biodegradable materials, controlled antigen release	Affects patient safety & regulatory approval	Testing biodegradable polymeric nanocarriers	Xie et al., 2021
8	**Stability & Storage Issues**	Risk of aggregation & degradation	Lyophilization, antioxidant coatings	Can reduce vaccine shelf life	Cryoprotectants to extend storage stability	Li et al., 2023
9	**Regulatory & Clinical Translation Barriers**	Lack of standardization for safety testing	GMP-compliant production, regulatory alignment	Slows clinical adoption	International regulatory collaborations for harmonized approval	Yu et al., 2020

Table 2: Challenges and Potential Solutions for Biomembrane-Based Nanovaccines.

Conclusion:

Biomembrane-based nanovaccines offer a promising advancement in cancer immunotherapy by enhancing antigen stability, immune evasion, and targeted antigen presentation. Their ability to mimic natural cell membranes allows for prolonged circulation, improved biocompatibility, and efficient antigen uptake by APCs, leading to strong and sustained immune responses. Despite these advantages, challenges such as scalability, immune clearance, regulatory approvals, and manufacturing complexity remain. Addressing these issues through automated bioprocessing, hybrid nanoplatforms, and stability-enhancing modifications will be crucial for clinical translation. Additionally, integrating nanovaccines with immune checkpoint inhibitors and personalized therapies may further improve efficacy. With continued advancements in nanotechnology and immunotherapy, biomembrane-based nanovaccines hold great potential for revolutionizing cancer treatment, offering a safe, efficient, and long-lasting strategy for mobilizing the immune system against tumors.

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