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Nanoparticle Vaccine Formulations for Dengue Virus

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Abstract

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Dengue virus (DENV) is one of the most prevalent mosquito-borne pathogens, with almost half of the global population at risk of infection. While most cases are mild, severe illness and even death is not uncommon. There are currently a lack of available antivirals and highly effective prophylactic vaccines available for DENV, leading to a significant gap in protection. While live-attenuated vaccines have been developed and briefly utilized, some have been found to increase the risk for developing antibody-dependent enhancement (ADE), a phenomenon that can worsen outcomes in those who are exposed to DENV after receiving the vaccination. Nanoparticle-based vaccine formulations provide numerous advantages over live-attenuated vaccines such as controlled release, dose-sparing, and ability to effectively encapsulate adjuvants and viral antigens, while simultaneously minimizing the risk for development of ADE. Numerous carrier systems have been developed, including polymeric, lipid, inorganic, and protein-based formulations. Each system has been found to induce unique antigen-specific immune activation that includes varying degrees of humoral and cellular immune responses. While there is still much research to be done, nanoparticle-based vaccine formulations offer a promising approach to combat the growing threat of DENV.

Introduction

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The dengue virus (DENV) belongs to the *Flaviviridae* family which comprises a group of positive-sense, single-stranded RNA viruses wrapped in an envelope (**Figure 1**). Within this family are four genera, and DENV finds its place in the *Flavivirus* genus, alongside other well-known pathogens such as Zika, Yellow Fever, West Nile, and Japanese Encephalitis viruses. Members of this genus share a common mode of transmission: they are vector-borne diseases spread through the bites of mosquitoes, particularly those of the *Aedes* genus.

DENV has become the most widespread mosquito-borne flavivirus,[1] and its reach continues to grow across the United States. Each year, an estimated 390 million people are infected worldwide, resulting in around half a million hospitalizations and 20,000 deaths, most of which occur in low-income tropical regions. Globally, 3.9 billion people remain at risk of infection, roughly half of the world's population. With no specific antiviral treatment available, the World Health Organization (WHO) has placed dengue vaccine development among its top global health priorities.

Remarkably, DENV now accounts for more annual infections than malaria or tuberculosis. In 2024 alone, the Americas and the Caribbean reported over 13 million cases, with long-lasting outbreaks occurring in Puerto Rico and the U.S. Virgin Islands, and sporadic cases appearing in parts of the U.S., such as California, Texas, Florida, Arizona, and Hawaii.[2] Unfortunately, rising global temperatures and increases in international travel and urbanization are only accelerating transmission. The growing threat was underscored when Puerto Rico declared a public health emergency in March 2024, during what would normally be its low-transmission season. Overall, DENV contributes to more than 1.14 million disability-adjusted life years (DALYs) lost each year which demonstrates its heavy burden on both health systems and economies.

DENV exists as four distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), each capable of infecting humans. Infections can range from asymptomatic or mild flu-like illness to severe and life-threatening disease. In its most dangerous forms, DENV can damage the blood and blood vessels, leading to coagulopathy (impaired clotting) and plasma leakage, a hallmark of

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dengue hemorrhagic fever (DHF). When this progresses further, patients may develop dengue shock syndrome (DSS), a critical condition marked by circulatory collapse and hypovolemic shock. Most cases of dengue fever (DF) are self-limiting, with a low mortality rate (<1%) when treated promptly. However, severe forms such as DHF and DSS can be deadly, with mortality rates of 2–5% even with treatment, and up to 20% without.[3]

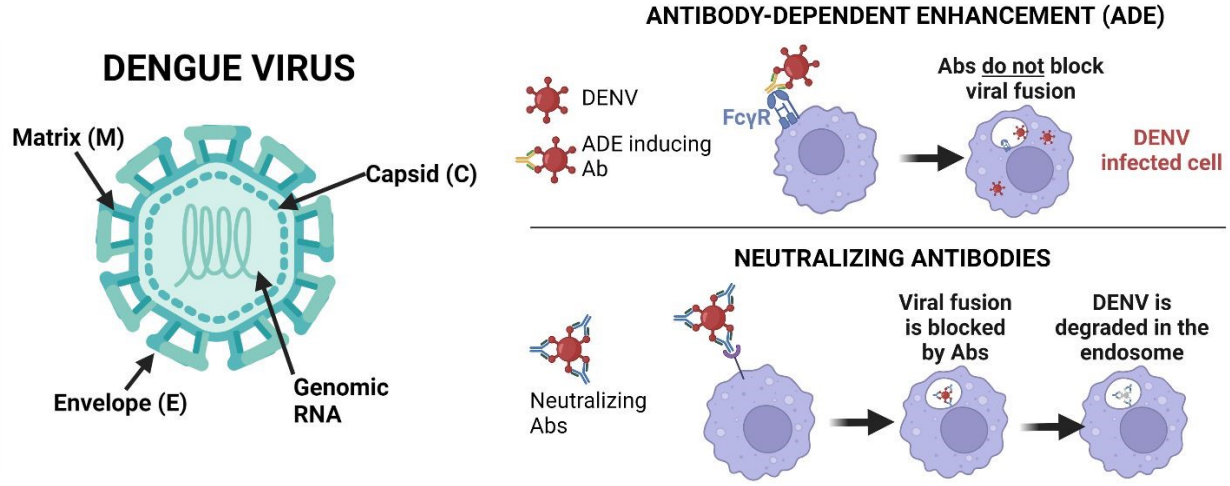

Figure 1. Schematic DENV with its structural proteins and antibody-dependent enhancement (ADE) with DENV infection. Ab = antibody

Infection from DENV is particularly dangerous because it circulates as four distinct serotypes and infection with one serotype provides only transient cross-protection, and a subsequent infection with a different serotype can trigger antibody-dependent enhancement (ADE; **Figure 1**).[4] ADE occurs when non-neutralizing antibodies from a prior infection facilitate viral entry into immune cells via Fcγ receptors, leading to increased viral replication, elevated viremia, and an exaggerated inflammatory response. This process is a key driver of severe DF manifestations, including DHF and DSS, highlighting the urgent need for a vaccine that can elicit balanced, durable protection against all four serotypes.

In DENV vaccine development, a detailed understanding of the viral structural proteins is critical for informed antigen selection. The DENV genome encodes three structural proteins (capsid (C), precursor membrane (M), and envelope (E)) in addition to seven nonstructural

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proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (**Figure 1**). Among these, the E glycoprotein is of particular importance, as it mediates viral attachment and membrane fusion, and represents the primary target for neutralizing antibodies and vaccine design.[5, 6]

DENV-infected cells naturally produce subviral particles (SVPs) composed of the M and E proteins but lack genomic RNA. Expression of M–E alone is sufficient for SVP assembly, secretion, and release.[7] Compared with native virions, SVPs are smaller in size and display approximately 30 E protein dimers, rather than the 90 present in infectious particles. Despite this reduction, SVPs retain the authentic E protein conformation on their surface, rendering them an attractive noninfectious subunit antigen for vaccine development.[8, 9] Notably, SVPs can be efficiently generated from a single plasmid encoding an M/E expression cassette, providing a practical platform for recombinant vaccine production.[10]

Several antibody and antiviral therapies for DENV are currently under development. In the antibody space, the Serum Institute of India has partnered with the Drugs for Neglected Diseases initiative (DNDi) to develop a monoclonal antibody targeting all four DENV serotypes, with Phase III trials underway in countries like India, Brazil, and Thailand.[11] Additionally, AbViro has developed a human monoclonal antibody designed to neutralize DENV (NCT06799741). On the antiviral front, Johnson & Johnson's JNJ-1802 has shown efficacy in preclinical studies and is progressing into Phase II clinical trials for the prevention and treatment of DENV[12, 13]. Similarly, NITD-688, developed through a collaboration between the University of Texas Medical Branch and Novartis, has demonstrated potential in halting DENV infection by targeting the virus's replication mechanisms[14]

Developing effective DENV therapeutics faces multiple challenges. ADE is a major concern and targeting non-structural proteins such as NS1 or non-virion epitopes may reduce this risk. Early administration of both antibodies and antivirals is critical, as delayed treatment is less effective once severe disease develops. However, this is challenging since often DENV infection

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is misdiagnosed as influenza or zika. Antibody therapeutics must provide potent, durable, and broadly neutralizing activity across all four serotypes, which is a demanding benchmark given the virus's genetic diversity. Cost and accessibility continue to remain major barriers to implementation, particularly for monoclonal antibodies in low- and middle-income countries where the disease is endemic. Adding to this, safety must be ensured across diverse populations, including seronegative individuals, children, and pregnant women, adding further complexity. Finally, regulatory approval requires demonstration of meaningful clinical endpoints, including reduction in viremia, prevention of severe disease, and mortality, ideally in real-world endemic settings. Collectively, these challenges underscore the need for effective preventative vaccines as the most sustainable strategy to combat DENV infection.

To date, three vaccines have led the field for prevention of DENV infection: Dengvaxia**,** QDENGA, and TV003/TV005 (**Table 1**). Dengvaxia, which uses non-structural proteins from attenuated yellow fever virus as its replication backbone and expresses the M and E proteins of all four DENV serotypes, initially demonstrated efficacy in Phase III trials and was licensed in several endemic regions. However, subsequent studies revealed an increased risk of severe disease in DENV-naïve individuals, prompting its discontinuation (CDC). QDENGA (Takeda), built on an attenuated DENV-2 backbone with chimeric M–E proteins from DENV-1, -3, and -4, has shown protection against DENV-2 but was withdrawn from FDA review in the United States. TV003/TV005, developed by the NIH and produced by the Butantan Institute, are live-attenuated tetravalent DENV vaccines designed to induce balanced immunity against all four serotypes in a single dose. Phase III data from Brazil (Butantan-DV) show promising efficacy (~80% overall) with protection in both seronegative and seropositive individuals, positioning it as one of the leading candidates for regulatory approval and broader deployment.[15] Together these vaccines are the primary preventative tool for DENV infection, but they rely on attenuated viruses that can replicate in the host and therefore pose potential safety concerns for vulnerable populations, including those living with HIV.[15, 16] In Nigeria, studies report that 44–62% of people living with HIV have

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detectable DENV antibodies, indicating prior exposure, which underscores the need for safer alternatives to live viral vaccines.[17, 18] Additionally, optimizing immune responses to minimize ADE (as observed with Dengvaxia) remains a key consideration for improving vaccine design. One promising approach is the use of synthetic or nanoparticulate carriers. Nanoparticulate carriers are advantageous due to their ability to allow precise control over antigen and adjuvant presentation, resulting in enhanced immune responses through directly targeting antigen presenting cells (APCs) or lymph nodes. Additionally, using nanoparticulate carriers can reduce the amount of antigen or adjuvant required to generate an effective immune response, leading to dose sparing that can reduce reactogenicity, and improve safety as well as overall cost.[19]

Table 1. Historically and currently available DENV vaccines and DENV vaccines in clinical trials.

Vaccine	Manufacturer	Antigen/Adjuvant	Date available	Shortcomings	Ref.
Dengvaxia	Sanofi Pasteur	Attenuated yellow fever virus backbone/expresses M & E proteins of all serotypes	2015-2024	Increased risk of ADE in DENV-naïve individuals	20
QDENGA	Takeda	Attenuated DENV-2 backbone/expresses chimeric M & E proteins from DENV-1,3,4	2022-present	Poor efficacy against DENV-3 and 4 in naïve individuals	21
TV003/TV005	NIH/Butantan Institute	Live-attenuated tetravalent vaccine of all serotypes	Not yet available; under Phase III clinical trials	Long-term safety still unknown	22
PepGNP-Dengue	Emergex Vaccines	9 capsid and non-structural MHC I peptides conjugated to gold nanoparticles	Not yet available; under Phase I clinical trials	Small phase 1 clinical trial size limits extrapolation of results	23

DENV Animal Models

Preclinical evaluation of DENV vaccine candidates relies heavily on animal models that can recapitulate aspects of viral infection, immune response, and protection observed in humans.

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However, replicating the full spectrum of human DENV disease in animals remains a major challenge, as no single model perfectly mimics both viral replication and clinical manifestations. Among available systems, mouse models are the most widely used due to their accessibility, genetic tractability, cost, and the breadth of available immunological tools (**Figure 2**). Although immunocompetent mice, such as BALB/c and C57BL/6, are not naturally susceptible to DENV infection, they remain invaluable to study correlates of protection. These models are commonly used to evaluate both humoral and cellular immune responses, including neutralizing antibody titers (50% Plaque Reduction Neutralization Test [$PRNT_{50}$] and Focus Reduction Neutralization Test [FRNT] assays), IgG subclass titers, T-cell proliferation, cytokine production with antigen recall (ELISpot, ELISA), and the generation of $CD4^+$ and $CD8^+$ memory responses (flow cytometry). Despite the absence of robust viral replication, such models provide critical insights into the immune mechanisms stimulated by vaccine antigens and adjuvants.

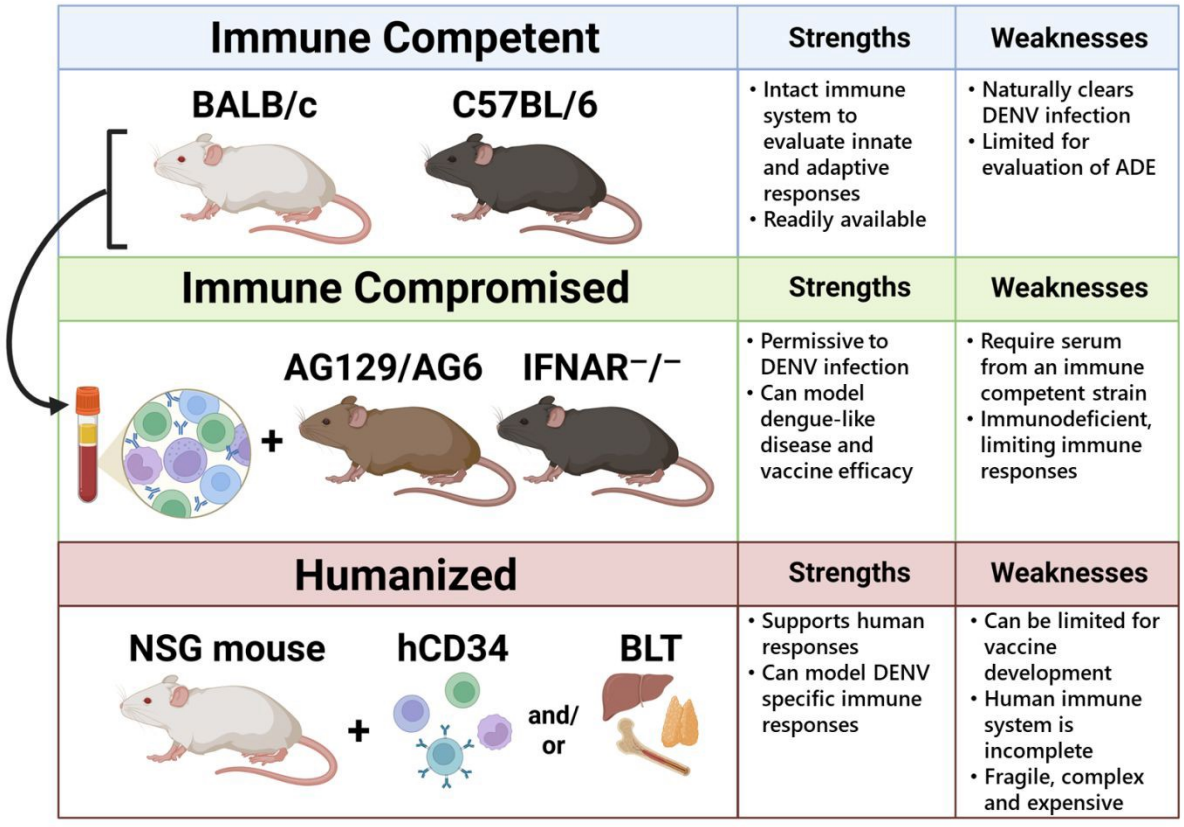

Figure 2. Mouse models used for evaluation of DENV vaccines.

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To more effectively evaluate protective responses elicited by DENV vaccines, several immunocompromised mouse models have been developed. The primary models used are AG129 and IFNAR$^{-/-}$ mice, both of which lack functional interferon receptors, a key component of antiviral defense, rendering them highly susceptible to DENV infection.[24] AG129 mice are the most widely used for DENV vaccine studies, as they lack both the interferon-α/β receptor (IFN-α/βR) and the interferon-γ receptor (IFN-γR), allowing efficient viral propagation. Whereas AG129 mice are on the 129/Sv background, AG6 mice are on the C57BL/6 background and also lack both IFN-α/βR and IFN-γR.[25] Similarly, IFNAR$^{-/-}$ mice have a disrupted interferon signaling system, permitting DENV infection and replication. To assess viral clearance and vaccine-mediated protection in these models, serum and/or immune cells from immunocompetent donor mice (e.g., C57BL/6) are transferred into infected immunocompromised recipients. Following transfer, the recipient mice can be challenged with DENV and monitored for survival (using body condition scores, weight loss, or other clinical indicators), as well as viral burden and immune responses, which can be quantified via viral RNA titers in tissues or immunohistochemistry.

Humanized mice are also used to evaluate DENV vaccines. To facilitate DENV infection, these mice are engrafted with human cells or tissues. In the development of a humanized mouse model for DENV infection, typically non-obese diabetic (NOD)-scid IL2Rγ^null (NSG) mice are used. Specifically, these mice lack functional T cells, B cells, and natural killer (NK) cells. This profound immunodeficiency makes them ideal for engraftment of human hematopoietic stem cells (HSC) or tissues which in turn allow for the development of a humanized immune system. One such model is the hCD34 model wherein hCD34^{+} HSC–engrafted mice are generated by transplanting human CD34^{+} stem cells into NSG recipients.[26] This leads to the development of human T cells, B cells, and myeloid lineages capable of mounting human-like immune responses. Similarly, BLT (bone marrow–liver–thymus) mice are created by implanting human fetal liver and thymus tissues along with CD34^{+} HSCs into NSG mice, producing a more complete and functional

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human immune system, including thymically educated T cells.[27] With DENV infection, these humanized mice strains will express human cytokines that can be evaluated as well as develop clinical symptoms of the disease and virus-specific immune responses. Despite their utility, humanized mice have several limitations, including high cost, an incomplete single-species immune system, fragility, and donor-to-donor variability, all of which can constrain their experimental use.

To bridge the gap between mouse models and human infection, non-human primates (NHPs) are also used to evaluate DENV vaccine formulations. Most commonly, rhesus macaques and cynomolgus monkeys are used as their immune system closely matches that of humans. In the NHP model, virus load (e.g. viremia), humoral responses (e.g. antibody titers, $PRNT_{50}$ and FRNT) and cellular responses (e.g. T cell activation) can be directly measured, most often through serum collection. While NHPs are valuable for evaluating DENV vaccines, they have limitations, including high cost, limited availability, and mild or subclinical disease that does not fully replicate human DENV infection.

Nanoparticle Formulations

Nanoparticulate carriers offer distinct advantages for DENV vaccine development by better balancing serotype antigenicity compared to soluble agents,[28] activating complement for enhanced viral clearance,[29] and facilitating cross-presentation, which can increase $CD8^+$ T cell responses critical for viral clearance.[30-32] Nanoparticles can also co-deliver adjuvants to skew the immune response toward a protective helper-type 1 (Th1) profile. DHF is strongly associated with a shift from a Th1- to a Th2-biased immune response, and higher levels of Th2 antibodies during secondary infections correlate with worsened disease outcomes, including enhanced ADE.[33-38] Therefore, effective DENV vaccines must induce a Th1-skewed immune response to protect against DHF. While live-attenuated viral vectors naturally provide Th1-promoting adjuvant activity,

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their safety limitations restrict widespread use, making particulate carriers with incorporated adjuvants an attractive alternative for subunit vaccines capable of eliciting balanced humoral and cellular immunity.

Nanoparticle formulations also offer the benefit of providing enhanced protection for younger and older populations, with both populations at an increased risk of DENV infection and severe disease.[42, 43] Children are particularly susceptible to developing severe disease due to having greater vascular permeability than adults, while older populations are more susceptible due to general age-related immune decline, other comorbidities, and lack of available healthcare.[42, 44-46] Additionally, the immune systems of infants is often immature, with newborns having active Th2 responses, yet deficient Th1 responses for the first several months of life.[47, 48]This is especially concerning in relation to DENV infection, as the active Th2 response can increase the risk for DSS and DHF. Nanoparticle formulations can be specifically designed to deliver Th1-biased adjuvants to enhance the Th1 immune responses in infants, leading to improved protection against DENV and other intracellular pathogens.[47] Similarly, older populations often suffer from poor cellular responses due to immunosenescence and thymic involution, where their immune systems begin to decline with age leading to a gradual decline in T cells.[49] By including adjuvants in the formulation, specifically those that are Th1-biased, older populations may also experience enhanced cellular immune responses against DENV.[50]

Several nanoparticle carriers have been developed to enhance DENV vaccine delivery and immune responses including those comprised of polymers, lipids, inorganic biomaterials and engineered protein constructs (**Tables 2A-B**). Polymeric nanoparticles have been used in the formulation of DENV vaccines, offering enhanced stability and controlled release compared to many other formulations.[28, 29, 32, 51-56] For DENV vaccine formulations, lipid-based nanoparticles include some of the first FDA-approved lipid systems, such as liposomes, as well as mRNA lipid nanoparticles (LNPs) that build on the success of recent LNP-based vaccines.[57-61] A diverse range of inorganic, metal-based biomaterials have also been explored in DENV vaccine development,

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including gold nanoparticles, calcium phosphate particles, and carbon nanotubes. Each provides a distinctive platform capable of covalently binding or adsorbing vaccine components.[23, 62, 63] Also, engineered protein-based carriers have emerged as powerful delivery systems. These include ferritin nanoparticles and virus-like particles that self-assemble into multivalent structures displaying modified DENV proteins, as well as polymeric IgG.

Table 2A: Nanoparticle formulations that have been used to evaluate pre-clinical DENV vaccines.

Material	Description	Antigen/Adjuvant	Outcome	Ref.
Polymeric				
Poly(lactic-co-glycolic acid) (PLGA)	80×320nm PLGA rods with protein absorbed to the surface	E protein	NP formulated vaccine had higher levels of neutralizing titer	28
Chitosan	Chitosan nanoparticles encapsulating UV inactivated DENV	Nonstructural protein 1	Increased humoral and cellular responses	32
Poly(butadiene)-Poly(ethylene oxide) Polymersomes	Amphiphilic block copolymer vesicles carrying DENV peptides and adjuvant	B cell and T cell E protein epitopes with MPLA	Strong IgG and Th1 responses	52
Polycaprolactone (PCL)	Cationic, pH-responsive nanoparticles for intranasal delivery of tetravalent antigens	Quadrivalent envelope domain III (EDIII) antigen	Induces humoral and cellular immunity when given intranasally	53
Lipid-based				
Liposomes	Liposome with cobalt in the membrane to bind to HIS-tag on proteins	Tetravalent stabilized E protein	Enhanced antigen uptake and Th1 responses	58
mRNA LNP	Potential mRNA or antigen delivery	Modified E protein	A strong humoral and protective response was generated	59
Inorganic				
Calcium Phosphate	A pool of 6 MHCI class associated peptides were absorbed on the surface of the CaP nanoparticles	Peptides from capsid and Non-structural proteins (NS4B, NS5, NS2A) N-acetylglucosamine (GlcNAc) was used as an adjuvant	Increase cellular responses	62
Carbon Nanotubes	E protein was covalently bound to multi-walled carbon nanotubes	DENV-3 E protein	Equivalent levels of antibodies to soluble protein was observed, but a higher level of neutralizing titers was noted with the carbon nanotube formulation	63
Protein Based Particles				
Ferritin	Four sequences of E protein were expressed on the surface of ferritin	E proteins were modified in the DII region that induces ADE and adjuvanted with alum, RS09 or c-di-GMP	Produced a significant humoral, cellular and protective response	64
VLP	A VLP with the E protein modified in the fusion loop with precursor membrane (prM)	A quadrivalent VLP with each serotype adjuvanted with alum	Produced high levels of serum IgG and neutralizing titers in non-human primates and NHP transferred serum protected mice from challenge	65
Polymeric IgG	DIII E protein is in variable region of IgG that is presented as a dendritic IgG array	Adjuvanted with alum	The addition of alum lead to increased antibody titers, neutralization and IFNγ production with antigen recall.	66

Table 2B: Nanoparticle formulations that have been used to evaluate clinical DENV vaccines.

Material	Description	Antigen/Adjuvant	Outcome	Ref.
Inorganic				
Gold Nanoparticles	Conjugated with nine DENV peptides	Capsid and non-structural MHC I peptides were used	Phase I peptide vaccine trial that show moderate cell responses	23

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scaffolds functionalized to present viral antigens. Collectively, these innovative formulations have demonstrated enhanced vaccine delivery and protection in animal models.

Polymeric formulations

For vaccine formulation, polymeric carriers can be used to encapsulate antigen and adjuvant within the matrix of the biopolymer as well as having the agents absorbed to the surface or covalently attached. Polymeric carriers offer several distinct advantages over lipid-based, inorganic, and protein-based systems for DENV vaccine delivery. Fundamentally, encapsulation within polymeric matrices protects labile antigens from enzymatic degradation and premature clearance, improving vaccine in vivo longevity to allow controlled release of encapsulated antigen and adjuvant. Their inherent stability facilitates long-term storage, especially when prepared as lyophilized formulations. Further, their potential for enhanced thermostability can mean a reduced reliance on cold chain logistics, which is an important consideration for DENV-endemic regions.[67, 68] Additionally, polymeric nanoparticles can be produced through scalable and reproducible techniques such as spray drying, enabling large-scale manufacturing under Good Manufacturing Practice (GMP) conditions. Another advantage of polymeric formulations is the wide variety of biocompatible and biodegradable polymers available, enabling precise tuning of physicochemical and immunological properties to achieve desired characteristics such as acid sensitivity and controlled antigen or adjuvant release. Importantly, polymeric carriers can co-formulate both antigen and adjuvant within the same particle, enhancing co-localized delivery; however, several studies have shown that separate formulation or compartmentalized delivery of these components can sometimes elicit stronger or more balanced immune responses.[69-72] Compared to inorganic carriers that may induce non-specific inflammation or persist in tissues, polymeric systems are often biodegradable and well-tolerated, offering a safer and more tunable platform. Nonetheless, challenges remain, including the potential for anti-carrier immune responses, such as the development of anti-PEG antibodies, which can affect biodistribution, effectiveness, and safety of vaccine boosts. Moreover, despite extensive preclinical success, no polymeric vaccine formulation has yet received regulatory approval, highlighting the need for continued optimization and clinical validation. Collectively, these features position polymeric nanoparticles as a promising yet still emerging platform for the development of stable, effective, and scalable DENV vaccine formulations. Several different constructs have been evaluated pre-clinically as a DENV vaccine including emulsion particles, particle replication in nonwetting

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templates (PRINT) particles and polymersomes (**Figure 3**).

MATERIAL	CONSTRUCT	STRENGTHS	WEAKNESSES
Polymer	PRINT PLGA Nanoparticles	• Polydispersity of 1 • Flexible for a variety of antigen and adjuvants	• Challenging scale-up • PLGA degrades into acidic by-products
	PCL or Chitosan Nanoparticles	• Ease of fabrication and engineering in the lab setting	• Emulsion denatures protein • PCL degrades into acidic by-products • Challenging scale-up
	Polymersomes	• Flexible encapsulation of hydrophilic/phobic cargo • Ease of engineering	• Reproducibility can be challenging • Difficult scale-up and quality control
Lipid	Liposomes	• FDA approved platform • Flexible encapsulation of hydrophilic/phobic cargo • Ease of engineering	• Reproducibility can be challenging • Difficult scale-up and quality control
	mRNA Lipid Nanoparticles	• FDA approved platform • Scalable production • Ease of engineering	• mRNA is cost-limiting • Extensive cold-chain storage • Public skepticism may hinder mRNA-LNP uptake
Inorganic	Calcium Phosphate Nanoparticles	• Naturally occurring mineral in the body • Low toxicity	• Stability can alter with pH • Reproducibility and scale-up can be challenging
	Carbon Nanotubes	• High surface area for functionalization of antigen or adjuvant	• Biocompatibility and toxicity concerns • Manufacturing reproducibility can be difficult to control
	Gold Nanoparticles	• Established surface chemistry • Highly tunable size • Good stability	• Biocompatibility and long-term clearance concerns • Manufacturing can be complex and expensive
Protein	Ferritin	• Controlled spacing of protein on particle surface • Good biocompatibility • Modular scaffold	• Antigen geometry is critical • Manufacturing and purification can be challenging
	Virus-like Particles	• FDA approved platform • Can present multivalent antigen	• Requires adjuvant co-delivery • Manufacturing and characterization can be challenging
	Polymeric IgG	• Highly ordered and repetitive format	• Less established platform • Manufacturing and characterization can be challenging

Figure 3. The various nanoparticle constructs that have been evaluated as DENV vaccine

Poly(lactic-co-glycolic acid) (PLGA)

PLGA is one of the most extensively studied biodegradable polymers for vaccine delivery due to its established biocompatibility, safety, and regulatory approval. It is used in several FDA-approved drug delivery systems, including Risperdal Consta® and Lupron Depot®, which leverage its ability to provide sustained and

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controlled release of encapsulated therapeutics. These same properties have made PLGA an attractive platform for vaccine applications, as it can encapsulate and gradually release antigens and adjuvants to enhance immune activation over time. Notably, the World Health Organization (WHO) developed a PLGA-nanoparticle tetanus vaccine that demonstrated promising immunogenicity; however, it was ultimately limited by high production costs, acidic degradation byproducts within the polymer matrix that destabilized the antigen, and manufacturing challenges that hindered large-scale deployment.[73]

As a vaccine platform, PLGA offers several strengths: it enables prolonged antigen release, protection of labile biomolecules from enzymatic degradation, and tunable degradation kinetics by adjusting lactic-to-glycolic acid ratios or polymer molecular weight. However, PLGA also presents notable weaknesses, including the potential for antigen denaturation during encapsulation (due to exposure to organic solvents or shear stress), the aforementioned acidic microenvironments generated during polymer hydrolysis that can also denature the encapsulate, and batch-to-batch variability in particle size and release kinetics. Additionally, its manufacturing scalability remains more complex compared to lipid or protein-based systems. Despite these challenges, PLGA remains a leading polymeric carrier platform in preclinical vaccine development, serving as a benchmark for next-generation biodegradable nanoparticle formulations aimed at achieving controlled, safe, and durable immune responses as a DENV vaccine.[28, 51]

One platform that overcomes some of the limitations of other PLGA nanoparticles vaccines has been PRINT nanoparticles. In contrast to most PLGA nanoparticle formulations that use emulsion or similar technology to generate the carrier, PRINT technology uses PLGA cast into a nanoparticle mold comprised of a highly hydrophobic polymer, allowing small particles to be formed.[74] This formulation has previously been evaluated clinically as an adjuvant to an inactivated virus influenza vaccine (#NCT01224262). To mitigate the potential antigen denaturation associated with conventional emulsion-based particle fabrication, the DENV E protein was adsorbed onto 80 × 320 nm PRINT-fabricated PLGA rods to generate the vaccine formulation.[28] A quadrivalent formulation with four different DENV serovar protein E absorbed on the rods, was given to BALB/c mice at 0, 21 and 63 days through a subcutaneous (S.C.) injection. The nanorod formulated vaccine elicited total IgG titers that were significantly different from protein alone, but not significantly different from the alum adjuvant control. However, in the evaluation of the neutralizing titers in serum collected 16 weeks after vaccination, the nanorod formulation elicited a 50% neutralization that was significantly greater than the soluble protein for DENV-1,

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DENV-2, and DENV-4, and was also significantly different compared to the alum control for DENV-1 and DENV-4 (**Figure 4**). In summary, the PRINT-fabricated PLGA nanorod vaccine represents an innovative approach that avoids antigen denaturation associated with emulsion-based methods and elicits robust, serotype-specific neutralizing antibody responses against multiple DENV serovars, outperforming soluble protein formulations and, for select serotypes, even the alum-adjuvanted control.

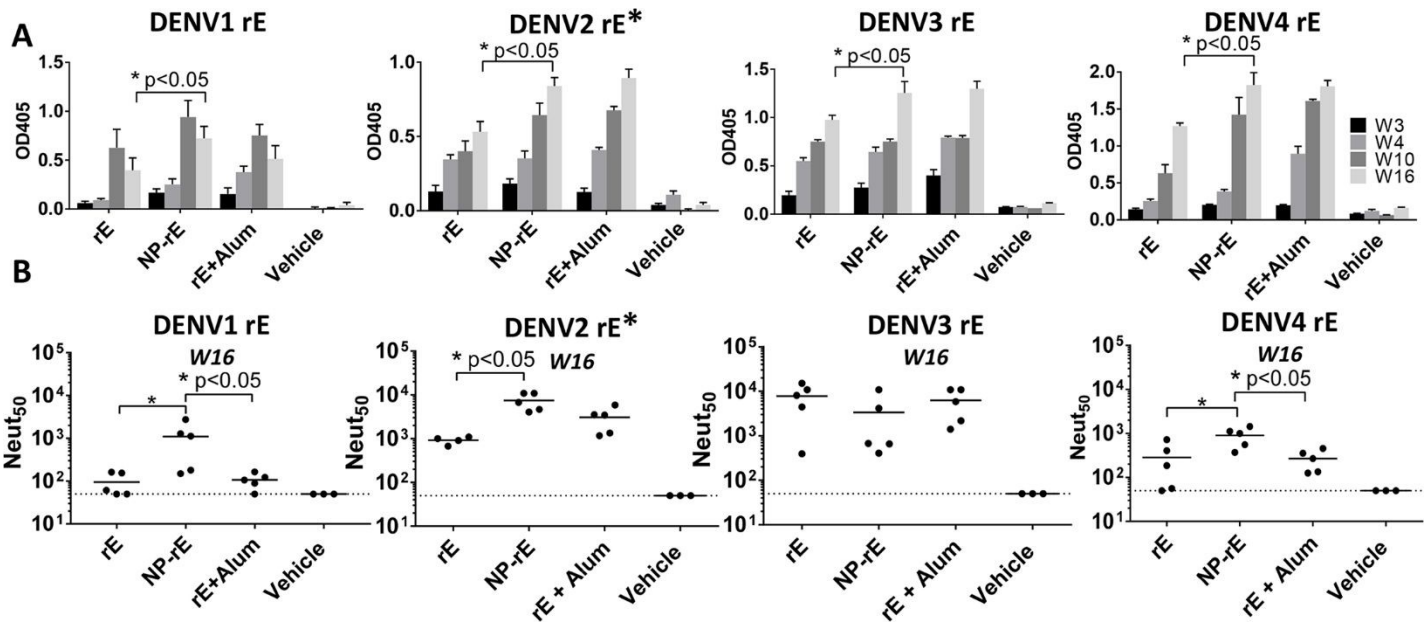

Figure 4. PRINT particles had DENV E protein from each serovar individually absorbed on the surface (5μg/each) and BALB/c mice were vaccinated on day 0, 21 and 63. rE is recombinant protein; NP-rE is the nanoparticle formulation; rE + alum is the positive control with alum; and vehicle is the PLGA nanorods alone without protein. (A) DENV1–4-specific IgG titers were measured at weeks 3, 4, 10, and 16. (B) Neutralizing activity of sera collected at week 16 was assessed using a virus neutralization assay, in which DENV was incubated with serially diluted mouse sera prior to infection of Vero cells. Neutralizing potency is reported as the serum dilution that achieved 50% viral neutralization ($Neut_{50}$). Reproduced from Metz et al. (*PLOS Neglected Tropical Diseases*, 2018)[28] published under a Creative Commons Attribution License (CC BY 4.0).

Polycaprolactone (PCL)

PCL is a biodegradable polyester that has garnered increasing attention as a vaccine delivery platform, including for DENV vaccine development. PCL is characterized by its hydrophobicity, and slow degradation rate, which together enable sustained antigen release over extended periods. Upon hydrolysis, PCL degrades into 6-hydroxycaproic acid, a non-toxic metabolite that enters normal metabolic pathways, contributing to its excellent biocompatibility and safety profile. Compared to other polyesters such as PLGA, PCL exhibits slower degradation kinetics and generates less acidic byproducts, reducing the risk of antigen destabilization within the carrier matrix.[75] PCL comprises FDA-approved Monocryl®, a bioresorbable suture material. Like PLGA, PCL can

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encapsulate or adsorb both hydrophilic and hydrophobic molecules, which makes it an attractive biomaterial for DENV vaccine formulations, particularly for applications requiring prolonged antigen exposure.

To enhance the performance of PCL as a vaccine carrier, hydrazine-modified PCL was developed to introduce acid sensitivity and a cationic surface charge for improved delivery of a DENV vaccine.[53] When nanoparticles are internalized by APCs such as dendritic cells, the phagosomal environment undergoes a natural pH drop to approximately 5, which can be exploited to trigger antigen release. Acid-sensitive polymers can therefore facilitate endosomal escape and enhanced antigen presentation to T cells, which is an advantageous property for vaccine applications.[30] The incorporation of hydrazine also imparts a positive surface charge, improving interaction with the negatively charged glycocalyx and mucosal surfaces, to promote mucoadhesion which is particularly relevant for mucosal vaccine delivery. Leveraging these properties, Vemireddy et al. formulated hydrazine-modified PCL nanoparticles encapsulating DENV envelope domain III (EDIII) proteins for intranasal (I.N.) administration. This route offers practical benefits over injection-based vaccination, including simplified delivery, needle-free administration, and reduced costs, providing key advantages in resource-limited, DENV-endemic regions.[76] A quadrivalent formulation containing EDIII antigens from all four DENV serotypes was administered intranasally to BALB/c mice on days 0 and 14. Interestingly, nanoparticles with higher degrees of hydrazine substitution elicited increased total serum antibody titers but lower IFN-γ responses upon antigen recall from splenocytes compared to unmodified PCL nanoparticles, suggesting that PCL–hydrazine nanoparticles preferentially enhanced humoral immunity without a corresponding increase in cellular responses. Continued optimization of PCL-based vaccine platforms, especially those incorporating stimuli-responsive or mucoadhesive functionality, may advance the development of stable, cost-effective, and broadly deployable DENV vaccines suited for endemic regions.

Chitosan

Chitosan is a naturally derived polysaccharide isolated from crustacean shells via the deacetylation of chitin. Its biocompatibility, biodegradability, and intrinsic mucoadhesive properties make chitosan particularly attractive for mucosal vaccine applications. The cationic nature of chitosan enables strong electrostatic interactions with the negatively charged mucosal surfaces and glycocalyx, improving residence time and facilitating antigen transport across epithelial barriers. Additionally, chitosan can act as an immunostimulatory

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adjuvant, promoting dendritic cell activation and the induction of both humoral and cellular immune responses.[77] When formulated into nanoparticles, chitosan offers controlled release capabilities, protection of encapsulated antigens, and compatibility with a range of antigens and adjuvants. These attributes have positioned chitosan-based nanocarriers as an appealing platform for needle-free, mucosal DENV vaccines,[29, 54-56] offering potential for enhanced immunity and improved accessibility in resource-limited, DENV-endemic regions.

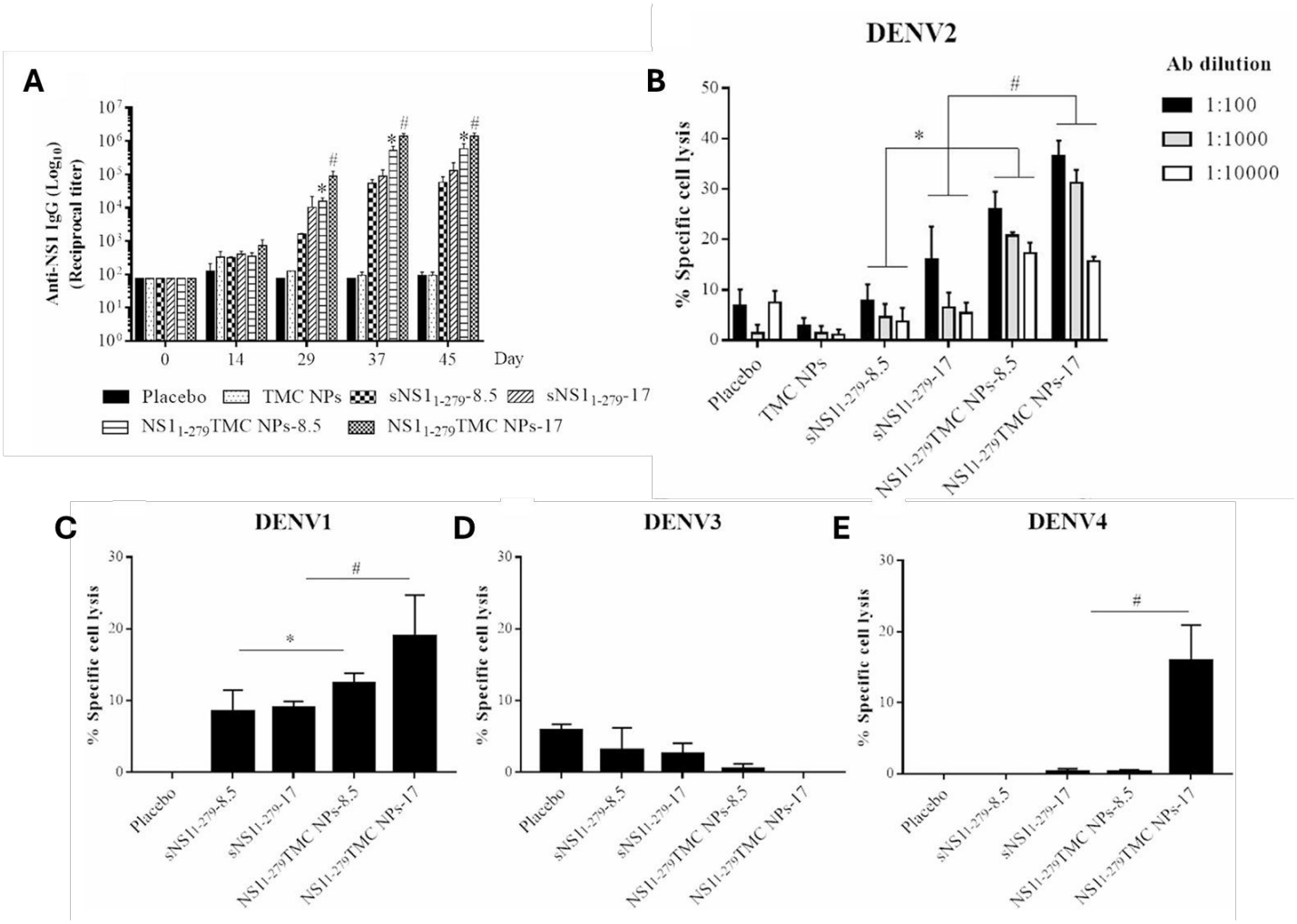

Figure 5. Mice were immunized with 8.5 or 17 μg per dose of either soluble NS1(sNS1$_{1-279}$) or NS1-loaded TMC nanoparticles (NS1$_{1-279}$ TMC NPs). (A) Anti-NS1 total IgG titers were quantified from sera collected on days 0, 14, 29, 37, and 45 post-immunization. (B–E) Complement-dependent cytotoxicity (CDC) activity of NS1-specific antibodies was assessed using DENV-infected BHK-21 cells. Cells were infected with DENV for 24 h and incubated with tenfold diluted pooled sera (for DENV-2; panel A) or 1:100 diluted sera (for DENV-1, -3, and -4; panels C–E) for 2 h at 37 °C, followed by addition of rabbit complement. Lactate dehydrogenase (LDH) release in culture supernatants was measured to determine CDC activity. Data represents the mean ± SD of three independent experiments. *, # denote statistically significant differences between sNS1 and NS1 TMC NP groups at 8.5 and 17 μg/dose, respectively ($p < 0.05$). Reproduced from Jearanaiwitayakul et al.[32] with permission from *Vaccine*, copyright 2020.

While most DENV vaccine formulations employ the E or M proteins as antigens due to their essential roles in viral assembly and infectivity, Jearanaiwitayakul et al.[32] explored an alternative approach using non-

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structural protein 1 (NS1), a highly conserved glycoprotein expressed during viral replication.[78] To deliver this antigen, NS1 was encapsulated within N,N,N-trimethyl chitosan (TMC) nanoparticles prepared via ionic gelation, a mild, charge-driven process in which cationic polymer and anionic crosslinker solutions are combined dropwise to form nanoparticles. BALB/c mice were immunized intraperitoneally (I.P.) with 8.5 or 17 μg of $NS1_{1-279}$ in either soluble or nanoparticle form on days 0, 15, and 30. Vaccination with the nanoparticle formulation induced significantly higher total IgG titers compared to the equivalent soluble NS1 doses (**Figure 5A**). Moreover, in assays assessing complement-dependent cytotoxicity (CDC) against DENV1–4-infected cells, sera from mice immunized with DENV-2 NS1-loaded TMC nanoparticles demonstrated the broadest cytotoxic activity, particularly against homotypic DENV-2 and heterotypic DENV-1 and DENV-4 strains (**Figure 5B–E**). In this assay, antibodies generated from vaccinated mice activate the complement cascade to lyse infected cells; however, protection against DENV-2 infection in vivo was not achieved with the nanoparticle formulation which may impact overall protection and enhanced ADE with this serovar. Overall, this study highlights NS1 as a promising, conserved antigen candidate for DENV vaccination and demonstrates the potential of TMC nanoparticles to enhance humoral responses, though further optimization is needed to achieve protective efficacy.

Poly(butadiene)- Poly(ethylene oxide) Polymersomes

Polymersomes are synthetic vesicular nanostructures formed from amphiphilic block copolymers, which self-assemble in aqueous environments into bilayer membranes analogous to liposomes (**Figure 3**).[79] Unlike lipid-based vesicles, polymersomes exhibit enhanced mechanical stability, tunable membrane thickness, and prolonged circulation times, making them attractive for biomedical and vaccine applications.[80] These enhanced features are afforded by the broad range of amphiphilic polymers that can be constructure with defined properties such as acid-sensitivity, and PEGylation to enhance circulation times. Their architecture enables the encapsulation of hydrophilic molecules (e.g. antigens, nucleic acids, or adjuvant) within the aqueous core, while hydrophobic compounds can be integrated into the polymer bilayer, allowing for versatile and controlled delivery systems. Among various block copolymers explored, poly(butadiene)-poly(ethylene oxide) (PBD–PEO) has been one of the most extensively characterized. The hydrophobic PBD block provides membrane robustness and flexibility, whereas the hydrophilic PEO block imparts steric stabilization and biocompatibility in physiological

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media.

PBD-PEO polymersomes have been used for the formulation of DENV peptides as a vaccine.[52] For this study, E protein epitopes were lipidated for incorporation in the polymersome shell. B cell ($E_{383-397}$: EPGQLKLNWFKKGSS), T cell ($E_{345-359}$: RHVLGRLITVNPIVT) and helper-T cell (Th) ($E_{352-368}$: LITVNPIVTEKDSPVNIE) epitopes had two copies of palmitic acid (C16) covalently attached to each peptide through the N-terminus and the sidechain of lysine. It was the intention of the researchers that this polymersome construct with incorporated peptides would then closely mimic the presentation of these proteins observed on the surface of DENV. However, one of the main challenges associated with using peptides rather than whole proteins as vaccine antigens is the variability in human leukocyte antigen (HLA) types among individuals, which necessitates the identification of multiple peptides capable of binding diverse HLA alleles. In addition, for a DENV vaccine, distinct peptide epitopes would be required for each viral serotype, further complicating vaccine design. Nonetheless, the polymersome formulation provides an excellent proof of concept platform for evaluation of this multiple-peptide E protein vaccine. To enhance the adjuvanticity of the peptides, toll-like receptor 4 (TLR4) agonist, monophosphoryl lipid A (MPLA) was used, as it's known to intercalate into bilayers such as with liposomes in adjuvant system 01 (AS01). For the study, BALB/c mice were vaccinated S.C. on day 0, 10 and 20 with a rigorous panel of experimental and control groups, including lapidated peptides alone, with the vehicle, or with the gold standard animal adjuvant, Complete Freund's Adjuvant (CFA). Among these groups, the CFA-adjuvanted group slightly outperformed the polymersome/MPLA/Peptide group in total serum IgG from day 27, however CFA is too potent for human use, illustrating the robust response generated by the adjuvanted polymersome formulation. The CFA group is not reported for antigen recall data, but IFN-γ is significantly higher for the adjuvant polymersome group compared to the other groups evaluated. Overall, the authors report an advanced pre-clinical DENV vaccine platform for eliciting a robust humoral and cellular response in mice.

Translational feasibility

Currently, PLGA and PCL are the only FDA-approved polymers that have been evaluated in pre-clinical DENV vaccines as delivery vehicles.[81, 82] While not FDA-approved, chitosan is a natural product and is widely considered Generally Regarded as Safe (GRAS) for use in the food industry. These approvals and labels are one of the first major hurdles that a prospective polymeric DENV vaccine must overcome to continue advancing towards the clinic. As described, polymers may suffer from high batch-to-batch variability when being

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synthesized, which can further complicate production, as a high degree of variability could lead to significant differences in immune responses between recipients. In order for polymers such as PLGA, PCL, and chitosan to be considered for future use in DENV vaccines, further research is needed to develop reliable and inexpensive means of large-scale manufacturing, while also continuing to characterize and evaluate potential antigen and adjuvant systems that may be incorporated into these polymers.

Lipid formulations

Lipid-based delivery systems have emerged as one of the most versatile and clinically validated synthetic platforms for vaccine development, with applications extending from traditional liposomes to mRNA LNP formulations used in COVID-19 vaccines (**Figure 3**). Liposomes are spherical vesicles composed of one or more phospholipid bilayers that can encapsulate both hydrophilic and hydrophobic molecules, offering flexibility in antigen and adjuvant delivery. Their biocompatibility and ability to mimic biological membranes have led to several FDA-approved drug formulations including Doxil® (doxorubicin liposomes) and AmBisome (amphotericin B liposomal). A liposomal hepatitis A vaccine (Epaxal®) was first licensed in 1994, approved in the European Union but discontinued in 2014.[83] More recently, mRNA LNPs have revolutionized nucleic acid vaccine delivery, as exemplified by the mRNA-based COVID-19 vaccines (Comirnaty® and Spikevax®), which use ionizable lipids to encapsulate and protect mRNA while facilitating cellular uptake and endosomal escape.

For DENV vaccines, lipid-based systems offer several advantages, including efficient antigen encapsulation, and protection from degradation. Their surface properties can be tuned for targeted delivery to APCs, and inclusion of immunostimulatory lipids or adjuvants can further enhance immunogenicity. However, challenges remain, including stability issues, manufacturing complexity, and the requirement for cold-chain storage, which limits use in resource-limited, DENV-endemic regions. Additionally, LNPs may induce reactogenicity or complement activation and anti-PEG responses, necessitating careful optimization of lipid composition and charge. Despite these challenges, the clinical success of lipid-based vaccines highlights their potential as adaptable, potent, and scalable platforms for future DENV vaccine development.[58-61]

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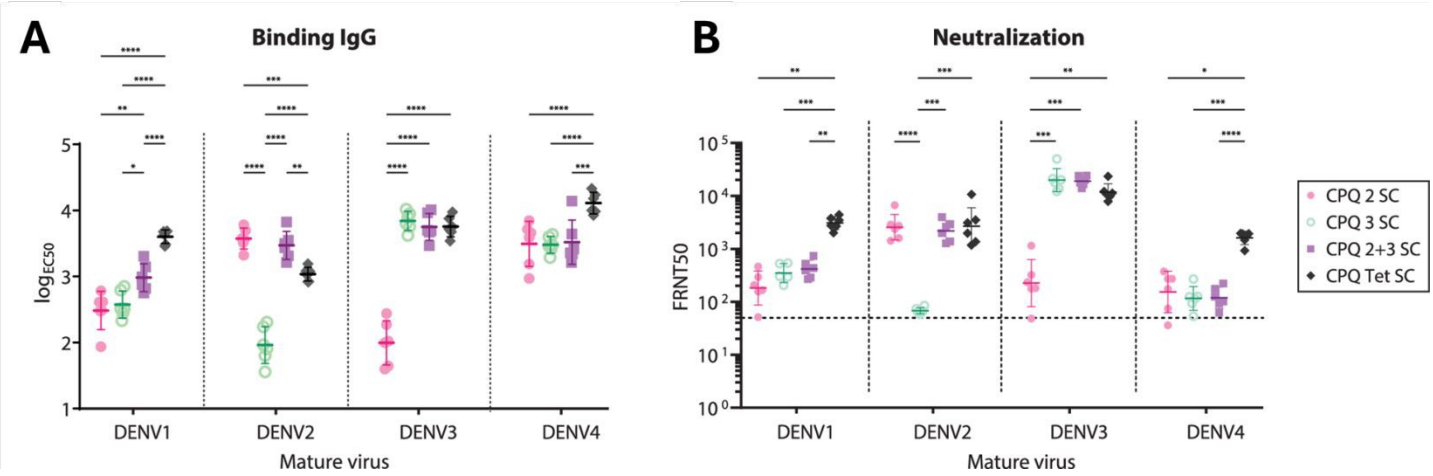

Figure 6. Mouse antibody response evaluation. Experimental groups included cobalt laden liposomes with monovalent DENV-2 or DENV-3 formulations (CPQ 2 SC, CPQ 3 SC), a bivalent DENV-2/DENV-3 formulation (CPQ 2+3 SC), and a tetravalent DENV1–4 formulation (CPQ Tet SC). Shown are placebo-subtracted binding (A) IgG titers and (B) neutralizing titers against mature DENV. The horizontal dotted line indicates the assay limit of detection. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). Reproduced from Phan et al. (*NPJ Vaccines*, 2025)[58] published under a Creative Commons Attribution License (CC BY NC ND).

Liposomes

A liposomal DENV vaccine formulation was developed incorporating stabilized E proteins from all four DENV serotypes.[58] In this approach, cobalt-containing liposomes were engineered to enable high-affinity binding between histidine-tagged antigens and cobalt ions within the lipid bilayer, facilitating the surface presentation of E proteins. While this design promotes efficient antigen display, cobalt's known cytotoxicity and genotoxicity at micromolar concentrations present a potential safety limitation.[84] In the study, BALB/c mice were immunized at weeks 0 and 4 with monovalent, bivalent, or tetravalent formulations. In a bivalent DENV-2/DENV-3 vaccine, stabilized E proteins elicited higher total IgG titers than their wild-type counterparts. Control formulations, including cobalt-free liposomes with soluble proteins or alum-adjuvanted proteins, generated significantly lower total IgG and neutralizing antibody levels than cobalt liposomes with stabilized E proteins. Notably, the tetravalent formulation induced strong total IgG and neutralization titers in day 84 sera, comparable to the bivalent formulations against their homologous serotypes (**Figure 6**). Collectively, these findings demonstrate that cobalt-based liposomal vaccines can effectively enhance humoral immune responses, highlighting their promise as a platform for DENV immunization despite safety considerations.

mRNA lipid nanoparticles (LNPs)

mRNA–containing LNPs have also been explored preclinically as a platform for DENV vaccination.[59] In

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one study, LNPs were formulated to deliver mRNA encoding either the wild-type DENV-2 E protein or a modified version containing a substitution in the N8 epitope to enhance cross-reactivity among serotypes. BALB/c mice were immunized intramuscularly (I.M.) on days 0, 14, and 28. Six weeks post prime immunization, sera from vaccinated mice exhibited robust anti-E total IgG titers, particularly against DENV-2, followed by DENV-1 and DENV-3, with minimal reactivity against DENV-4. Across all serotypes, the modified E protein elicited higher antibody titers than the wild-type construct, although neutralization titers were comparable between the two. To assess ADE, DENV-2 infection was evaluated using supernatants from K562 cells incubated with serially diluted sera from the immunized mice. The modified E protein induced antibodies that demonstrated neutralization levels similar to the mock control, while sera from wild-type E–immunized mice showed higher viral titers, indicative of enhanced ADE. In a passive transfer experiment, two-day-old BALB/c mice were inoculated with a mixture of DENV-2 and sera from vaccinated animals. Survival was monitored over 21 days, revealing higher survival rates in groups receiving sera from modified E–vaccinated mice compared to those given sera from wild-type or mock-vaccinated controls. Collectively, these findings highlight the potential of mRNA–LNP vaccines to induce strong and protective humoral immunity against DENV, while demonstrating that rational antigen modification may be key to minimizing ADE and improving vaccine safety across serotypes.

Translational feasibility

Lipid based formulations have a proven track-record of success when it comes to translating pre-clinical therapies to the clinic, as demonstrated by numerous FDA-approved lipid formulations and the recent success of the COVID-19 mRNA LNP vaccines. However, one of the most significant limitations to commercializing a DENV vaccine using a lipid formulation is the extensive cold chain requirements. DENV is predominantly endemic to tropical climates, particularly in resource-limited countries that do not possess the necessary healthcare and cold chain infrastructure. This is especially problematic considering that some lipid formulations, such as the Pfizer-BioNTech LNP COVID-19 vaccine, require storage at -20 °C.[85] While lipid based formulations have significant potential, additional research must be done to reduce the reliance on the cold chain, as well as cost-saving manufacturing practices.

Inorganic Formulations

Inorganic nanomaterials have been applied as platforms for DENV vaccine delivery due to their tunable

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physicochemical properties, stability, and capacity for multivalent antigen display. Several inorganic carriers that include calcium phosphate nanoparticles (CaP NPs), gold nanoparticles (AuNPs), and carbon nanotubes (CNTs) have been evaluated for DENV vaccine applications (**Figure 3**). Collectively, these materials can improve antigen stability, promote efficient delivery to APCs, and elicit strong humoral and cellular immune responses. Despite these advantages, several challenges limit the translational potential of inorganic-based DENV vaccines. While materials such as gold and calcium phosphate are FDA-approved for diagnostic or imaging applications, none have been approved for therapeutic or vaccine use, largely due to concerns regarding biocompatibility, long-term retention, and potential toxicity. CNTs have raised safety concerns related to inflammatory responses, oxidative stress, and bioaccumulation. Moreover, large-scale and reproducible synthesis of these nanomaterials under GMP conditions remains technically demanding. Therefore, while inorganic nanocarriers offer unique advantages for DENV vaccine design, their clinical translation will depend on addressing safety, scalability, and regulatory challenges to ensure acceptable risk–benefit profiles for human use.

Calcium Phosphate

CaP NPs are biocompatible, biodegradable inorganic nanocarriers composed primarily of calcium and phosphate ions in biomimetic mineral forms (amorphous calcium phosphate or poorly crystalline hydroxyapatite) that can be surface-functionalized to bind proteins, peptides or nucleic acids. They have been widely explored pre-clinically in drug delivery and vaccine applications because they protect cargo, enhance uptake by APCs and display an acceptable safety profile.[87] Importantly for immunization, CaP NPs dissolve in the acidic environment of endosomes/lysosomes, releasing Ca^{2+} and phosphate ions. The release of these ions can enhance cytosolic delivery by perturbing the endolysosomal membranes. Further, they raise local intracellular Ca^{2+} and engage Ca^{2+}-dependent signaling pathways that drive dendritic cell maturation (upregulation of MHC and co-stimulatory molecules) and cytokine production, thereby initiating an innate immune response.[88]

Conserved DENV T-cell epitopes were formulated into a nanoparticle-based vaccine by adsorbing the peptides with *N*-acetylglucosamine (GlcNAc) onto CaP NPs.[62] The six conserved epitopes selected comprised HLA-A2–restricted MHC I peptides derived from the capsid and non-structural proteins NS4B, NS5, and NS2A, which had been previously identified by immunoproteomic analysis of DENV-infected human cells. GlcNAc, a peptidoglycan monomer derived from bacterial cell walls, can interact with the NOD2 pathway, enhancing both

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the stability of the receptor and downstream immune signaling.[89] HLA-A2$^+$ transgenic mice were used to evaluate immunogenicity since the peptide sequences were specific to that HLA type. As with the polymersome DENV peptide formulation, these MHC I–restricted epitopes would be expected to elicit responses primarily in HLA-A2$^+$ individuals, rather than across the broader human population. Nonetheless, the CaP NP multi-peptide formulation induced robust cellular immune responses, with significantly higher IFN-γ production in antigen recall ELISpot assays. Interestingly, the lower peptide dose (10 μg peptide/150 μL CaP NP) produced a stronger response than the higher dose (50 μg peptide/150 μL CaP NP), and this effect was further enhanced by increased GlcNAc concentration. Because the formulation contained MHC I–restricted peptides, humoral responses were not reported; however, pronounced T-cell activation was clearly demonstrated. Overall, these findings highlight the potential of CaP NPs as an effective delivery platform for T-cell–targeted DENV vaccines, capable of promoting strong cellular immunity through antigen-specific and adjuvant-mediated mechanisms.

Carbon nanotubes (CNTs)

CNTs have attracted considerable interest in biomedical research due to their unique structural, electrical, and surface properties. Structurally, CNTs are cylindrical nanostructures composed of rolled sheets of graphene, existing either as single-walled CNTs or multi-walled CNTs that consists of multiple concentric graphene cylinders. In vaccine applications, CNTs have been explored as antigen carriers and immunostimulatory scaffolds capable of enhancing antigen uptake by dendritic cells and promoting both humoral and cellular immune responses.[90] Their needle-like morphology facilitates cellular penetration and endosomal escape, while their surfaces can be readily functionalized with peptides, proteins, or polysaccharides to tailor immune activation. However, despite these advantages, CNTs face significant challenges including their potential cytotoxicity, persistence in tissues, and inflammatory responses remain major concerns, particularly for non-functionalized nanotubes that can accumulate in organs such as the liver and lungs. Chemical functionalization and purification can mitigate some of these issues, but reproducibility, scalability, and long-term biocompatibility remain key barriers to clinical translation.[91]

Multi-walled CNTs have been evaluated as a preclinical DENV vaccine platform.[63] In this formulation, the DENV-3 E protein was covalently conjugated to the surface of CNTs via diimide-activated amidation. BALB/c mice were S.C. immunized with the CNT–E protein conjugate, formulated with CFA for the prime and Incomplete

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Freund's Adjuvant (IFA) for booster doses administered at 21, and 42 days. When sera were analyzed on day 56, total anti-E IgG titers were comparable between mice receiving the CNT formulation and those given soluble E protein, although the neutralizing antibody titers were slightly higher in the CNT-immunized group. The modest differences between groups are likely due to the potent adjuvanticity of CFA used in both prime formulations, which can mask formulation-specific effects. However, upon ex vivo restimulation of splenocytes with DENV-3 E protein, significantly greater lymphocyte proliferation and proinflammatory cytokine production were observed in the CNT-vaccinated group compared to the soluble protein control. Overall, the CNT-based vaccine elicited robust humoral and cellular immune responses, demonstrating its potential as an effective nanocarrier platform for DENV antigens.

Gold nanoparticles (AuNPs)

AuNPs have emerged as versatile platforms in biomedical research due to their chemical stability, biocompatibility, and tunable physicochemical properties. In vaccine development, AuNPs have been widely explored as antigen carriers and adjuvants capable of enhancing antigen uptake by dendritic cells and promoting robust humoral and cellular immune responses.[92] Their surfaces can be readily modified through thiol–gold (Au–S) chemistry, which enables stable conjugation of biomolecules such as peptides, proteins, nucleic acids, and polysaccharides.[93] This strong and simple surface chemistry allows AuNPs to serve as highly customizable scaffolds for antigen presentation and immune modulation. Beyond vaccines, AuNPs are utilized in biosensing and imaging applications owing to their unique optical and plasmonic properties, which provide high sensitivity and strong contrast in diagnostic assays.[94] However, while several gold-based contrast agents (e.g., AuroVist) have been evaluated in preclinical imaging studies, no AuNP formulations are currently FDA-approved for clinical imaging use in humans. Despite their promise, AuNPs face challenges related to long-term biocompatibility, and potential tissue accumulation.

AuNPs have been evaluated clinically as a vaccine platform for DENV. In this formulation, nine MHC I–restricted peptides were identified from DENV-infected, HLA-typed human cell lines.[23] These peptides, derived from capsid and nonstructural (NS1–NS5) proteins of DENV serotypes 1–4, were covalently conjugated to 1.6 nm AuNPs via thiol chemistry with GlcNAc and α-galactose. For the high-dose formulation, 7.5 nmol of total peptide was administered intradermally (I.D.) with 44.5 μg of gold, 19.4 μg of GlcNAc, and 15 μg of α-galactose

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on days 0 and 21. Twenty-six participants were enrolled, including 10 each in the low- and high-dose groups and three each in vehicle control groups. Adverse events were graded according to clinical standards, where Grade 1 indicates mild, Grade 2 moderate, and Grade 3 severe reactions requiring medical intervention. Grade 1 local site reactions occurred in all peptide-containing groups regardless of dose, while systemic events were more frequent in these groups and occasionally reached Grade 2 severity. One participant, each in the high-dose cohort experienced a Grade 3 systemic event in both the peptide and vehicle groups. As anticipated for a vaccine designed to elicit primarily T cell–mediated immunity, humoral responses were minimal. Unexpectedly, however, two participants in the high-dose vehicle control group, which lacked antigen, demonstrated total serum anti-DENV IgG titers consistent with seroconversion. T cell responses were variable among groups, though low-dose peptide vaccination induced detectable memory T cell populations in peripheral blood (**Figure 7**). Overall, the study demonstrated that AuNP-based peptide vaccines are safe and capable of eliciting modest cellular immune responses, supporting their continued evaluation as platforms for T cell–targeted immunization.

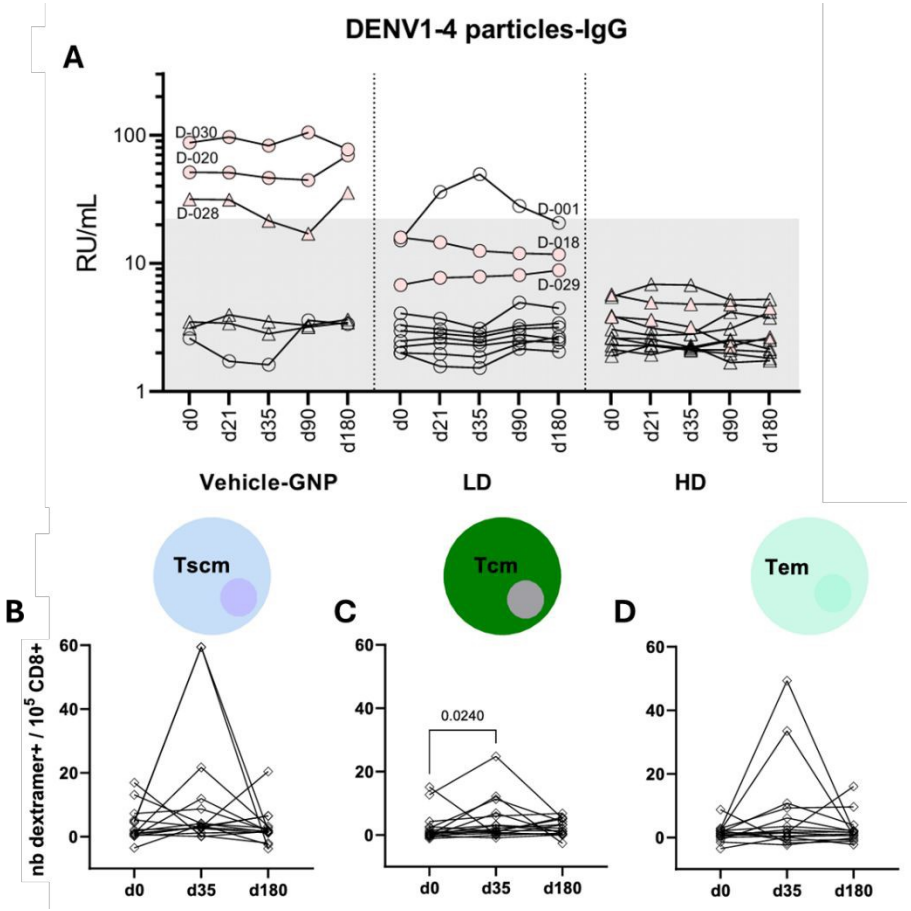

Figure 7. AuNPs were used to vaccinate humans on day 0 and 21 I.D.. (A) Total anti-DENV serum titters for vehicle, low-dose peptide (LD) and high-dose peptide (HD) groups on various days. (B) are early memory T cells (Tscm; CD45RA+CCR7+CD95+), (C) central memory T cells (Tcm; CD45RA−CCR7+), and (D) effector memory

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T cells (Tem; CD45RA−CCR7−) on various days in the serum post vaccination. Reproduced from Miauton et al. (*EBioMedicine*, 2024)[23] published under a Creative Commons Attribution License (CC BY 4.0).

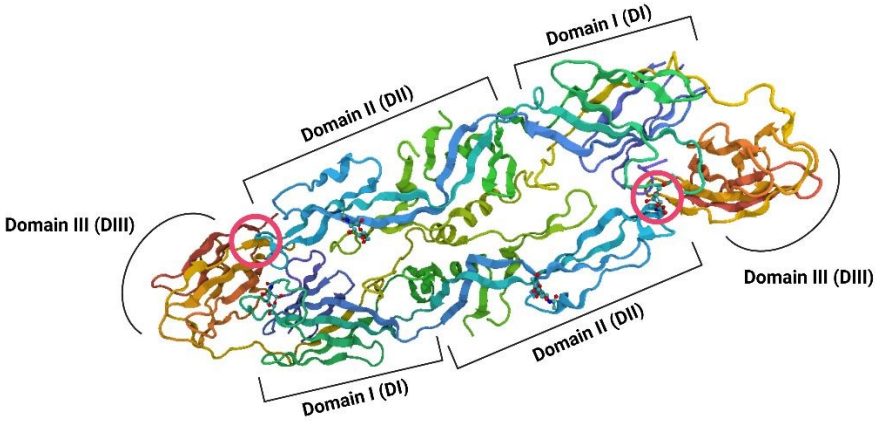

Figure 8. Regions of a generic DENV E protein indicating the three domains and the fusion loop region indicated in the pink circle.

Translational Feasibility

As discussed previously, the translation of inorganic formulations for DENV vaccines are currently hampered due to significant safety concerns. Additionally, many of these inorganic formulations require complicated synthesis methods that would become increasingly difficult to scale-up for mass manufacturing purposes. In order for inorganic formulations to become viable options for use in DENV vaccines, a significant amount of additional research is needed to improve safety and general biocompatibility, as well as simplifying formulations to reduce the manufacturing burden. Nevertheless, AuNPs are currently being evaluated in a Phase I clinical trial, and if successful may clear the way for other inorganic formulations to be evaluated.[23]

Protein Based Formulations

Protein-based antigens offer a safe, well-characterized, and versatile platform for eliciting targeted immune responses. Unlike live-attenuated or inactivated vaccines, which expose the immune system to the full pathogen, protein subunit vaccines present defined antigenic components that can be engineered to optimize immunogenicity and safety. This precision allows for the exclusion of pathogenic or cross-reactive epitopes, reducing the risk of adverse effects such as ADE. For example, in the DENV E protein, the fusion loop and domain II (DII) regions are key targets for engineering, as they are recognized by cross-reactive antibodies associated with ADE (**Figure 8**).[95, 96] These engineered proteins can be further enhanced by being expressed in tandem with carrier proteins to form antigen presenting protein nanoparticles.

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The manufacturing and production speed of protein-based vaccines is largely determined by the choice of expression system, such as insect, plant, or yeast cell lines. Advances in these platforms have dramatically reduced development timelines as exemplified with hemagglutinin (HA) based influenza vaccines. The Fraunhofer Institute produced an H1N1 HA influenza vaccine within 35 days, while Medicago achieved similar results in 19 days for an H5N1 vaccine.[97, 98] Other groups have successfully generated H5N1 vaccine antigens in under three weeks.[99] Moreover, modern protein manufacturing technologies can yield approximately ten million doses of GMP-grade protein antigen within 30 days, at an estimated cost of roughly $50 per gram (equivalent to about 50,000 20-µg doses, or ~$0.001 per dose).[100-102] These timelines are comparable to another rapidly produced platform mRNA LNPs however, protein based vaccines are significantly less expensive with the mRNA alone costing over $1 per 5-µg dose (the Moderna vaccine, for instance, contains 50 µg of mRNA).[86] Translating protein production advances noted with influenza to DENV could lead to inexpensive vaccines for resource limited settings. DENV protein-based nanoparticles include modified native proteins like ferritin or IgG as well as viral proteins like virus-like particles (VLPs). While protein subunit vaccines benefit from improved safety and scalability, protein antigens alone are typically not immunostimulatory enough to generate a significant immune response, requiring the inclusion of adjuvants.[39] As discussed earlier, severe clinical manifestations of DENV infection, such as DHF, often occur as a result of Th1-biased responses transitioning towards a Th2 response that promotes the production of non-neutralizing antibodies. For effective DENV vaccine development, adjuvants that induce a significant Th2-biased response should be avoided in favor of Th1-biased adjuvants. However, the most commonly used FDA-approved vaccine adjuvants are Th2-biased (aluminum salts), while Th1-biased adjuvants such as CpG 1018 or AS01B/AS04 are used to a lesser extent. It is apparent that additional research is needed to develop safe and effective Th1-biased adjuvants that can be included in future DENV vaccine formulations.[40, 41]

Ferritin nanoparticles

Ferritin nanoparticles are self-assembling protein nanocages composed of 24 subunits that form a highly symmetrical, hollow structure capable of displaying multiple copies of an antigen on their surface.[103] Derived from a naturally occurring iron-storage protein, ferritin nanoparticles have been found to be biocompatible, stable and easy to manufacture. Its uniform nanoscale size (approximately 12–20 nm) facilitates lymphatic trafficking and uptake by all cells, including APCs.[104] However, fusion of antigens to ferritin subunits can sometimes alter

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antigen folding or particle assembly, and repeated immunization may induce anti-ferritin antibodies that could limit booster responses.[105] Additionally, ferritin has shown to produce a robust CD4+ T follicular helper cell response in numerous inbred mouse strains, which may result in an immunodominance of ferritin over the vaccinating antigen.[106] Despite these limitations, ferritin nanoparticles represent a robust and versatile platform for subunit vaccines, enabling precise antigen presentation which with DENV proteins may lead to a confirmations that can limit ADE.

To form a ferritin based DENV vaccine, the DII region of the E protein were replaced by sequences of 'GGGGS' and 'S'.[64] A quadrivalent vaccine was formed with the engineered E protein functionalized ferritin nanoparticles. For the studies, BALB/c and AG6 mice were vaccinated on day 0 and 28 with the quadrivalent formulation S.C. and challenged with DENV-2 or DENV-3 given intravenously (I.V.). In BALB/c mice, vaccination induced higher total serum anti-DENV1–4 IgG titers, enhanced neutralizing activity, increased memory B cell formation, and reduced antibody-dependent enhancement compared to the vehicle control. Although knocking out interferon receptors in AG6 mice would lessen generation of a protective immune response with vaccination, these mice were vaccinated and monitored for humoral and protective immunity. When evaluating viral load in vaccinated mice challenged on day 42 and evaluated 3 days post infection, DENV-2 and DENV-3 infected mice had reduced viral load in the blood compared to vehicle control. Further out to 21 days post infection mice had 100% survival, whereas the vehicle control for DENV-3 had 20% survival at day 21 and DENV-2 infected mice succumbed to infection on day 7. The vaccine was then evaluated in BALB/c mice with adjuvants including alum, TLR4 agonist RS09 and STING agonist c-di-GMP. Of the three adjuvants, c-di-GMP had the most balanced Th1/Th2 antibody response, lowest ADE and highest neutralization and total serum IgG. Together, these results demonstrate that ferritin-based DENV vaccines can elicit potent, protective, and balanced immune responses, particularly when paired with appropriate adjuvants such as c-di-GMP.

Virus like particles (VLPs)

VLPs are proteins that assemble into nanometer particles that mimic the exterior structure of viruses.[107] They are typically produced by expressing one or more viral structural proteins (for example DENV E and M proteins) in heterologous systems such as yeast, insect cells using baculovirus vectors, mammalian cells, or plants under appropriate conditions to form nanoscale particles. Several FDA-approved vaccines, such as the

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human papillomavirus (HPV) vaccines Gardasil and Cervarix and hepatitis B vaccine Heplisav-B utilize VLPs. To elicit robust humoral and cellular immune responses, adjuvants are incorporated into these vaccine formulations: Gardasil contains alum, Cervarix includes the AS04 system (TLR4 agonist MPLA and alum), and Heplisav-B utilizes a CpG 1018. However, challenges remain in achieving consistent large-scale production of VLPs, ensuring structural homogeneity across serotypes, and maintaining correct epitope presentation. Despite these hurdles, DENV VLPs represent one of the most promising next-generation approaches for safe and broadly protective DENV vaccination.

VLPs have been used to generate pre-clinical formulations of DENV vaccines.[65, 108-110] To evaluate a VLP–based DENV vaccine, the fusion loop of the E protein was modified to reduce ADE. Initial VLP constructs were produced in Freestyle 293F cells co-expressing the precursor membrane (prM) and E proteins containing an F108A mutation in the fusion loop, yielding 35–50 nm spherical particles for each serotype.[110] For evaluation in NHPs, the EDIII, stem, and transmembrane regions of the DENV-2, -3, and -4 constructs were replaced with the corresponding regions from the DENV-1 E protein to enhance vaccine production.[65] NHPs were immunized I.M. at weeks 0, 4, and 8 with a tetravalent alum-adjuvanted formulation, which elicited robust total IgG and neutralizing antibody responses against all four serotypes, with seroconversion sustained beyond week 50. Upon viral challenge, vaccinated animals exhibited significantly reduced viremia lasting over two weeks. Moreover, passive transfer of immune serum into AG129 mice conferred complete protection against DENV-2 challenge, with 100% survival through day 20 post-infection, whereas control mice succumbed to infection by day 15. These results demonstrate that the engineered tetravalent VLP vaccine induces durable immunity and provides effective protection against DENV challenge in preclinical models.

Polymeric IgG

IgG is the predominant antibody isotype in mammalian serum, playing a central role in adaptive immunity through mechanisms such as neutralization, opsonization, and complement activation. Structurally, the Y-shape of IgG consists of two antigen-binding Fab regions and a constant Fc domain that interacts with Fc receptors and complement proteins.[111] Leveraging the inherent modularity of IgG, researchers have developed polymeric and multimeric IgG-based constructs that enhance antigen display, improve B cell receptor cross-linking, and potentiate humoral immune responses (**Figure 3**). These polymeric IgG scaffolds can be designed to present

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repetitive antigenic arrays, mimicking viral architectures. However, despite these advantages, polymeric IgG vaccines face several challenges. The large molecular size and complex architecture can hinder expression yield, proper folding, and stability during production and storage. Additionally, Fc-mediated interactions may lead to off-target immune activation or altered biodistribution, and excessive Fc clustering can trigger unwanted inflammatory responses.[112] Furthermore, the immunogenicity of the Fc region itself may complicate repeated administration with Fc-mediated immune responses.

A polymeric IgG platform has been evaluated preclinically as a candidate DENV vaccine.[66] In this design, the DIII sequence of the E protein was inserted into the variable regions of either mouse IgG2a-Fc or human IgG1-Fc to generate hexameric and pentameric polymeric constructs. BALB/c mice were immunized S.C. at weeks 0, 2, and 4 with either plant-derived (tobacco-expressed) or mammalian (CHO cell–expressed) polymers. Both plant- and CHO-derived constructs demonstrated antigen binding by ELISA, and the plant-derived particles specifically bound to Fcγ receptor–positive J774 macrophages but not to receptor-negative 3T3 fibroblasts, confirming receptor-mediated targeting. Serum from vaccinated mice showed comparable antibody titers for both expression systems, which were further enhanced with the adjuvant alum. Interestingly, while alum-adjuvanted consensus EDIII protein elicited antibody titers similar to the polymeric constructs, the latter induced significantly higher neutralizing activity. Splenocyte recall assays revealed increased IFN-γ production in groups receiving plant- or CHO-derived polymers, with or without alum, indicating a stronger cellular immune response compared to the consensus EDIII protein group. Together, these findings suggest that polymeric IgG scaffolds can enhance both humoral and cellular immunity against DENV.

Translational Feasibility

Beyond the context of DENV, protein-based formulations have been successfully translated into effective, FDA-approved vaccines (E.g., Gardasil, Cevarix, Heplisav-B). The ability to mass-produce protein-based formulations and antigens with relative ease and in a cost-effective manner places protein-based formulations at the forefront of options for the development of DENV vaccines. When developing protein-based vaccines for DENV, one of the primary considerations needs to be the inclusion of adjuvants that enhance the immune response without increasing the risk for DSS/DHF. Additionally, significant efforts need to be made to ensure that these formulations are thermostable to an extent to improve distribution in low-resource settings. If these challenges can be addressed, then protein-based formulations for DENV vaccines are a highly promising

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technology.

Conclusions and Future work

A diverse range of vaccine platforms has been explored in the preclinical development of DENV vaccines, including polymeric, lipid-based, inorganic, and protein subunit constructs. These alternative platforms aim to overcome the limitations of live-attenuated vaccines, which, despite clinical success, have been associated with ADE and limited applicability in immunocompromised populations. Across the range of vaccine candidates examined in this review, humoral responses, particularly serum antibody and neutralization titers, have been the most consistently measured indicator of immunogenicity, while comparatively fewer studies have evaluated cellular immune responses or in vivo protection following viral challenge. Establishing standardized preclinical evaluation protocols, including evaluation of ADE and the consistent use of AG129 or AG6 mouse models for protection assessment, would enable more meaningful comparisons among vaccine candidates and accelerate translational progress.

Advances in protein engineering, such as modifications in the E protein fusion loop or DII, have successfully reduced ADE potential and generated safer, more immunogenic antigens suitable for incorporation into modern delivery systems like nanoparticles or polymeric scaffolds. Interestingly, it has been observed that certain individuals who are infected with DENV on multiple occasions can develop potent broadly neutralizing antibodies (bnAbs) against the E dimer epitope, which can effectively neutralize all four DENV serotypes. Future rational antigen design may aim to exploit this epitope, as well as closely related epitopes, in an effort to develop a DENV vaccine that induces bnAbs against all serotypes.[113] The addition of rationally selected adjuvants which can include alum, TLR agonists, and other pattern associated molecular patterns (PAMPs), have also been shown to augment both humoral and cellular responses across diverse vaccine platforms. These strategies collectively highlight the importance of integrating antigen design, delivery optimization, and adjuvant formulation to achieve a balanced, durable, and broadly protective DENV immune response.

One aspect of nanoparticle-based formulations that surpasses traditional vaccine formulation approaches is the ability to target specific immune cell populations to enhance subsequent antigen-specific immune responses. Nanoparticle-based formulations can be optimized to actively target immune cells, such as dendritic cells, through the functionalization of different targeting ligands, or through passive targeting of APCs

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in a size-dependent manner.[114] Specific targeting of dendritic cells and other APCs can lead to increased internalization of vaccine formulations and innate immune system activation, leading to the migration of dendritic cells to the local draining lymph nodes where they can more effectively initiate an adaptive immune response. Future strategies should focus on designing formulations that preferentially traffic to specific immune cell types, which may lead to more efficient immune responses, dose-sparing of antigens and adjuvants, and reduce or eliminate the need for booster doses.

From a translational perspective, cost-effectiveness and accessibility remain critical considerations, particularly in resource-limited settings where DENV burden is highest. Innovative delivery approaches, such as microneedle patches, have demonstrated protection in AG129 mouse models and hold promise for scalable, low-cost vaccine deployment.[115] Re-emerging adjuvant systems like immune-stimulating complexes (ISCOMs) are also being revisited, having shown safety and efficacy in pre-clinical studies.[16] Beyond enhancing immunogenicity, adjuvants can contribute to antigen dose sparing and broaden protection across diverse populations.

However, several parameters remain underexplored in preclinical DENV vaccine development that are essential for clinical translation. One key factor is endotoxin characterization, particularly for nanoparticle or recombinant protein formulations. Endotoxin contamination can inadvertently enhance immunogenicity through TLR4 activation, leading to misleading efficacy results. Because sterile filtration alone is insufficient to remove endotoxins, quantitative assays such as plate reader–based tests should be routinely implemented to ensure formulation purity. Another critical consideration is the evaluation of anti-carrier immune responses. As seen with LNP formulations, pre-existing or induced anti-PEG and anti-lipid antibodies have been linked to reduced mRNA expression and diminished vaccine efficacy. Similar immune responses against polymeric, lipid, metal, or protein carriers could compromise long-term performance, especially in vaccines requiring multiple booster doses. Furthermore, future studies should include evaluation in obese, aged, and immunocompromised models to better predict performance across real-world populations.

In conclusion, while significant progress has been made in the rational design of DENV vaccines, which include antigen engineering to delivery and adjuvant optimization, further success will depend on harmonizing preclinical evaluation methods, addressing overlooked formulation variables such as endotoxin and carrier immunogenicity, and prioritizing platforms that are both scalable and globally accessible. Together, these efforts

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will accelerate the translation of next-generation DENV vaccines capable of providing safe, broad, and durable protection against all four serotypes.

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Conflict of Interest Statement

None.

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Data availability statement

This is a review paper, there is no data to make available.