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Edible DNA Origami Nanostructures: Food-grade Platforms for Oral Vaccine Delivery and Functional Foods Targeting Mucosal Immunity

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Abstract

Edible DNA origami nanostructures have emerged as an innovative, food-grade approach for oral vaccine delivery, addressing critical challenges associated with traditional vaccination methods. This review comprehensively explores the design, functionalization, and application of DNA origami platforms tailored for food-based mucosal immunization. We systematically examined the foundational principles of DNA origami technology, focusing on its intrinsic advantages such as biocompatibility, precise antigen arrangement, and potential for targeting mucosal immunity via the gastrointestinal tract. Through a critical synthesis of recent peer-reviewed literature, we identified successful strategies for integrating DNA origami vaccines into functional food matrices, including encapsulation techniques, antigen-adjuvant co-delivery, and mucosal targeting approaches. Key findings indicate that DNA origami nanostructures effectively enhance the immunogenicity and stability of orally administered vaccines, induce robust mucosal IgA responses, and show superior flexibility and precision compared to conventional edible nanocarriers. However, challenges such as manufacturing scalability, stability optimization in food and biological environments, and regulatory considerations remain significant hurdles. Addressing these challenges through ongoing interdisciplinary research is essential for translating edible DNA origami platforms into viable immunotherapeutic products. Overall, this review highlights the transformative potential of DNA origami-based edible vaccines to revolutionize preventive health strategies, improve vaccine accessibility, and establish a new paradigm for immunization through functional foods.

Keywords

DNA origami, Edible vaccines, Oral delivery, Functional foods, Mucosal immunity

Introduction

Oral vaccination through functional foods represents a promising strategy to induce protective immunity at mucosal surfaces, which are the entry points for many pathogens. Traditional injectable vaccines often fail to elicit strong mucosal immune responses, leaving individuals susceptible to infections at sites like the gastrointestinal tract). By contrast, vaccines delivered via the oral route can stimulate both localized mucosal immunity and systemic immunity, providing a first line of defense where pathogens enter [1]. Edible vaccines-such as those produced in transgenic plants-have been explored as a needle-free, accessible approach to immunization, offering advantages like low cost, ease of administration, and the potential for broader acceptance [2]. Indeed, early trials of plant-based oral vaccines (e.g., potatoes expressing antigens for diarrhea) demonstrated that consuming antigen-containing plant tissue can raise immune responses in humans [3]. However, edible plant vaccines also face significant challenges: the antigen dose can vary between fruits or plants, the stability of antigens within raw

foods is uncertain, and the need to eat large quantities or raw produce for an effective dose is impractical [4]. Issues such as variable dosing, degradation during storage or cooking, and regulatory hurdles for genetically modified foods have thus far limited the widespread adoption of traditional edible vaccines [5]. These limitations highlight the need for new "food-grade" delivery platforms that ensure consistent dosing, stability in the gastrointestinal environment, and robust immunogenicity [6]. Crucially, DNA, a biopolymer naturally present in all foods, is generally recognized as safe to ingest, with humans routinely consuming hundreds of milligrams to grams daily from ordinary diets without adverse effects. As a result, DNA nanostructures offer intrinsic biocompatibility and biodegradability, distinguishing them from many synthetic nanomaterials. Animal studies indicate that DNA origami nanoparticles (NPs) are non-toxic even at high doses, eliciting only mild and transient immune responses that resolve over time [7]. Importantly, these nanostructures are metabolized or excreted without lingering toxicity which may support their suitability as safe, food-grade carriers for oral vaccine delivery [6]. The combination of edibility (as a naturally occurring nutrient) and structural programmability positions DNA origami as a promising "food-grade" nanocarrier for oral vaccines and functional foods [8]. Recent advances have shown in preclinical studies that DNA origami NPs can be engineered to mimic viral particles and present antigens in highly organized patterns to the immune system [9]. Recent studies demonstrate that DNA origami vaccines displaying the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike receptor-binding domain (RBD) can elicit strong neutralizing antibody responses and confer protective immunity in animal models [10, 11]. These findings underscore the potential of DNA origami as a versatile, food-grade nanoplatform for oral vaccines, offering precise control over antigen presentation and multivalency that may offer advantages in antigen presentation and immunogenicity, though direct comparative studies are still limited [12]. Figure 1 provides a schematic overview of the oral edible DNA origami vaccine delivery pathway, illustrating the sequential steps from nanostructure manufacture and encapsulation within food or supplement matrices, through storage and ingestion, to gastrointestinal transit, uptake by mucosal immune cells, and subsequent immune activation.

This review provides a comprehensive overview of edible DNA origami nanostructures as emerging vehicles for oral vaccine delivery and immune-modulating functional foods. We first explain the fundamentals of DNA origami technology and its unique advantages for food and nutraceutical applications. We then discuss strategies for designing and functionalizing DNA origami to carry antigens and adjuvants, and how these can be incorporated into foods or oral dosage forms. Key considerations of stability in gastrointestinal fluids and food matrices are examined, along with comparisons to other edible nanocarriers (e.g., polysaccharide NPs) that have been used to target mucosal immunity. We also explore the ways in which DNA origami could be harnessed to stimulate mucosal immune responses, and the challenges that must be addressed for future clinical translation. By critically analyzing recent high-impact studies and pioneering efforts in this interdisciplinary field, we aim to assess the promise of DNA origami nanostructures as food-grade platforms for next-generation oral vaccines and functional foods targeting mucosal immunity. Fundamentals of DNA origami technology in food applications

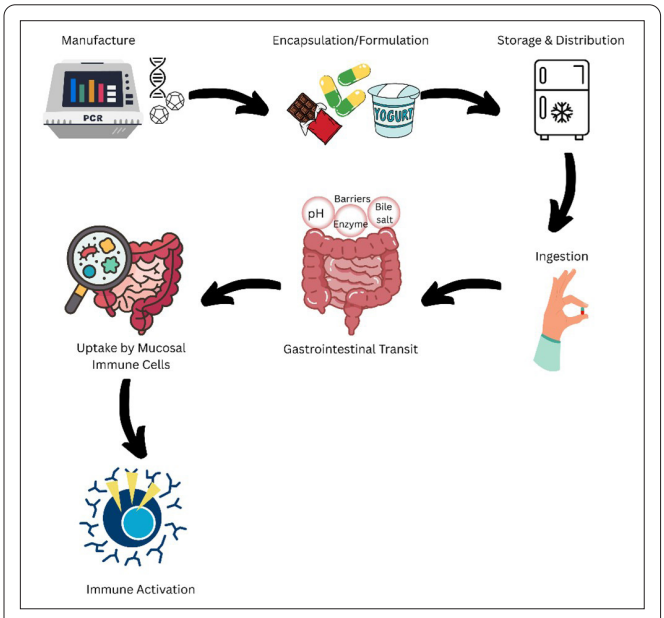


Figure 1: Schematic overview of the oral delivery pathway for edible DNA origami nanostructures. After manufacture and encapsulation/formulation (e.g., as supplements or functional foods), nanostructures are stored and distributed, ingested, and pass through the gastrointestinal tract, encountering pH shifts and digestive enzymes. Surviving structures are taken up by mucosal immune cells, leading to immune activation.

DNA origami involves the folding of DNA into predetermined shapes, analogous to paper origami, through the mechanism of complementary base pairing [13]. In the seminal work by Rothemund [14], a long single-stranded viral DNA (the "scaffold") was folded with the help of ~200 short synthetic oligonucleotides ("staples") to create nanoscale shapes like smiley faces and stars. Since then, the technique has advanced to produce an impressive variety of two-dimensional and three-dimensional nanostructures, including nanotubes, polyhedral, and dynamic devices. The precision and addressability of DNA origami are highly precise and addressable-each staple strand can be designed to position with nanometer accuracy, allowing the incorporation of functional elements at exact sites on the structure [15]. Moreover, the folding is a self-assembly process that occurs under benign conditions (aqueous solution, moderate temperature) and yields monodisperse NPs with highly predictable geometry. From a food and biomedical standpoint, DNA origami offers several intrinsic benefits [16]. First, DNA is highly biocompatible and non-toxic. It is a fundamental biological macromolecule that cells enzymatically degrade into nucleotides; any DNA origami ingested would ultimately be broken down into nutrients or excreted without harmful residues [17]. Animal studies have reinforced this safety profile, for example, Lucas et al. [7] found no significant toxicity in mice injected with high doses of DNA origami, and no evidence of organ damage or persistent inflammation. Such biocompatibility is a critical advantage over many synthetic NPs and suggests DNA origami could be suitable for use in foods or oral medicines. Second, DNA origami is

programmable and unable. Because the scaffold and staples are sequence-designed, virtually any shape or size in the tens to few hundreds of nanometers range can be constructed [18]. This programmability means DNA nanostructures can be tailored to optimally interact with biological systems. For instance, object size and geometry influence cellular uptake and biodistribution. DNA origami can be made in wireframe or compact designs with controllable dimensions, offering a great opportunity to optimize factors like uptake by Peyer's patch microfold (M) cells or drainage to lymph nodes [19]. Compared to natural food particles or plant cells (which have fixed sizes and compositions), DNA origami allows a modular design approach to meet specific delivery goals. Third, DNA as a material has predictable and consistent structural properties [13]. Double-stranded DNA has a well-defined diameter (~2 nm) and persistence length (~50 nm), and base-pairing yields reliable secondary structures. These features make DNA an ideal nanoscale "building block" – as noted by Ghosal et al. [20], the highly conserved A-T and G-C base pairing leads to completely predictable self-assembly, and the resulting structures can be reproduced with nanometer-scale precision. Unlike many food biopolymers (proteins, polysaccharides) that can be heterogeneous or sensitive to processing, DNA origami constructs are homogeneous and robustly defined by sequence [21]. Additionally, DNA nanostructures can be prepared under mild, aqueous conditions (often at room temperature), avoiding harsh chemicals and making them "environmentally friendly and toxicity-free" to produce [22]. This is advantageous for scaling up manufacturing in a food-grade or pharmaceutical context, as it may not require toxic reagents or solvents.

DNA origami offers high functional adaptability. The surfaces of DNA nanostructures are decorated with staple strand ends that can be readily modified with functional groups (e.g., biotin, amine, thiol) for conjugating a wide array of molecules [23]. In vaccine applications, this translates to the ability to display multiple copies of an antigen in specific orientations and spacing, akin to the repetitive antigen array on viruses (which is known to strongly stimulate B cells). In food science, the same principle allows immobilizing nutraceutical compounds or flavor molecules on DNA carriers in a controlled way [24].

In the context of edible applications, an implicit advantage of DNA origami is that DNA is a normal component of diet. Every cell in plant- or animal-derived food contains DNA; estimates suggest a human meal can contain up to a gram of DNA from meat or vegetables without ill effect. Consuming DNA origami structures, even in the microgram to milligram range for a dose, would add only a negligible fraction to the daily nucleic acid intake and is unlikely to pose novel safety concerns beyond those of ordinary food [25]. Furthermore, any DNA that does enter the body via the gut is subject to degradation by digestive enzymes and innate immune mechanisms. Encouragingly, studies indicate that DNA origami may be even less immunostimulatory than free DNA because of its folded architecture-the immune system has evolved to recognize certain DNA motifs (like unmethylated CpG dinucleotides) as danger signals, but DNA origami can potentially mask these motifs sterically or through base-pairing [19]. A 2022 *in vivo* study found only modest immune activation from DNA origami, not strong enough to be dangerous or to break immune tolerance to self-DNA. This suggests that DNA origami can often evade unwanted immune detection, a desirable property for a vaccine carrier (where we want immune response to the antigen delivered, not against the DNA scaffold itself) [26].

Design and functionalization of edible DNA origami for vaccine delivery

Designing DNA origami for vaccine delivery involves both the structural design of the DNA nanoparticle and its biochemical functionalization with immunologically active molecules. The goal is to create an edible nanoparticle that can carry antigens (and adjuvants) through the harsh conditions of the gastrointestinal tract, target relevant immune cells in the mucosa, and ultimately trigger a mucosal immune response, which is the main target of oral vaccines [12]. Figure 2 illustrates the main requirements for designing edible DNA origami nanostructures: biocompatibility, pH stability, edible formulation, structural precision, and food-grade safety. These criteria underpin their suitability as next-generation platforms for oral vaccine delivery and mucosal immune targeting. Several key design principles have emerged from recent research in DNA origami vaccines and nanomedicine:

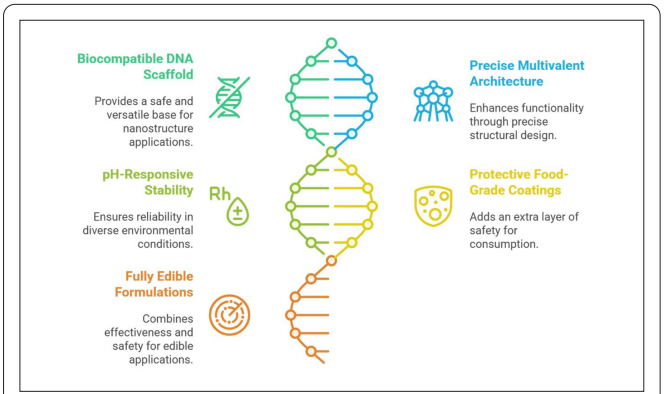


Figure 2: Key characteristics and design strategies of edible DNA origami nanostructures.

Virus-like particle design

A successful vaccine often mimics the structural features of pathogens to exploit the immune system's natural recognition pathways. Viruses, for example, are typically 20 - 200 nm in size and present arrays of antigens on their surface with high density and repetitive geometry [27]. DNA origami can be engineered to resemble these virus-like particles (VLPs) in size and antigen organization [9]. Feng et al. [28] demonstrated this approach by constructing an icosahedral DNA origami ~90 nm in diameter-closely matching the size and morphology of many viruses (including SARS-CoV-2). The icosahedral DNA nanostructure was used as a display platform for the coronavirus RBD antigen, arranged in multivalent clusters. By tuning the antigen spacing and copy number on the origami, the researchers identified configurations that maximized B-cell activation *in vitro* [28]. The optimized DNA origami vaccine induced stronger and longer-lasting antibody respons-

es in mice than soluble antigen and even achieved immune responses comparable to a conventional mRNA vaccine in some metrics [29]. Other studies, such as Veneziano et al. [30], similarly used wireframe DNA origami to arrange HIV envelope proteins in virus-like patterns, finding that nanoscale antigen organization dramatically influences B cell receptor clustering and activation [30]. These findings form a "design rule" for DNA origami nanovaccines – by presenting antigens in a multivalent, repetitive array on a nanostructure of the right size (tens of nm), one can greatly enhance immunogenicity compared to monomeric antigens.

Multivalent antigen attachment

DNA origami structures include hundreds of staple strand endpoints that can serve as attachment sites for antigens. all food-grade reagents and processes must be considered when adapting these methods for edible vaccine applications [31]. For example, protein antigens can be attached via biotin–streptavidin bridges: the DNA origami is synthesized with biotinylated staples at desired positions, and the antigen is prepared with a streptavidin or biotin tag so it will bind to those sites [32]. Oktay et al. [10] used a complementary strategy by genetically fusing a peptide tag to the SARS-CoV-2 RBD antigen, which allowed site-specific anchoring of ten RBD trimers onto a DNA origami nanopegboard [10]. This resulted in a DNA nanoparticle displaying 30 copies of RBD in a defined arrangement, along with tethered CpG oligonucleotides as adjuvants. The multivalently decorated origami elicited a robust neutralizing antibody response and protected mice from live virus challenge. The ability to co-display multiple antigens or antigen copies is particularly useful for vaccines that might require presenting several epitopes (for example, a cocktail for a polyvalent vaccine) or mimicking viral spikes that are trimeric. In principle, DNA origami can also carry different antigens on the same particle (creating a multi-valent or multi-pathogen vaccine), simply by assigning different sets of staple strands for different molecules [33].

Incorporation of adjuvants

To provoke a strong immune response via the oral route, incorporating immunostimulatory adjuvants is often necessary. DNA origami itself can serve as a scaffold for certain adjuvants. A notable example is the use of unmethylated CpG motifs, which are short DNA sequences that activate toll-like receptor 9 (TLR9) in immune cells [34]. CpG oligodeoxynucleotides are approved adjuvants in some vaccines (e.g., Hepatitis B) due to their ability to boost Th1-type immunity. DNA origami vaccines have capitalized on this by including CpG sequences either within the scaffold or attached to it [35]. Ghosal et al. [20] note that CpG oligos have been covalently attached to DNA nanocarriers to simultaneously deliver antigen and provide immunostimulation [20]. Oktay et al. [10] similarly included CpG sequences on their SARS-CoV-2 RBD origami, essentially building the adjuvant directly into the nanoparticle [10]. When considering oral vaccines, CpG DNA is attractive because TLR9 is expressed by antigen-presenting cells in gut-associated lymphoid tissue (GALT); DNA origami carrying CpG could potentially trigger those receptors upon uptake [36]. However, it's worth noting that chemical modification of CpGs (e.g., phosphorothioate backbone for stability) can reduce their immunostimulatory potency and even cause side effects. Thus, using the DNA origami as a protective carrier for unmodified CpG motifs could strike a balance, preserve adjuvant activity while prolong its presence in tissues without resorting to chemical stabilization that might add toxicity.

Targeting ligands for mucosal tissues

Edible DNA origami vaccines can benefit from targeted delivery to key cells such as M cells in Peyer's patches, which actively take up particulates from the gut lumen, or to dendritic cells and macrophages that populate the intestinal mucosa [37]. Targeting can be achieved by decorating the DNA nanostructure with ligands recognized by receptors on these cells. A compelling example is the use of β-glucan (from yeast cell walls) as a targeting ligand. β-1,3-glucans bind to Dectin-1, a receptor abundantly expressed on intestinal M cells and macrophages [38]. Liu et al. [39] leveraged this biology by coating DNA nanoparticle vaccines with yeast cell wall fragments rich in β-glucan. They first formulated protamine–DNA particles carrying a plasmid DNA vaccine and then attached nanometer-sized yeast cell wall pieces onto the surface via electrostatic interactions [39]. The β-glucan-coated NPs showed enhanced uptake by dectin-1^+ antigen-presenting cells and M cells in vitro and in vivo, as well as improved stability in simulated gastric conditions [40]. Mice orally immunized with these yeast-coated DNA origami NPs generated high levels of RBD-specific intestinal IgA and robust T-cell responses, whereas uncoated NPs were much less effective [41]. This study provides a blueprint for functionalizing edible nanovaccines: by using food-derived targeting ligands (yeast is obviously an edible organism and β-glucan itself is a dietary fiber supplement), one can direct the DNA origami to the desired mucosal immune inductive sites.

Encapsulation of antigens and enzymes

While surface display is ideal for B-cell antigens, some vaccine approaches might encapsulate immunogens or enzymes within protective carriers to shield them from degradation. DNA origami can form hollow cage-like structures that encapsulate cargo internally [42]. Studies have shown that proteins (including enzymes) can be placed inside DNA origami boxes or barrels, which can protect them from proteases and preserve their activity [43]. For instance, a DNA origami cage loaded with an enzyme was found to retain enzymatic function and resist proteolytic digestion better than the free enzyme. In a vaccine context, one might encapsulate a delicate antigen (or even mRNA for a transcript that produces antigen) inside a DNA origami shell to get it through stomach acid and enzymes, then allow it to be released or presented once the structure is taken up by an immune cell. Encapsulation can be achieved by designing the origami to self-assemble around the cargo (e.g., DNA origami boxes that open and close) [44].

Edible form factor and formulation

To integrate DNA origami vaccines into foods or oral dosage forms, designers must consider the overall form. DNA origami itself is a nanoscale object typically prepared in buffer.

For edible use, it might be dried or embedded in a matrix. One design approach is to incorporate DNA origami into biopolymer microspheres or films that are recognized as safe [45]. For example, encapsulating DNA origami within alginate or chitosan microcapsules could create a composite wherein the DNA nanostructures are released in the intestine. Alginate, a polysaccharide from seaweed, forms gels that survive stomach pH but dissolve in neutral pH – an alginate-encapsulated DNA origami would remain intact through the stomach and release payload in the small intestine (a concept similar to enteric coating) [6]. Likewise, chitosan (derived from crustacean shells) is mucoadhesive and has been used to coat NPs for oral delivery. DNA origami coated with a thin layer of chitosan might adhere to mucosal surfaces longer and promote uptake [46]. In designing such formulations, care must be taken that the assembly and functionalization of the origami are not disrupted. Encouragingly, DNA origami can remain stable when freeze-dried with appropriate buffers or sugars (e.g., trehalose), which suggests it could be incorporated into dry food products or powder form vaccines without loss of structure upon rehydration [47]. The modular design of DNA origami also means the same underlying nanostructure could be decorated in different ways for different applications: for example, one could have a base 100 nm origami cage and in one formulation attach influenza antigens for an oral flu vaccine, while in another attach probiotic-derived proteins for a gut health intervention [48].

Oral delivery of vaccines via functional foods

Oral vaccine delivery through functional foods is an appealing strategy to improve vaccine accessibility and compliance. By formulating vaccines into edible products – such as yogurts, beverages, snack bars, or capsules containing food-grade matrices – immunization could be achieved without injections, potentially reaching populations with limited healthcare access or needle aversion [49]. Several paradigms have been investigated: transgenic edible crops expressing vaccine antigens (as discussed, e.g. potatoes, tomatoes, bananas), probiotic vectors (live bacteria like *Lactobacillus* engineered to produce antigens), and encapsulation in food-derived biopolymers (like starch, proteins, or polysaccharide NPs) [5]. DNA origami nanostructures can be viewed as a new addition to this toolbox – they can be integrated into functional foods either by incorporation into a food matrix or by co-administration with edible carriers that protect and target them [20].

One practical approach to oral vaccine functional foods is to use fermented or probiotic foods as carriers. For instance, a freeze-dried formulation of DNA origami vaccine could be added to a probiotic yogurt or kefir, effectively creating a symbiotic product: the dairy or fermented matrix might buffer stomach acidity while the DNA origami delivers the antigen [50, 51]. Additionally, probiotic bacteria themselves might assist; *lactobacilli* have been used as live oral vaccines by displaying antigens on their surface [52].

Another avenue is incorporating DNA origami vaccines into conventional oral dosage forms that are nonetheless food compatible [52]. For example, capsules made of plant-derived cellulose or gelatin could be filled with a powder of DNA origami mixed with stabilizers (like trehalose, which is often used to stabilize biologicals in dry form). These capsules can be enteric-coated (with food-safe polymers like shellac or pH-sensitive methacrylate copolymers) so they pass through the stomach intact and dissolve in the intestine, releasing the DNA origami to interact with gut immune tissue [8]. This approach is similar to that used for oral typhoid and cholera vaccines that use enteric capsules. The difference is the payload: instead of live attenuated organisms, it would be nanostructured DNA assemblies with antigens [53]. Because DNA origami is nanoscale, once released, they have the potential to penetrate mucus than larger microparticles. In fact, a recent preclinical report found that DNA origami's shape can affect its diffusion through intestinal mucus-in a study by Tollemeto et al. [54], rod-like and wireframe DNA origami structures were observed to have distinct diffusion rates in porcine intestinal mucus, indicating topology influences how well these particles can move in the mucosal layer [54].

Functional food matrices can also play a protective role. As mentioned, the yeast cell wall is one innovative functional coating that improved oral DNA vaccine performance [55]. Yeast derivatives are already used as functional food supplements (e.g. β-glucan fibers for immune health), which suggests a possible synergy: a functional food product could contain yeast β-glucan along with the DNA origami vaccine, essentially using the β-glucan as both an immune-modulator and a shield for the nanoparticle. Similarly, components like milk proteins could be employed. Milk has natural buffering capacity and contains proteins (caseins, whey) that can form complexes with polyionic molecules [56]. One could envisage encapsulating DNA origami within casein micelles – casein, the main protein in milk, self-assembles into colloidal particles that have been studied as nutrient carriers [57]. DNA origami might adsorb to casein micelles (due to the micelles' positive charges at acidic pH) and be carried through the stomach more gently when ingested in a dairy product. Upon reaching the higher pH of the intestine, casein micelles disassemble, potentially releasing the DNA origami [57]. Another concept is using prebiotics and dietary fibers as co-delivery agents. Certain polysaccharides like inulin, pectin, or resistant starch can form gels or particles that aren't digested until reaching the colon. While the small intestine (Peyer's patches) is the primary induction site for oral vaccines, if one wanted to target gut immunity further down (e.g., for colorectal pathogens), formulating DNA origami with coatings of pectin (which survives small intestine but is degraded by colonic bacteria) could release the vaccine in the colon [58, 59]. In general, matching the functional food matrix properties to the target site is important: acid-resistant but bile-soluble matrices for small intestine targeting vs. matrices stable through small intestine but fermentable in colon for large intestine targeting [60]. Figure 3 illustrates the stepwise mechanism of mucosal immune activation following oral administration of edible DNA origami nanovaccines. After ingestion, the DNA origami nanostructures are shielded by protective, food-grade carriers as they traverse the acidic gastric environment. Upon reaching the intestine, the nanovaccines are taken up by specialized M cells within the Peyer's patches. These antigens are

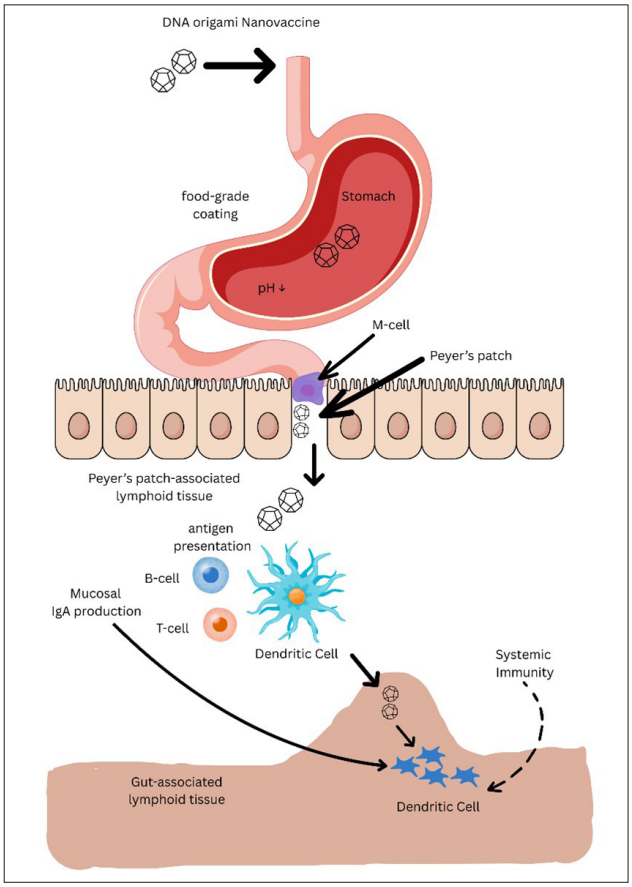


Figure 3: Schematic representation of the mucosal immune activation pathway following oral delivery of edible DNA origami nanovaccines. After ingestion, DNA origami is protected by a food-grade carrier as it transits the stomach's acidic environment. Upon reaching the small intestine, nanostructures are transported across the epithelium via M cells into Peyer's patches. There, dendritic cells process and present antigens to B and T cells, resulting in mucosal IgA production and contributing to both local and systemic immunity. And illustrates the key stages and cellular interactions required for effective oral vaccine function [28, 61, 62].

subsequently presented by dendritic cells to B and T lymphocytes in the associated lymphoid tissue, promoting mucosal IgA production and systemic immune responses. The pathway highlights key barriers and cellular interactions critical for the efficacy of oral nanovaccine delivery systems.

It's also noteworthy that many edible vaccine strategies leverage the gut's tolerogenic environment to some extent-which might sound counterintuitive since tolerance is what we must overcome to get an immune response [5]. But functional foods sometimes include immunomodulatory ingredients that tip the balance. For example, vitamin A (retinoic acid) in diet influences gut immunity by promoting IgA responses and gut-homing of immune cells. A functional food vaccine could be fortified with vitamin A or probiotics known to enhance vaccine responses (there is evidence that certain *Lactobacillus* strains can act as adjuvants by stimulating innate immunity in the gut) [63]. Another interesting component is immunonutrition elements like arginine or dietary nucleotides, which are added to infant formulas to support immune development. Thus, an oral DNA origami vaccine delivered via a nutrient-rich food might both immunize and provide a subtle nutritional immunomodulation.

Stability and behavior of DNA origami nanostructures in biological fluids and food matrices

One of the central challenges in deploying DNA origami nanostructures for oral delivery is ensuring that they remain sufficiently stable from the point of manufacture, through storage (potentially within a food product), and during transit through the harsh environment of the digestive tract [64]. DNA is susceptible to denaturation by heat and extreme pH, and to degradation by nucleases (DNases) present in biological fluids. As a result, substantial research has focused on understanding and improving the stability of DNA origami nanostructures under these diverse conditions [65]. In biological fluids such as serum or tissue culture media, naked DNA origami tends to be rapidly attacked by nucleases. However, several studies have shown that these folded nanostructures often exhibit greater resilience than linear or duplex DNA, likely due to their compact, multi-stranded architecture [26]. For example, a typical DNA origami might survive several hours in the presence of DNase, whereas an unstructured DNA of comparable length would be degraded much faster. Furthermore, different shapes of DNA origami display different stabilities; for instance, triangular origami structures persisted longer in mouse circulation than rod-like designs, a difference attributed to structural compactness and interactions with serum proteins [66].

The gastrointestinal tract presents a multifaceted challenge: DNA origami must withstand extremely low pH in the stomach (pH ~1–2), digestive enzymes, and then a transition to neutral or basic pH in the small intestine, where pancreatic DNase I and bile salts can further destabilize the nanostructure [67]. While the double-helical domains of DNA origami are stable in moderately acidic conditions, very low pH can cause depurination and strand breakage if exposure is prolonged [68]. Additionally, essential Mg^2+ ions required for origami stability may be lost or precipitated in acidic environments, potentially reducing structural integrity. To address these challenges, several stabilization strategies have been developed. pH-responsive coatings-such as enteric polymers that dissolve at higher pH-can protect DNA origami through the stomach, while buffering food matrices (e.g., dairy products or bicarbonate-based buffers) can help neutralize stomach acid locally [69]. Increasing crossover density within the DNA origami design has also proven effective, as it makes the nanostructure more rigid and less accessible to enzymatic attack [70]. Interactions with food components add further complexity. When DNA origami is mixed with real food substances, proteins, polysaccharides, and lipids may adsorb onto the nanostructure, forming a "biomolecular corona" that can either stabilize or destabilize the origami. For example, serum proteins have been shown to protect DNA origami from enzymatic degradation [71]; similar effects may arise from food proteins or polysaccharides. Encapsulation within edible microgels or liposomes provides another layer of protection against harsh digestive conditions until release in the intestine [72].

Temperature stability is equally important, particularly if the functional food product is stored at ambient temperature. DNA origami can generally maintain its structure up to approximately 40 °C in the presence of Mg^2+, but high-

er temperatures can induce denaturation or aggregation [73]. Lyophilization (freeze-drying), especially in the presence of sugars, has been shown to enhance the thermal stability of DNA origami, supporting their inclusion in shelf-stable food products without the need for refrigeration [74]. Once DNA origami reaches the intestinal environment, it needs to persist for several hours to allow uptake by mucosal immune cells. While direct data in gut fluid are limited, *in vitro* and serum studies suggest that well-stabilized DNA origami can remain intact long enough for effective delivery [75]. After uptake, DNA origami is generally degraded in lysosomes, releasing antigenic peptides for immune presentation-a desirable outcome for vaccine applications [20]. For applications requiring cytosolic delivery, endosomal escape mechanisms-such as the incorporation of pH-sensitive polymers-can be considered. To ensure efficacy, DNA origami must remain viable throughout food processing, storage, and distribution. This includes resistance to mixing, mild heating, dehydration, and variable storage conditions [76]. Overall, while the stability of DNA origami nanostructures in biological fluids and food matrices poses significant challenges, recent advances in design and formulation provide promising solutions for their use in edible vaccine platforms.

Edible nanocarriers and food-grade delivery systems

The concept of using nanocarriers for oral delivery is not entirely new-a variety of nanoparticle systems have been studied to improve the oral bioavailability of drugs and the efficacy of oral vaccines. These include polymeric NPs (e.g., Poly(lactic acid)/poly(lactic-co-glycolic acid (PLGA), and CS), lipid-based carriers (liposomes, solid lipid NPs, and emulsions) inorganic NPs (like calcium phosphate or silica, which are less common in foods but have been considered), and biologically derived NPs (like exosomes from milk or plant viruses) [77]. When considering food-grade delivery systems, emphasis is placed on materials that are safe to ingest, digestible or at least eliminable, and ideally already approved as food additives or supplements. Table 1 summarizes key edible nanocarriers explored for oral vaccine delivery and functional foods, highlighting their material origins, immunogenicity, and major applications.

Chitosan NPs

Chitosan, derived from chitin in crustacean shells, is a cationic polysaccharide that is widely used in oral drug and vaccine delivery research. It is generally regarded as safe and is actually used in some dietary supplements [86]. Chitosan NPs have mucoadhesive properties because their positive charges interact with the negatively charged mucosal surface, leading to prolonged retention in the gut and enhanced uptake of associated antigens [87]. Over the past decade, chitosan NPs have been widely used to deliver vaccine antigens mucosally in veterinary and biomedical studies. They can encapsulate proteins or DNA, protecting them from degradation. For instance, Renu et al. [79] report that oral or nasal vaccines formulated with chitosan NPs induced robust secretory IgA and systemic immune responses in poultry and pigs. The chitosan NPs enhances immunogenicity by targeting the cargo to mucosal antigen-presenting cells – specifically, M cells, dendritic cells, and macrophages in Peyer's patches, leading to strong local and systemic immunity [79]. Chitosan's appeal lies in its dual role as a delivery vehicle and an adjuvant; it can open tight junctions transiently to allow paracellular transport and itself can activate immune cells (it's known to have some immunostimulatory effect by triggering pattern recognition receptors like TLR2) [88]. DNA origami offers far more precision in structure and the ability to arrange antigens in defined patterns, whereas chitosan NPs are essentially with less controlled antigen distribution with antigens either encapsulated or surface-adsorbed randomly [89]. However, chitosan NPs are generally easier and less expensive to manufacture at scale compared to DNA origami, which requires precise DNA synthesis. In terms of safety, both are biocompatible chitosan degrades into glucosamine units which are nutrients, DNA origami degrades into nucleotides.

Polymeric micro/NPs (PLGA, Alginate)

PLGA is a biodegradable polymer used in many drug delivery systems and is FDA-approved for certain uses. It has been used for oral vaccines but less so in foods because it's synthetic (though its monomers lactic and glycolic acid are metabolic products). Alginate and pectin are plant polysaccharides often used in foods (gelling agents) and can form microcapsules for oral delivery [90]. For example, alginate beads can encapsulate antigens or probiotics and protect them until they reach the intestine. A 2023 study encapsulated inactivated influenza vaccine in alginate and observed good mucosal responses in mice [91]. DNA origami could potentially be

Table 1: Comparison of major edible nanocarriers for oral vaccine delivery.

Nanocarrier type	Material origin	Key properties	Immunogenicity	Typical applications	Ref.
DNA origami	Synthetic DNA	Programmable, food-grade, multivalent, biocompatible, customizable size	High (with antigens/adjuvants)	Oral vaccines, functional foods	[28, 78]
Chitosan **NPs**	Crustacean shells	Mucoadhesive, biodegradable, mild adjuvant effect	Moderate–high	Oral vaccines, drug delivery	[79, 80]
Alginate beads	Brown algae (seaweed)	Acid-resistant, dissolves at neutral pH, food-approved	Moderate	Antigen/probiotic delivery	[81, 82]
Liposomes	Phospholipids (soy/egg)	Biocompatible, fusogenic, can encapsulate proteins/nucleic acids	Moderate–high	Vaccine, nutrient delivery	[83]
Plant VLPs	Plant viruses	Robust, natural particulate, genetically programmable	High	Oral/mucosal vaccines	[84]
Milk exosomes	Cow's milk	Natural vesicles, oral bioavailability, scalable	Moderate	RNA/protein delivery	[85]

loaded into such alginate beads as well, benefiting from the pH-dependent gel dissolution: alginate remains solid in low pH (stomach) and dissolves at neutral pH (intestine). The downside is alginate capsules are often a few hundred microns in size, which might reduce direct uptake-but they release their nanoscale contents in the gut [91].

Lipid-based carriers

Lipid NPs gained fame in mRNA COVID-19 vaccines (though those are injected). For oral use, liposomes have been explored for decades. Liposomes made from phosphatidylcholine (from soy or egg) are generally considered safe (used as emulsifiers in foods) [92]. Orally, liposomes can fuse with cell membranes or be endocytosed. However, bile salts in the intestine can solubilize liposomes, so they need protective coatings. One relevant concept is the "lipid digestible nanocarrier" – formulating antigens in emulsions that form smaller particles upon digestion to aid absorption (like how fatty meals help absorb certain nutrients) [93]. DNA origami might not mix well with hydrophobic lipid interiors but could be displayed on the surface of liposomes via anchors (like cholesterol-modified DNA strands that insert into lipid bilayers) [94]. Indeed, DNA-lipid conjugates have been used to attach DNA nanostructures to lipid membranes for constructing hybrid systems [95]. A DNA origami anchored to a liposomal surface could present antigens while the liposome provides a large, protective and targetable carrier.

Biogenic NPs

This category includes VLPs from plants or bacteria, and exosomes or extracellular vesicles from foods (like nanovesicles from cow's milk or plants) [96]. Plant virus-based VLPs, such as Cowpea mosaic virus, have been given orally in animal models and can survive passage through the gut, inducing immune responses [97]. These naturally occurring nanostructures are robust, but their antigen cargo is limited to what you can genetically or chemically attach. DNA origami could complement by offering a customizable structure that mimics these VLPs. Milk exosomes are nanoscale vesicles that have evolved to be taken up by gut cells (infants absorb maternal milk exosomes). Researchers have considered using milk-derived exosomes to deliver drugs or RNA orally [98]. It's conceivable to load DNA origami into such vesicles – though loading efficiency and scale would be challenging. Still, the idea of using nature's own NPs in synergy with DNA origami is intriguing for future "bio-hybrid" edible vaccines.

Safety and regulatory perspective

A crucial aspect for any edible delivery system is regulatory approval. Materials like chitosan, alginate, gelatin, etc., are already used in foods or supplements, smoothing their path. DNA origami would likely be classified as a biologic or "biotechnology-derived" ingredient. Regulators will consider the source (if from synthetic DNA, no infectious risk; if from a virus scaffold, ensure purity and absence of replicative virus). Given that DNA is a normal part of diet and the sequences used do not code for any harmful proteins (they're mostly nonsense or antigen pieces), one can argue for its safety. However, large-scale manufacturing of DNA origami for human use may need to ensure no endotoxins (if produced in bacteria) and no toxic contaminants from synthesis [99]. The cost of DNA synthesis has dropped, but making, say, 100 mg of DNA origami (which could be needed for millions of doses) is still expensive compared to fermenting a batch of chitosan or growing a field of potatoes for an edible vaccine [100]. Thus, economic feasibility is as much a consideration as technical feasibility. Approaches like enzymatic rolling circle replication to produce scaffold, or using optimized bacterial production for staple strands, might be necessary to scale up DNA origami economically.

Comparative effectiveness

Traditional oral vaccines like attenuated viruses have the advantage of replicating (amplifying the antigen) but come with safety risks. Non-replicating approaches (subunits in carriers) are safer but historically less immunogenic. Nanocarriers aim to bridge that gap by focusing the antigen to the right place and protecting it from destruction. A chitosan NP oral vaccine vs. a DNA origami oral vaccine might each have pros and cons. Chitosan NP could induce strong IgA due to mucoadhesion, but DNA origami might induce a more controlled response with less risk of tolerance because of its precise antigen display optimizing B cell activation. Also, DNA origami can incorporate multiple signals (antigen + CpG + targeting ligand) in one construct rationally, whereas with chitosan one often mixes antigen and adjuvant and relies on co-delivery by chance. The ability to fine-tune the spatial arrangement of immunostimulatory components on DNA origami is a unique strength that few other edible nanocarriers have [101].

One should also consider immunogenicity of the carrier itself. Chitosan and alginate are mostly immunologically inert or mildly adjuvanting, which is fine. DNA origami, being DNA, could in theory break tolerance if not careful – though as discussed, it seems not strongly immunogenic on its own. Still, repeated dosing might risk anti-DNA antibodies (like those in autoimmune diseases). However, the studies so far in mice did not report any anti-DNA antibody development [102]. Additionally, using sequence designs that avoid TLR9 over-activation (avoiding excessive CpG content unless intended as adjuvant) can minimize innate responses to the DNA itself.

Mucosal immunity targeting potential and applications

Mucosal immunity is the immune protection that operates at mucosal surfaces (gastrointestinal, respiratory, urogenital tracts, etc.), primarily mediated by secretory IgA antibodies, tissue-resident memory T cells, and innate elements like mucosal macrophages and dendritic cells. Vaccines that can induce mucosal immunity are highly desirable for diseases where pathogens enter through those routes (e.g., enteric infections like rotavirus, cholera, norovirus, or respiratory infections like influenza and SARS-CoV-2). Targeting mucosal immunity means formulating vaccines that, when administered at mucosal sites (oral, nasal, sublingual), elicit robust local immune responses (IgA, mucosal T cells) as well as systemic immunity (IgG in circulation) [103].

One key advantage of mucosal vaccination is the concept of dual immunity: as Thakur and Foged [104] explain, a successful mucosal vaccine induces immune responses both at the

mucosal surface and systemically. For example, an oral polio vaccine (Sabin) not only generates IgG in the blood (protecting against paralysis) but also IgA in the gut (preventing infection and transmission). Traditional injected vaccines usually elicit strong IgG and circulating T cells, but very little IgA in the mucosa [105]. This is why someone vaccinated intramuscularly for a respiratory virus might still get a mild infection in the nose (virus can replicate superficially before IgG reaches it)-whereas a mucosal vaccine could potentially neutralize the virus at the entry point.

To achieve mucosal immunity via oral route, the vaccine must engage the specialized immune structures in the gut, namely the GALT. GALT includes the Peyer's patches in the small intestine, isolated lymphoid follicles, and mesenteric lymph nodes [58]. Peyer's patches are essentially immune sampling stations: they have M cells that ferry antigens from the gut lumen to the patch, where underlying dendritic cells, B cells, and T cells can mount responses [58]. Targeting GALT is crucial-if an oral vaccine antigen simply gets digested and absorbed as nutrients, it will induce tolerance or nothing at all. If it reaches GALT in the right form, it can initiate a productive immune response [106]. DNA origami nanostructures are well-suited to target mucosal immunity for several reasons:

Particulate nature: The immune system in Peyer's patches has evolved to respond to particles (like microbes). M cells preferentially take up particles in the ~50 – 2000 nm range. DNA origami typically is 20 – 100 nm in size, which is within the sweet spot of efficient M cell uptake. Their size and repetitive surface can stimulate B cells directly in the patches (for T-independent antigens or at least for initial B cell activation) [106].

Customizable targeting: As discussed, ligands like β-glucans can direct origami to M cells. Other targeting moieties could include integrin-binding peptides (M cells express α_vβ_3 integrins that some pathogens target), or chemokine motifs to attract dendritic cells [107]. By functionalizing DNA origami, one can ensure it not only is taken up but perhaps even directs which immune cell type processes it.

Promotion of tolerogenic vs immunogenic outcome: The gut has a tendency towards tolerance (to avoid reacting to food proteins and commensals). An oral vaccine must overcome this. Typically, strong adjuvants or particulates are needed to flip the switch towards an active immune response (as opposed to oral tolerance which can happen with soluble antigen feeding). NPs like DNA origami provide danger signals by their particulate form (dendritic cells often interpret particulate antigen as pathogenic, especially if accompanied by PRR agonists like CpG) [108]. Additionally, CpG or other TLR ligands on origami provide a direct activation signal to dendritic cells, encouraging them to stimulate T cells rather than induce tolerance. Thakur and Foged [104] note that adjuvants for mucosal vaccines are often essential due to the need to overcome the default tolerogenic environment. DNA origami can carry those adjuvants as part of its structure (e.g., unmethylated CpG DNA, or potentially RNA motifs, or could even be combined with mucosal adjuvant molecules like cholera toxin subunit if chemically attached) [75].

Induction of IgA: The hallmark of mucosal immunity is secretory IgA, which is dimeric IgA that transcytoses into the lumen and neutralizes pathogens without causing inflammation. Studies of oral nanoparticle vaccines (including chitosan NPs and others) commonly measure antigen-specific IgA in feces or intestinal fluid as a readout [109]. For instance, in the yeast glucan-coated DNA nanoparticle vaccine study, they observed high RBD-specific secretory IgA in the intestines of vaccinated mice-a strong indicator that the vaccine effectively engaged mucosal B cells in Peyer's patches (which class-switch to IgA) [110]. Achieving high IgA titers is important for blocking infections at the portal of entry. The precise geometry of antigens on DNA origami (multivalent display) likely helps robust IgA production by clustering B cell receptors effectively in the Peyer's patch germinal centers (as B cells there will preferentially respond to repetitive antigens, a phenomenon known from immunology that highly ordered antigens can directly cross-link BCRs and provide a strong activation signal) [58].

Mucosal T cell responses: Effective mucosal vaccines also generate T cells that home to mucosal sites. Tissue-resident memory T cells (T_RM) in the lungs or gut can provide rapid local protection. Oral DNA origami vaccines delivering protein antigens should generate CD4+ T helper cells in Peyer's patches that help B cells produce IgA, and possibly CD8+ T cells if some antigen crosses into the MHC I pathway (either via cross-presentation or if antigen gets into the cytosol of dendritic cells) [1]. The yeast-coated DNA origami vaccine, for example, triggered extensive cellular immunity in the intestinal mucosa (indicated by assays of T cell activity). This suggests that T cells were primed effectively. Achieving good T cell responses orally is non-trivial; it likely requires that the antigen be presented by dendritic cells in the context of co-stimulatory signals (i.e., an activated DC thanks to an advert). DNA origami's ability to carry CpG provides such co-stimulation triggers via TLR9 [35].

Potential applications of mucosal targeting DNA origami functional foods span a range of diseases. Obvious ones are gastrointestinal infections (e.g., norovirus, rotavirus, cholera, enterotoxigenic *E. coli*), where an oral vaccine can produce local antibodies to neutralize pathogens in the gut lumen. Edible DNA origami vaccines for these could be delivered in an infant formula or a weaning food to immunize children in endemic regions. Another set of targets are respiratory viruses like influenza or coronaviruses-while one might consider nasal delivery for those, an oral vaccine that induces strong IgA in both gut and respiratory tract is possible due to the common mucosal immune system. It's known that gut immunization can sometimes confer immunity in the respiratory tract (because activated B and T cells in GALT can migrate to other mucosal sites, guided by imprinting factors like vitamin A metabolite that cause them to express homing receptors). Thakur and Foged [104] mention that immune responses elicited at one mucosal site can disseminate to distal mucosal surfaces. Thus, an oral vaccine might protect the lungs or reproductive tract by generating a pool of IgA-secreting cells that home generally to mucosa-associated lymphoid tissues (Table 2).

Table 2: Edible DNA origami nanostructures for oral therapeutic and functional food applications.

Cargo/Application	DNA origami formulation	Oral delivery outcome	Ref.
Insulin (diabetes)	DNA-based pH-responsive hydrogel microcapsule	Protected insulin from gastric degradation; released in intestine; lowered glucose in diabetic rats, comparable to injections; no toxicity	[111]
Vildagliptin (I)	DNA nano-triangles, Eudragit L100 polymer-coated (enteric NP)	~95% drug encapsulation; sustained release (~15 h); improved glycemic control in mice; no pancreatitis or toxicity	[112]
Vildagliptin (II)	DNA nanocubes + poly-L-lysine and alginate coating	~90% drug loading; pH-dependent release (~13 h); better GLP-1, glycemic control, reduced dosing, minimal side effects	[113]
Probiotics	DNA-polymer hydrogel matrix encapsulating live bacteria	~10× improved probiotic survival in gastric acid; effective during cold storage; food-grade and safe	[114]

Despite the promise, there are hurdles. One is the dose-mucosal vaccines often require higher antigen doses than injections because so much is lost or not taken up. Edible vaccines might need to be given in gram quantities of material, which is fine if the cost is low but for DNA origami that could be expensive [5]. Another is individual variability-gut conditions differ from person to person (pH, microbiota, etc.), which might affect how well a given formulation works. Some people might have robust M cell uptake, others less so; the microbiome might degrade or even capture the DNA origami (certain gut bacteria produce DNases or might bind DNA for nutrition) [115]. Still, has the potential to increase vaccine reach and compliance, especially in populations with barriers to traditional injection-based immunization.

Challenges and Future Directions

The development of edible DNA origami nanostructures for oral vaccine delivery, while promising, comes with a set of challenges that must be navigated through future research and innovation. In this section, we outline the major hurdles-scientific, technological, and regulatory – and discuss potential strategies to overcome them [116]. We also highlight future directions that could broaden the applicability and effectiveness of this approach.

Manufacturing and scale-up

One of the most immediate challenges is producing DNA origami nanostructures in the quantities and cost structure required for widespread vaccine use. Traditional DNA origami assembly uses synthetic oligonucleotides for staples and often the M13 bacteriophage genome (~7 kb) as a scaffold [100]. While these are readily made at microgram to milligram scale for laboratory studies, producing kilograms of DNA (which might be needed for millions of oral vaccine doses) is non-trivial. Biotechnological solutions will be needed – for example, enzymatic DNA synthesis and amplification. Rolling circle amplification could generate long single-stranded scaffolds in large quantity from a short circular template, potentially providing scaffold material cheaply (provided the template design allows for the desired origami folding) [21]. For staples, approaches like asymmetric polymerase chain reaction or the use of modified bacteria or yeast to produce single-stranded DNA have been explored.

Stability vs delivery trade-off

We discussed stability in the gastrointestinal tract extensively. A challenge is that measures to increase stability (like more crossovers, covalent bonds, heavy coatings) can sometimes reduce the bioavailability of the antigen [117]. For instance, if DNA origami is made too rigid or heavily coated, it might not disassemble adequately inside antigen-presenting cells to release antigenic peptides for presentation. There is a fine balance: the origami should be stable enough to reach immune cells but then be processable by those cells. Future research could focus on developing stimuli-responsive DNA origami nanostructures that remain stable during gastrointestinal transit but selectively disassemble or release their cargo in response to intracellular triggers-such as endosomal pH changes or specific enzymes-within immune cells. Such smart designs, already being explored in drug delivery, could enhance antigen release and improve vaccine efficacy [118].

Immune response optimization

While initial studies show strong immune responses are achievable, there's much to optimize. The Nature Communications study systematically varies antigen spacing and clustering, but only for one antigen (SARS2 RBD). Different pathogens might require different arrangements-for instance, a densely packed array might work for one antigen, but another might need to be spaced out to avoid steric hindrance of antibody binding [119]. Also, if multiple antigens are displayed (say for a multivalent vaccine against several viruses), Future research will likely use high-throughput techniques and computational modeling to navigate this design space. DNA origami offers so many degrees of freedom that empirical optimization could be laborious; computational immunology models might help predict B-cell epitope spacing needs or T-cell epitope processing from DNA carriers [120]. Additionally, the longevity of mucosal immunity induced by edible DNA origami vaccines needs study. Systemic vaccines often require boosters; mucosal IgA can wane relatively quickly after oral vaccines. It will be important to determine if DNA origami vaccines induce strong memory B-cell pools in the Peyer's patches or if periodic re-dosing is needed.

Safety and tolerability

DNA origami appears safe in animal studies [29, 34, 41, 113, 121]. However, when moving to humans, thorough safety evaluation is required. One theoretical concern is whether repeated exposure to exogenous DNA might trigger anti-DNA autoimmune reactions or precipitate conditions like systemic lupus erythematosus in susceptible individuals. Lucas et al. [7] saw no significant anti-DNA immune reaction in mice, but

human immune systems might differ. Monitoring anti-DNA antibody titers in any trials would be prudent. Another concern is inflammation in the gut: a very potent oral adjuvant (like too much CpG) could cause local inflammatory responses, possibly manifesting as transient diarrhea or gut discomfort. Dose-escalation studies in preclinical models will have to find a dose that is immunogenic but not overtly reactogenic [7]. Encouragingly, DNA is gentler than some other adjuvants (like cholera toxin, which, while effective, causes diarrhea). The materials used must also be non-allergenic and free of unwanted components.

Regulatory and acceptance issues

Regulatory bodies play a major role classifying what an edible DNA origami vaccine is (a food, a drug, or a biologic). It might be regulated as a vaccine (biological product) even if delivered in a food format, because its intended use is disease prevention. This could complicate bringing it to market, as it might need to meet both food safety regulations and drug efficacy standards. Clear demonstration of safety, consistency, and efficacy in controlled trials will be important to approval. Public acceptance is another aspect. Some consumers might be wary of this due to misconceptions, equating it with genetic engineering or unnatural substances. Clear public communication will be essential to address misconceptions regarding DNA nanotechnology in foods. The term "edible vaccine" itself, while exciting to scientists, has historically faced some skepticism-for instance, concerns about dosage control (how to ensure someone ate enough of the vaccine), or about vaccine stability in varying conditions of cooking or digestion. These concerns can be addressed by careful product design (e.g., a measured dose format like a capsule or sachet ensures correct intake and instructing to take with cold or room temp beverage rather than hot to avoid denaturation, etc.).

Personalization and next-gen concepts

Looking ahead, DNA origami could potentially be tailored to individual or demographic needs. For example, for populations with certain HLA types, one might include specific T-cell epitopes on origami to ensure coverage [122]. Or for an immunosenescent elderly population, one might incorporate stronger adjuvants or more copies of antigen to overcome weaker responses. There is also the interesting concept of DNA origami as oral immunotherapy for allergies or autoimmunity by inducing tolerance intentionally-ironically, this would be the opposite goal (designing it to promote tolerogenic presentation) [123]. Another potential direction involves exploring alternative nucleic acid architectures, such as RNA origami or hybrid DNA-RNA structures, which may offer distinct immunostimulatory properties if adequately stabilized for oral use. While mirror-image L-DNA origami is highly resistant to nuclease degradation, its non-natural backbone may hinder immune processing and antigen presentation, making natural D-DNA the preferable scaffold for oral vaccine applications [124].

Integration with other technologies

Future edible vaccines might not rely solely on DNA origami. We could see multi-component systems-for example, a scenario where plant-based production and DNA origami merge where plants could be engineered to produce many scaffold strands or staples, or even to assemble simpler DNA nanostructures *in vivo*. Or DNA origami could be combined with microneedle patches for oral cavities (some studies use patches that dissolve in the mouth-technically still mucosal but not gastrointestinal track). This would combine synthetic biology and DNA nanotech. It's far-out, but not impossible that the bacteria can be made to display proteins (like antigen anchors) that could potentially bind a DNA origami via biotin-streptavidin or complementary base pairing. Then you feed the bacteria to the person (like a probiotic), and it sets up shop and acts as a vaccine depot. This could maintain long-term mucosal immunity without repeated dosing. However, many safety concerns and controls are required with this [26].

Delivery of other functional molecules

While vaccines are the focus, edible DNA origami could also deliver immunomodulatory compounds as part of a functional food – e.g., delivering cytokine mimics or siRNAs to modulate gut immune responses (for inflammatory diseases) [5]. If the DNA origami platform proves safe and ingestible, it could be repurposed beyond vaccines, to general oral nanomedicine (for instance, oral gene therapy of gut epithelium by carrying mRNA or Cas9 RNPs, although crossing into cells actively would be needed).

Conclusion

Edible DNA origami nanostructures represent a transformative frontier at the intersection of nanotechnology, immunology, and food science, with the potential to reshape oral vaccine delivery and functional food design. This review has highlighted the unique advantages of DNA origami-including programmability, biocompatibility, and structural precision-as a food-grade platform for presenting antigens and immunostimulatory molecules. Key studies demonstrate that these nanostructures can be engineered for enhanced mucosal and systemic immune responses, outperforming conventional edible vaccine strategies. The modularity of DNA origami enables precise antigen display, incorporation of adjuvants, and functionalization for targeted delivery, all within an edible format compatible with functional foods and oral dosage forms. However, the translation of this technology faces challenges such as large-scale, food-grade manufacturing, maintaining stability through gastrointestinal transit, and ensuring robust, long-lasting immune memory. Regulatory pathways, public acceptance, and safety will also require careful consideration. Despite these hurdles, ongoing advances in DNA synthesis, oral delivery systems, and structural immunology provide promising solutions. Future research should focus on optimizing antigen presentation, scalable production, and clinical validation of immunogenicity and safety. There is significant potential for multivalent vaccines, personalized formulations, and new applications beyond infectious diseases-including allergy and autoimmunity modulation. Overall, edible DNA origami nanostructures offer a highly adaptable and innovative approach to oral vaccination, with far-reaching implications for global health and accessible immunization. With contin-

ued interdisciplinary effort, these platforms may soon enable effective, convenient, and equitable vaccine strategies that meet the needs of diverse populations worldwide.

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None.

Conflict of Interest

None.

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