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Review Article

CHITOSAN-BASED NON-PARTICULATE VACCINE DELIVERY, CHALLENGES, AND FUTURE DIRECTIONS

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ABSTRACT

Vaccination, a cornerstone of modern medicine, faces challenges with traditional injection methods, including pain, safety concerns, and cold-chain distribution issues. This review explores the potential of chitosan-based non-particulate vaccine delivery systems as a patient-friendly and effective alternative. Chitosan, a biocompatible and biodegradable polysaccharide, offers inherent mucoadhesive properties and a positive charge, facilitating interaction with mucosal surfaces and negatively charged antigens. Non-particulate systems (solutions, films, and hydrogels) offer advantages such as simplified manufacturing, antigen stability, and controlled release. This review focuses on the properties of chitosan relevant to non-particulate vaccine delivery, including physicochemical characteristics (molecular weight, degree of deacetylation, viscosity) and biological properties (biocompatibility, biodegradability, antimicrobial/antiviral potential). It examines the formulation considerations for chitosan-based hydrogels, films, and solutions, highlighting their mechanisms of action and applications in mucosal vaccine administration. The review further addresses current challenges, such as dosage control and long-term stability, and discusses future opportunities for innovation in this rapidly evolving field, with an emphasis on recent trends in nasal and oral vaccine delivery.

Keywords: Chitosan, Non-particulate vaccine delivery, Vaccine administration, Drug delivery systems, Vaccine efficacy.

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INTRODUCTION

Vaccination stands as a cornerstone of modern medicine, a powerful tool in preventing infectious diseases and saving countless lives [1]. Although the conventional vaccine delivery technique, which is through injections, is effective, it comes with several challenges, including pain, lesser safety for those administering the vaccines through reduced risk of needle-stick injuries, storage and transportation of vaccines, particularly in developing countries where cold-chain distribution is difficult. This has created the emphasis on the other methods of delivering the vaccines, and recently, the non-intramuscular injection methods have received wide popularity as a more patient-friendly, accessible, and potentially safer way for administering the vaccines [2]. The quest for these next-generation vaccines is focused on improving efficacy while minimizing the drawbacks associated with current injection-based methods.

Compared to other polymers that are currently undergoing experimental testing for efficient drug and vaccine delivery, chitosan appears to be one of the most suitable options. Chitosan is a natural, non-toxic biopolymer poly saccharide produced by the deacetylation of chitin, the second most abundant natural biopolymer after cellulose [3]. Its structure comprises a linear chain of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units, exhibiting a remarkable array of properties that make it suitable for biomedical applications [4]. Chemical structures of chitosan and its derivatives (Fig. 1). Chitosan is biocompatible and biodegradable, meaning it is well-tolerated by the body and breaks down naturally. Moreover, it possesses inherent mucoadhesive properties, allowing it to adhere to mucosal surfaces, and a positive charge, which facilitates interactions with negatively charged molecules, including many biological agents [5]. These properties have led to its established use in various biomedical applications, from wound dressings to drug delivery systems (Fig. 2).

Although interest in chitosan as a carrier for particulate systems such as nanoparticles (NPs) has been focused a lot of attention, the use of chitosan in non-particulate systems deserves consideration. Non-particulate delivery system include solution, film and hydrogel that are the systems that do not make use of encapsulation or aggregation of the vaccine antigens [6]. These simpler systems suggest several potential benefits for vaccine delivery, including easier and more efficient manufacturing, potential stability of antigens, and, in the case of some hydrogels, controlled distribution of the antigens. However, in non-particulate systems, the vaccine antigen may be more readily contacted with mucosal tissues, which are involved in initiation of at least part of the immunogenic response [7]. For example, research exploring mucosal delivery routes such as nasal and sublingual administration have highlighted the potential for non-particulate formats, particularly given the challenges of producing nanoparticulate systems on a large scale with rigorous quality control.

The synergy between chitosan's inherent properties and the advantages of non-particulate delivery platforms makes their combination a compelling approach for innovative vaccine strategies the mucoadhesive properties of chitosan might also help to prolong the exposure of non-particulate vaccines at mucosal surfaces and facilitate efficient uptake by antigen presenting cells as well as local immune responses [8]. The positive charge could enable the reaction with negatively charged antigens and further improve the vaccine's stability, especially at poorly controlled temperatures. Furthermore, chitosan has some peculiarities, as it is biocompatible and biodegradable, and thus can be used for non-particulate formulations. Thus, administration of the antigen through chitosan-based gels can be modified in such a way that will afford the same important stimulation of the immune response but using fewer administrations. The potential of chitosan-based non-particulate systems has been revealed in few studies of recent times, especially in the nasal and oral vaccine administration, in provoking mucosal immunity [9,10].

This review article therefore focuses specifically on chitosan-based non-particulate vaccine delivery systems, exploring their advantages over traditional and particulate approaches, highlighting current research,

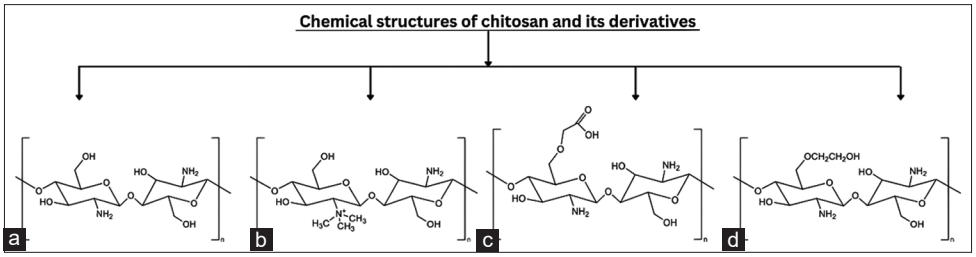

Fig. 1: Chemical structures of chitosan and its derivatives. (a) Chitosan, (b) Trimethyl chitosan, (c) Carboxymethyl chitosan (d) Glycol chitosan. Created in canva

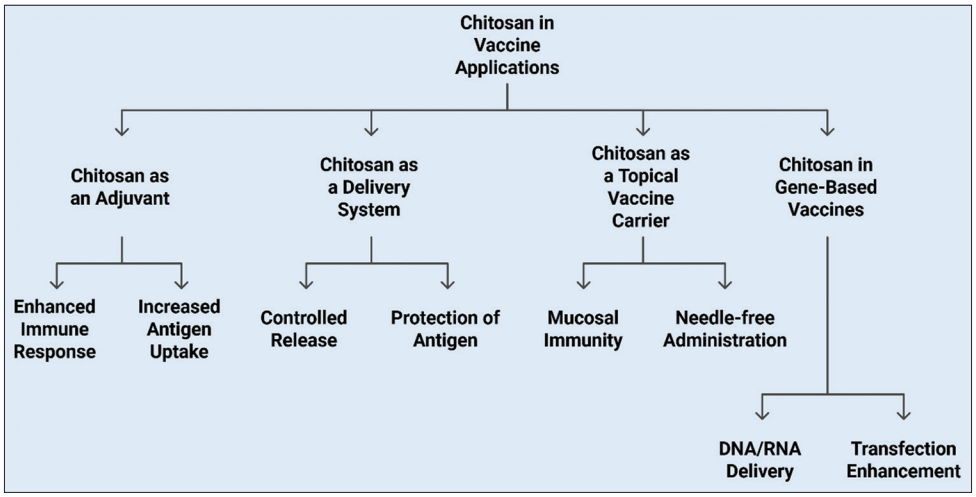

Fig. 2: Schematic representation of different vaccine applications of chitosan and its derivatives. Created in canva

and identifying existing challenges and future possibilities. We will begin by exploring the mechanisms behind chitosan's interaction with biological tissue and antigens, setting the stage for subsequent discussions on the diverse non-particulate formulations, including solutions, films, and hydrogels, and how these formulations are being used for vaccine delivery. The review will discuss advantages, from improved mucosal administration to improved stability of vaccines. Following a review of the literature, we will delve into the current challenges in this field, such as dosage control and long-term stability, and propose solutions. Finally, we will present the future potential of non-particulate chitosan vaccine delivery, focusing on the latest trends and opportunities for innovation in this rapidly evolving field. The next sections aim to provide a thorough evaluation of the current knowledge base to better understand the potential of this method in the vaccine field.

CHITOSAN PROPERTIES RELEVANT TO NON-PARTICULATE VACCINE DELIVERY

The efficiency of chitosan as the delivery vehicle, especially in non-particulate systems, is dependent on the property inherent to chitosan. The reader is reminded that these properties not only delineate its functioning in the biological context but also code its capacity for stabilization, for protection, and delivery of vaccine antigens. Knowledge of these properties is thus crucial for the effective formulation of chitosan-based vaccines.

Physicochemical properties

Three main parameters in chitosan, specifically molecular weight (MW), degree of deacetylation (DD), and viscosity have been found to play a significant role in the behavior and effectiveness of the delivery system. MW, often between a few kDa and over 1,000 kDa, influences the degree of chain entanglement of the polymer and thus its viscosity, solubility, and permeability. Chitosans with higher MW thus possess higher viscosity and solution or gel thickness that may serve to advantage when encapsulation and controlled release or retention at mucosal sites is desired [11]. Conversely, lower MW chitosans often demonstrate improved solubility and greater mobility, which can be advantageous for applications requiring rapid drug or antigen release. Similarly, the DD, referring to the percentage of glucosamine units in the polysaccharide chain, influences chitosan's positive charge density and its solubility. An increase in DD leads to revelation of free amino groups, thus an enhancement of the positive charge making the peptide solubility to improve as the solution becomes more acidic. These distinctions affect the behavior of the material with other molecules and the cellular membranes, which affects the total delivery capability. Viscosity as influenced by MW and DD is an important determinant of formulation and administration of the feedstuff [12,13]. For example, high viscosity solutions might be ideal for topical or mucosal applications where retention is key but unsuitable for spray administration, which demands lower viscosities.

The presence of positively charged amino groups on chitosan's backbone is a pivotal characteristic that sets it apart from many other biomaterials. This positive charge enables the compounds rich in the biological environment, especially the negative charged ones, to have strong electrostatic attractions to the charged molecule. The main constituent of the mucosal layer is mucin with highly charged and glycosylated groups that can form good bonding interfaces to chitosan. This type of spraying contributes to the formation of electrostatic attraction because of chitosan, which makes it have good mucoadhesive properties, making it to attach well to mucosal tissues. The increased adhesion enhances the extent of time the formulation spends at the site of application, facilitating greater uptake of antigens by immune cells present in the mucosal tissues [14]. Moreover, the positively charged chitosan can also combine with negatively charged vaccine antigens, and this shows the ability to enhance the stability of the antigen within the delivery system and its encapsulation. A premiere has been devoted to the enhancement of MW and DD of chitosan to meet the delivery

of the vaccine, the stability of the formulation, and the biological response [15].

Biological properties

Chitosan biocompatibility and biodegradability give it a definite edge in vaccine delivery over other drug delivery systems. It befits the immunological interface to have certain thresholds of biocompatibility that would allow repeated interactions with the body's tissues without provoking toxicological reactions associated with repeated administrations in schemes of vaccination. Chitosan is biodegradable, and this is through the assistance of enzymes that are naturally produced in the body and once chitosan is in the body it is broken up into harmless byproducts that are easily processed by the body. This eliminates long-term accumulation problems and greatly minimizes the hazards that come with using non-biodegradable synthetic products. Such properties prove chitosan as a safe and stable material to be used as an adjuvant in the formulation of vaccines [16].

Furthermore, antimicrobial and antiviral potentials of chitosan are beneficial for the physical and chemical stability of vaccines. Against bacterial and fungal contamination during manufacturing and storage of formulations, it has the capacity to protect vaccines via its antimicrobial attributes that come in handy where compliance to the cold chain is an issue. The antiviral properties, especially effective against some enveloped viruses, further enhance the formulation, potentially reducing the risk of contamination and thereby increasing its efficacy and safety profile. Ongoing research aims to maximize these properties to enhance both vaccine efficacy and formulation robustness using chitosan-based systems [17,18].

Formulation considerations

This biopolymer can also be subjected to different modifications if the improved performance of chitosan-based delivery system for vaccines is desirable. Further chemistry can be performed to modify chitosan properties regarding solubility, mucoadhesion, and the interaction with tissues through grafting with the polymers of polyethylene glycol or conjugation with targeting ligands. The changes allow an enormous level of control over chitosan in terms of its property alterations to match the requirements of delivery, for instance, enhanced solubility or ability to deliver substances to particular cells of the mucosal membrane [19]. Optimizing these factors leads to the design of more effective chitosan delivery systems.

The behavior of chitosan in non-particulate formulations is significantly influenced by pH, ionic strength, and temperature. Chitosan solubility increases with increasing the pH, which means that the formulation pH must be controlled for polymer stability. In addition, ionic strength and fluctuations in temperature similarly influence the charge, viscosity, and overall stability of the polymer. Further, these factors should be managed by the best formulation approaches, including the right buffers and stabilisers, in a bid to complement the efficiency and stability of non-particulate chitosan-based vaccines, especially when stored or transported. More current investigated work has been dedicated to preparing thermo-sensitive chitosan hydrogels for vaccines, which may also a trigger effect; this can be an upcoming hot area in the field of investigation [20].

CHITOSAN-BASED NON-PARTICULATE VACCINE DELIVERY SYSTEMS

The use of chitosan in vaccine delivery is gaining traction due to its inherent advantages. Non-particulate approaches, on the other hand, are concerned with chitosan in solution or gel form or the form of moisture-resistant chitosan film representing pharmacokinetics/absorption, distribution vehicles. The difference is observed to have a major influence on how the vaccine works with the host's immune response.

Chitosan hydrogels

Hydrogels are the dosage forms that consist of a large amount of water obtained due to the processes of cross-linking of highly water-soluble polymers. Different cross-linking agents known as gelling agents are used to control the release characteristic of the hydrogels [21]. Thermosensitive hydrogels are among the most thoroughly researched types of hydrogels. These materials exist in a liquid state at the ambient temperature, but when implanted into the body, they change their phase to the solid phase because of an increase in temperature. This transformation is mainly a result of the decrease in dielectric constants and the removal of electrostatic interaction between the polymer and crosslinker as well as the formation of hydrophobic interactions between polymer chains as dehydration increases because of heating (Fig. 3[1]) [14,22].

Hydrogels are proving to be versatile delivery vehicles for immunotherapy agents administered locally. Their efficiency in various administration routes, including intratumoral or peritumoral delivery, intra-peritoneal subcutaneous administration, intracranial administration, intravesical administration, and pulmonary administration has been considered (Figs. 3 and 4) [23-27].

Chitosan hydrogels: Properties and mechanisms

The ability of chitosan to act as a hydrogel-forming agent is due to its cationic nature of it; polyelectrolyte complexes with concurrent anionic polymers are formed by chitosan. These complexes can also greatly increase the stability of the antigens which are encapsulated, preventing breakdown in extreme conditions as are found in the Gastrointestinal Track [28]. Furthermore, the capability of chitosan to form NPs offers other possibilities in the controlled and site-specific delivery of antigens [29,30]. Research has highlighted various preparation methods for chitosan NPs, which can be tailored to optimize encapsulation efficiency and release profiles [31]. As shown in (Fig. 4), the physicochemical characteristics, such as cross-linking and hydrophobic/hydrophilic balance of these chitosan-based systems, are found to have a great influence over their drug delivery potential [32].

Microneedles (MNs)

The global landscape in terms of vaccine delivery is in the process of changing the conventional method of injection with hypodermic needles to other methods of delivery that are more acceptable to the patients as well as more effective. Among these innovations, MNs have turned out to be a promising and unique platform, especially when made of biocompatible and biodegradable material such as chitosan. Chitosan is correctly chosen as a base material for MNs fabrication because it is a natural polysaccharide derived from chitin, which has biocompatibility, biodegradability, mucoadhesive, and the ability to encapsulate and release a drug characteristic. Out of these, chitosan MNs are highly promising for painless transdermal vaccine delivery if certain drawbacks of standard administration techniques are considered. Conventional methods have their advantages, but implementing them can cause people stress and anxiety, and requires skilled professionals for the injections, which is a problem in mass vaccination campaigns as well as in developing countries. These problems are solved in chitosan MNs by the formation of micro-scale channels passing through the stratum corneum of the skin, thus ensuring the quick and direct delivery of the vaccine antigens to the target epidermal and dermal layers containing the immune cells. In particular, the effective delivery of the vaccine to specific individuals is important for developing specific immunity. In general, the MNs penetrate only to tens to hundreds of micrometers into the skin, thus avoiding nerve endings and blood vessels and thereby patients' pain and bleeding, which are characteristic of hypodermic injections [33,34]. In ddition, the characteristic physicochemical properties of chitosan enable better uptake of antigens by the antigen-presenting cells (APCs), including Langerhans cells and dermal dendritic cells [35]. This reduces the bitter experiences users are likely to have with the MNs while at the same time promoting successful and efficient uptake of the antigen by the targeted cells due to the positive charges of chitosan and the negative charge of the cell membranes [36,37]. APCs are present in large number on the skin in the form of Langerhans cells and dermal dendritic cells (Fig. 4).

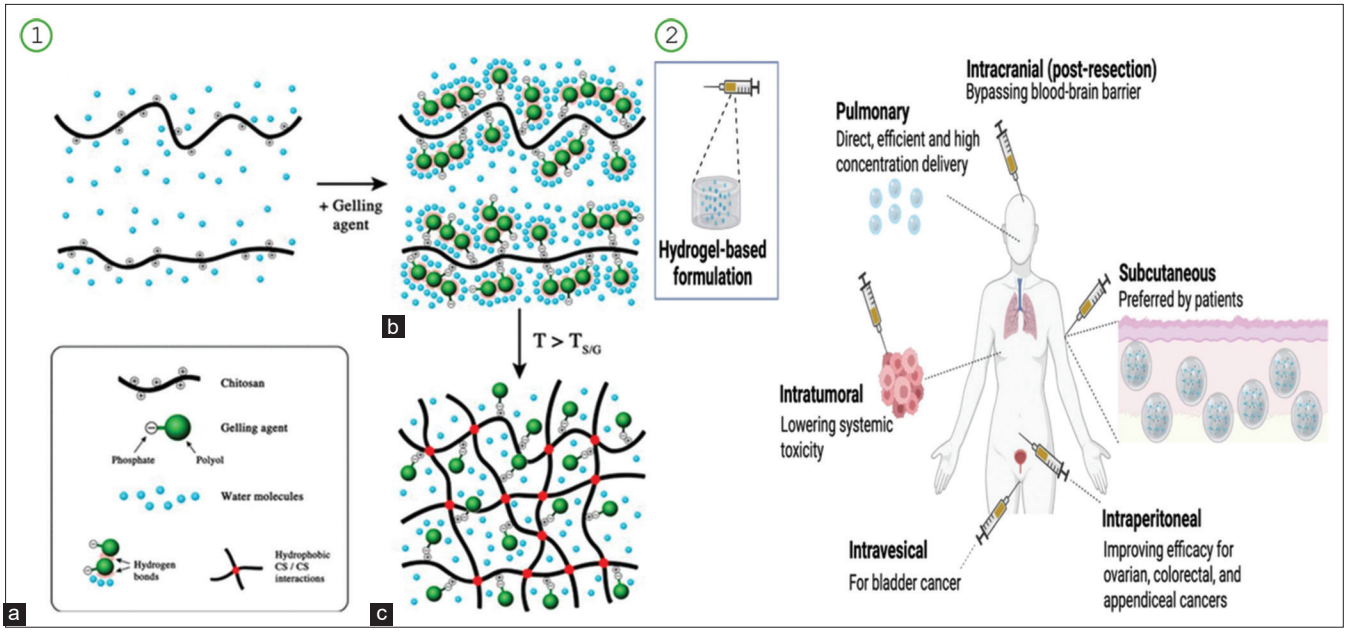

Fig. 3: (1) The process of gel formation in thermosensitive chitosan hydrogels is influenced by rising temperatures. This can be described in three stages: (a) the initial chitosan solution; (b) a combination of the chitosan solution and gelling agent at room temperature; and (c) the development of chitosan hydrogels when the temperature exceeds the sol/gel transition temperature (TS/G). This information is adapted with permission from Supper S, Anton N, Seidel N, Riemenschnitter M, Schoch C, and Vandamme T, who conducted a rheological study on chitosan/polyol-phosphate systems, examining how the polyol component affects the thermo-induced gelation process. Langmuir. 2013; 29 (32): 10229–37. DOI: 10.1021/la401993q. Copyright 2013 American Chemical Society. (2). Hydrogels are proving to be versatile delivery vehicles for immunotherapy agents administered locally (i.e., not intravenously). Research has explored their use for a range of administration routes, including intratumoral or peritumoral, intraperitoneal subcutaneous, intracranial, intravesical, and pulmonary delivery. This adaptability makes hydrogels suitable for targeting different tumor types and locations [24]. © 2023 The Author(s). Published by Elsevier Ltd

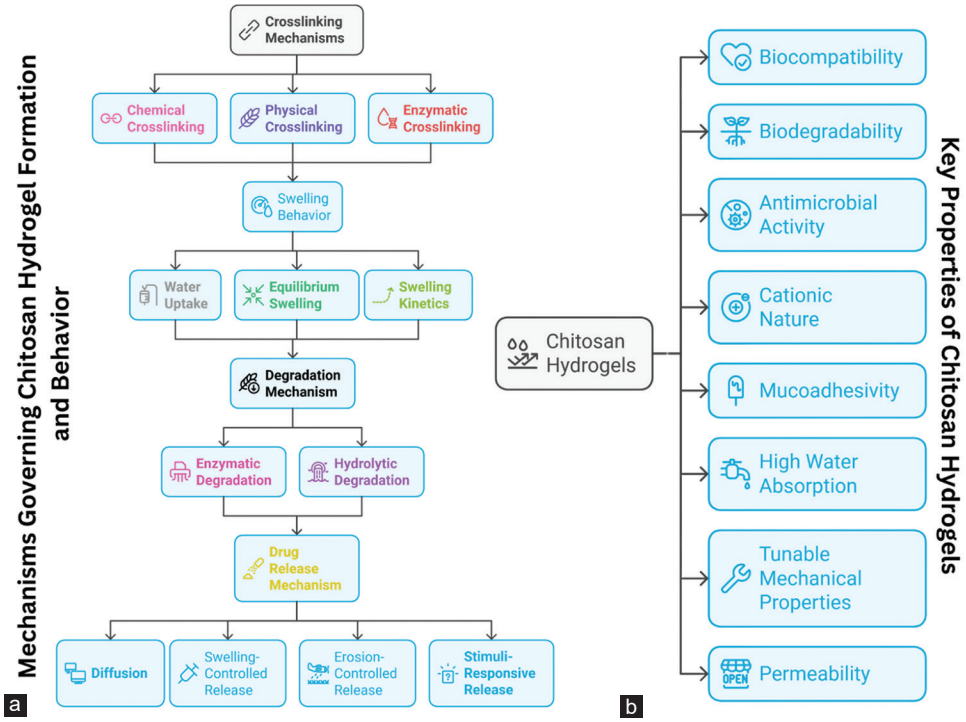

Fig. 4: A visual representation of the mechanisms governing chitosan hydrogel formation and behavior (a), alongside the key properties of chitosan hydrogels (b)

Furthermore, the immune activation mechanisms following microneedle administration, including the interaction of dendritic cells with T-cells and B-cells, and the production of antibodies, are illustrated in Fig. 5.

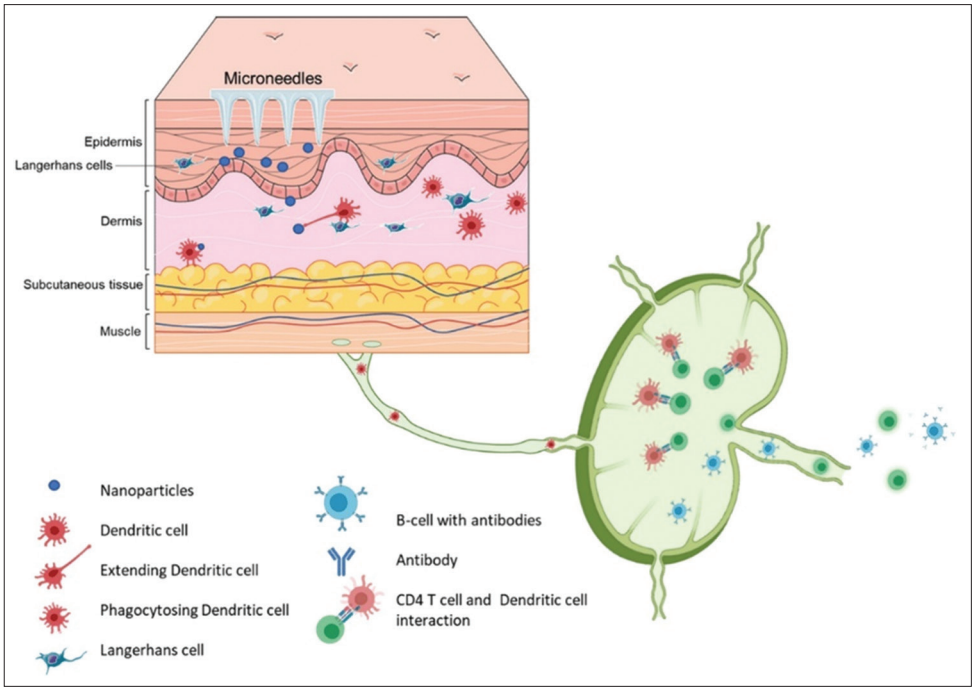

Fig. 5: Illustration of immune cells activation after using the microneedle vaccines. © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/) [38]

Chitosan is biodegradable and therefore, the MNs will dissolve within the skin, which removes the cleaning process and reduces the chances of having infection or foreign body reactions. Chitosan MNs can be applied in vaccine delivery of different types of antigens as follows: Inactivated viruses, live attenuated viruses, subunit, and DNA vaccines. There are several methods to load the antigen into chitosan MNs, including the dissolution of the antigen in the chitosan solution before micro-fabrication of the MNs, or the physical coating of the fabricated MNs with the antigen [39,40]. Furthermore, the future of chitosan MNs technology is aimed at creating more sophisticated MNs [41]. For example, the encapsulation of antigens in NPs, which can be further encapsulated into chitosan MNs, which can potentially further increase the vaccine efficacy [42,43]. The biosensors integration into MNs is also under consideration to self-monitor the vaccines and the immune response produced by them. Such technologies are expected to revolutionize the process of vaccine delivery in the near future when they are used in conjunction with one another [44,45]. A detailed look at the advantages and disadvantages of chitosan MNs for vaccine delivery is summarized in Table 1.

Conjugate vaccines

Chitosan is one of the attractive candidates for conjugate vaccine production because of its biocompatibility and biodegradation characteristics as a natural chitin derivative. Here, chitosan is chemically conjugated with antigen, reducing the ease of degradation and increasing the immunogenicity of the vaccine. Compared to previous techniques of employing adjuvant antigen combinations, this technique has several important advantages, mainly due to the enhancement of the presentation of antigens to the immune system. Another advantage of employing chitosan in vaccine formulation is that it is capable of eliciting depot effect at the site of immunization. Chitosan solutions were found to be capable of providing an excessive deposition of antigens at the injection site with a high percent of the dose remaining 4 days after compared to saline solutions. For example, measuring the percentage of the protein antigen remaining at the injection site after 8 h and up to 7 days, <9% of the soldered in saline, while more than 60% of the soldered in chitosan. This can not only extend the time during which antigens are made available but also make steady immune activation possible [52,53]. Chitosan's mucoadhesive properties further contribute to its effectiveness as a vaccine carrier. The positively charged amino groups of chitosan interact electrostatically with negatively charged mucus and epithelial surfaces, facilitating better adhesion and absorption of the antigen [54]. This characteristic is especially beneficial to mucosal vaccines, as improved uptake can enhance mucosal immunity. For example, using chitosan-based vaccines with intranasal application registered strong Th1 and Th2 responses, which is significant for protection against diverse pathogens [55]. Also, it is not only a good adjuvant but also substitutes and improves other adjuvants when used concurrently. CpG chitosan formulation has been demonstrated to elicit a higher Th1 and Th17 compared to a free CpG [55]. The Th17 response is particularly important for combating infections as it aids in recruiting neutrophils and stimulating antimicrobial peptide secretion. This synergistic effect underscores the versatility of chitosan as both a standalone adjuvant and a co-adjuvant in vaccine formulations [56].

The immunological mechanisms behind chitosan's effectiveness include its ability to promote dendritic cell maturation and enhance T lymphocyte responses. Chitosan NPs encapsulating various antigens have demonstrated significant increases in cytokine production, including interferon gamma and interleukin-17, indicative of strong cellular immune responses [57].

Conjugate vaccines employing chitosan may be considered one of the important achievements during the development of new-generation vaccines. Moreover, because chitosan improves the stability of antigen, the durability of the immune response, and the efficiency of antigen presentation, the mentioned chitosan-based formulations should improve the usefulness of the vaccines. While further study efforts are being made to determine precisely how these benefits occur, chitosan could be even more important in aiding the creation of vaccines for a variety of viral and bacterial infections. The incorporation of chitosan into the vaccine not only solves problems related to classical adjuvants but also expands new possibilities for stimulating both humoral and cellular immunity in various fields.

Table 1: Comparison of microneedle types for vaccine delivery [38,46-51]

Feature	Solid microneedles	Coated microneedles	Dissolving microneedles	Hollow microneedles
Mechanism	Create microchannels in the skin; vaccine applied after removal	Microneedles coated with the vaccine, release upon insertion	Microneedles containing the vaccine, dissolve in the skin	Delivers vaccine via a microfluidic channel, remains in the skin
Material	Metal (e.g., stainless steel, titanium), silicon, polymers	Metal, silicon, or polymer base; vaccine coating	Biocompatible materials (e.g., polymers, sugars)	Metal (e.g., stainless steel), silicon, polymers, glass
Vaccine delivery	Topical application after needling	Direct release of vaccine from coating	Direct release of vaccine as microneedles dissolves	Directly injects liquid vaccine into the skin
Needle structure	Rigid and pointed	Rigid and pointed	Sharp, pointed, or blunted	Hollow channel within the needle
Insertion depth	Typically, shallow (epidermis, upper dermis)	Typically, shallow (epidermis, upper dermis)	Typically, shallow (epidermis, upper dermis)	Adjustable depth within the skin (epidermis, dermis)
Pain level	Generally, less painful than hypodermic needles	Generally, less painful than hypodermic needles	Generally, less painful than hypodermic needles	Generally, less painful than hypodermic needles
Advantages	Relatively simple to manufacture; Reusable (with proper sterilization)	Direct vaccine delivery; Potentially faster delivery	Direct delivery; Biocompatible; no sharps waste; potential for easier manufacturing	Precise control over drug delivery; Can deliver larger volumes; Potential for automated injection
Disadvantages	Requires a second step for vaccine application; limited vaccine loading	Challenges in coating uniformity and stability; limited vaccine loading	Challenges in mechanical strength; limited shelf life due to moisture sensitivity; vaccine stability during manufacturing	More complex manufacturing; potential for clogging; more costly
Application	Vaccine enhancement, skin penetration	Direct vaccination	Direct vaccination	Precise drug delivery
Examples of vaccines	Various types, including subunit vaccines	Various types, including subunit and DNA vaccines	Various types, including protein and DNA vaccines	Various types, including liquid vaccines
Clinical status	Some have seen clinical testing for skin treatments	Preclinical and early clinical studies underway	Several clinical trials; some commercially available	Emerging technology; undergoing clinical evaluation
Manufacturing complexity	Relatively low	Moderate	Moderate	High

CHALLENGES IN CHITOSAN-BASED VACCINE DELIVERY

Chitosan, a biopolymer derived from chitin, has garnered attention as a potential vehicle for vaccine delivery due to its biocompatibility, biodegradability, and intrinsic immunogenic properties. However, several challenges must be addressed to optimize its use in vaccine formulations.

Targeted delivery and controlled release

The major drawback of chitosan-based-systems for the delivery of vaccines has been the difficulty to achieve both targeted delivery and controlled release of antigens. Studies show that chitosan NPs can hold the contents of a vaccine; however, there is an opportunity for development to modify the physicochemical specifications of these particles [14]. The stability of these NPs, particularly in the context of different storage conditions, also poses a challenge [58].

Immune response modulation

The immune responses regulation activity of chitosan formulations is important regarding its functioning. Research shows that for chitosan NPs to be effective, they need to activate both systemic and mucosal responses especially for respiratory pathogens vaccines [59]. However, ensuring that these NPs can successfully activate the immune system without causing adverse reactions remains a significant hurdle [60].

Blood-brain barrier (BBB) penetration

The delivery of vaccines to the central nervous system (CNS) is particularly challenging due to the BBB, which restricts the passage of large molecules [61]. The release of chitosan-based systems capable of crossing this barrier is crucial, particularly in relation to the vaccines targeting neurodegenerative illnesses [29]. Future research should focus on modifying chitosan NPs to enhance their uptake in CNS tissues.

Stability of mRNA vaccines

The COVID-19 pandemic has amplified the requirement of a steady delivery system for mRNA vaccines. The ultracold storage requirement of mRNA vaccines could be a logistical issue, but chitosan-based formulations might solve this problem [62]. However, there is an urgent need to investigate the stability of chitosan in maintaining the integrity and functionality of mRNA under various conditions [61].

Safety and toxicity concerns

However, in general it can be said that, on the one side, chitosan revealed many desirable properties, while, on the other side, there are still concerns regarding its safety and possible toxicological effect. Current research indicates that chitosan is regarded as safe but the differences in origin and consistency differences from batch to batch are random [63]. Comprehensive studies are needed to establish the safety profiles of chitosan-based vaccine delivery systems in clinical settings.

FUTURE DIRECTIONS

The future directions for chitosan-based non-particulate vaccine delivery systems focus on several critical areas.

First, there is a need to optimize formulation techniques to improve the encapsulation efficiency and stability of vaccines. This involves refining chitosan nanoparticle fabrication methods to ensure that the integrity of the antigens is preserved during preparation and storage [55]. Second, the need to find new approaches to deliver the vaccines that do not involve needles is necessary since patients are unlikely to stick to the recommended practice. New delivery methods including MNs and nasal sprays have the advantage of allowing people to use the vaccines on their own which is an improvement in the delivery systems since it's more conveniently possible for people from various backgrounds to get vaccinated [57,63].

Third, there is such a thing as improving the ability of chitosan formulations to deliver substances through the skin, as expanding the possibilities of their use dramatically. Recent developments in altering chitosan with fluorocarbon moiety seem to offer solution to problems such as enhanced TPA for biomacromolecules including antibodies and

antigens. This approach minimizes the toxic effects on the body while at the same time leading to more powerful immune reactions than the conventional technique of injections [64]. Fourth, addressing the challenges associated with cold chain logistics for vaccine distribution is vital. Chitosan's inherent properties may contribute to developing formulations that exhibit improved stability at ambient temperatures, making them more suitable for global vaccination efforts, especially in low-resource settings [13].

However, further studies on the factors through which chitosan triggers immune responses will serve as a basis in the more effective use of chitosan-based vaccines. At present, knowing the biological properties of chitosan with respect to immune cells can allow for the development of more efficient adjuvants that would heed the immunostimulatory capacities of the polymer while avoiding the undesirable immunomodulatory effects [65,66].

Last but not the least, there is need to determine the best strategies for chitosan-based formulations so that the existences can be taken to the clinical settings. All these challenges will require multi-dimensional efforts from the academic institutions, industry as well as the respective regulating agencies. Over time, more research is expected that CAST based non particle delivery systems will be central in developing new vaccine technologies especially regarding emerging diseases that require faster development of new vaccines [66].

CONCLUSION

Non-particulate vaccines based on chitosan are innovative improvements over conventional methodologies of vaccination, there are numbers of advantages with chitosan-based non-particulate vaccine delivery systems over conventional methods of immunization. Chitosan has been established to have several good features that make it well suited for use as a vaccine delivery system: biocompatibility, biodegradability and mucoadhesive properties. These systems help in enhanced mucosal delivery and stability of the antigen which is a characteristic requirement for stimulating strong immune responses. Today's studies focus on the abilities of non-particulate delivery systems such as solutions, films, and hydrogels that can contribute to the simplification of manufacturing processes as well as the effective delivery of antigens. However, obstacles in dosage control and long-term stability must be overcome to maximize the potential of these systems for mass application. In the future, there will be a need to invest in the studies of further alteration of chitosan properties, as well as the creation of new formulations for chitosan-based vaccines. It could be said that incorporating these modern strategies can greatly change the future of the vaccine delivery system to become more convenient for patients in the future.

AUTHOR'S CONTRIBUTIONS

Arshiya Saiyyad: Conceptualized the project, developed the methodology, and drafted the manuscript. Poonam Lal: Contributed to data collection, reviewed the manuscript, and provided supervision.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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ETHICAL APPROVAL

Nil.

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