HHS Public Access

Author manuscript

Expert Rev Vaccines. Author manuscript; available in PMC 2019 January 14.

Published in final edited form as:

Expert Rev Vaccines. 2018 March ; 17(3): 217–227. doi:10.1080/14760584.2018.1435279.

Future of human *Chlamydia* vaccine: potential of self-adjuvanting biodegradable nanoparticles as safe vaccine delivery vehicles

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Abstract

Introduction: There is a persisting global burden and considerable public health challenge by the plethora of ocular, genital and respiratory diseases caused by members of the Gram-negative bacteria of the genus *Chlamydia.* The major diseases are conjunctivitis and blinding trachoma, non-gonococcal urethritis, cervicitis, pelvic inflammatory disease, ectopic pregnancy, tubal factor infertility, and interstitial pneumonia. The failures in screening and other prevention programs led to the current medical opinion that an efficacious prophylactic vaccine is the best approach to protect humans from chlamydial infections. Unfortunately, there is no human *Chlamydia* vaccine despite successful veterinary vaccines. A major challenge has been the effective delivery of vaccine antigens to induce safe and effective immune effectors to confer long-term protective immunity. The dawn of the era of biodegradable polymeric nanoparticles and the adjuvanted derivatives may accelerate the realization of the dream of human vaccine in the foreseeable future.

Areas covered: This review focuses on the current status of human chlamydial vaccine research, specifically the potential of biodegradable polymeric nanovaccines to provide efficacious *Chlamydia* vaccines in the near future.

Expert commentary: The safety of biodegradable polymeric nanoparticles-based experimental vaccines with or without adjuvants and the array of available chlamydial vaccine candidates would suggest that clinical trials in humans may be imminent. Also, the promising results from vaccine testing in animal models could lead to human vaccines against trachoma and reproductive diseases simultaneously.

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Declaration of interest

R Sahu, R Vermat, S Dixit, S Duncan, SR Singh and V.A Dennis received grants from the National Institutes of Health (NIH) and National Science foundation (NSF). J.U Igietseme received grants from the Centers for Disease Control and Prevention (CDC) and NIH. C.M Black received grants from the CDC. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose. The conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.

Keywords

Chlamydia; vaccines; biodegradable polymeric nanoparticles; nanovaccines; immunity

1. Introduction

1.1. *Chlamydia* diseases as a public health challenge and need for a vaccine

1.1.1. *Chlamydia* and human diseases—The Gram-negative intracellular bacterial species of the genus *Chlamydia* are of high clinical interest and pose considerable global public health concerns. All *Chlamydia* spp. (e.g. *C. trachomatis, C. psittaci, C. pneumoniae,* and *C. pecorum*) have common developmental cycle, comprising two prominent morphologically distinct forms, the infectious elementary body (EB) stage, and an obligate intracellular, non-infectious and vegetative form, the reticulate body. Among the common species, *C. trachomatis,* a major pathogen in humans, is composed of approximately 15 serovars (serotypes) or genovars (genotypes), designated as A through K and L1 –L3, based on the antigenic or sequence variation in the major outer membrane protein (*OmpA*) [1–5]. These different chlamydial species cause ocular, genital, and respiratory infections whose major complications include blinding trachoma, reproductive dysfunctions, and respiratory diseases with considerable morbidities and exerting huge socioeconomic burdens on human healthcare.

Trachoma is a major human ocular disease caused by *C. trachomatis* serovars A, B, Ba, and C, and it is the most common preventable blinding disease; it is of epidemic proportion in several developing nations in Africa, South East Asia, and the Middle East. There is a global estimate of 150 million *C. trachomatis* infected people, of which 6 million are severely visually impaired or irreversibly blinded by trachoma [6,7]. Unlike trachoma that is presently mostly prevalent in developing societies, human genital *C. trachomatis* infections and their clinical outcomes are endemic in both industrialized and under-developed nations and therefore constitute a major worldwide concern. In fact, the epidemiologic data from essentially all international disease monitoring, control, and prevention agencies, including the WHO and CDC have ranked genital *C. trachomatis* infections as the most common bacterial cause of sexually-transmitted diseases (STDs) worldwide since the late 1970s [8–11]. Genital infection by the different oculogenital serovars of *C. trachomatis* (specifically serovars D through L) accounts for over 100 of the 500 million annual new STDs globally out of which females are disproportionately affected (~60%) [8,9,11]. Diseases caused by genital chlamydial infection include self-limiting urethritis in both males and females, cervicitis in women, and epididymitis and proctitis in men; in addition, pelvic inflammatory disease (PID), chronic pelvic pain, ectopic pregnancy, and tubal factor infertility (TFI) are major long-term complications of untreated female genital chlamydial infection, and PID may precede the onset of the other reproductive complications [12–14]. Besides, neonatal ocular chlamydial infection may occur during birth by mothers harboring a genital infection, and the infected infants may develop conjunctivitis and respiratory disease that could progress to pneumonia. Furthermore, Reiter′s syndrome is a complication of genital chlamydial infection with self-limiting arthropathic (joint disease) manifestation.

Human respiratory infections by *Chlamydia* spp. are mostly associated with *C. pneumoniae,* which are rampart, with approximately over 60%–80% of most American, European, and Asian societies being exposed. The infection presents as mild to acute respiratory diseases, such as pharyngitis, bronchitis, and even pneumonia which accounts for over 10% of community-acquired pneumonia [15]. It remains uncertain whether there is a causal association between *C. pneumoniae* infection and certain chronic diseases such as atherosclerosis and some age-related autoimmune diseases on the basis of initial correlative data [16– 18] because the links are yet to be substantiated clinically and experimentally. A psittacosis-like disease that may in rare cases become systemic or may evolve into fatal pneumonia in humans has been associated with exposure to the zoonotic *C. psittaci* [19], an occupational hazard for workers in the poultry and farming industry, and persons exposed to infected avian species [20]. Thus, although different species of *Chlamydia* may cause disease in humans, perhaps the highest burden of chlamydial diseases that have caused much of the public health concerns are caused by *C. trachomatis*. Most human prevention and control strategies as well vaccine research are focused on *C. trachomatis* diseases.

1.1.2. Control and prevention strategies—The history, global prevalence, and distribution of trachoma indicated that improvements in sanitary and hygienic conditions could substantially control the disease by preventing transmission of ocular chlamydial infection through person-to-person, flies, and fomites. Thus, in 1993 the WHO led the implementation of the SAFE strategy with the goal to eradicate trachoma by 2020 through Surgery (S) for cases of trichiasis, Antibiotic (A) treatment of active disease, Facial (F) cleanliness for personal hygiene, and Environmental (E) improvement through provision of clean water supply and toilets that reduce the flies acting as vectors in the areas [7,14,21]. After two decades of implementation of the WHO′s SAFE initiative, some achievements have been made in controlling trachoma worldwide; however, there are significant pockets of trachoma-endemic regions around the world, especially in developing societies, partly because several countries have not responded adequately to the E portion of SAFE and the slow pace of implementation in other countries due to socioeconomic, political, or sociocultural reasons. Under these challenging circumstances, a one-shot strategy is needed to eradicate trachoma from the human population.

The control of genital chlamydial infections and the complications has presented serious challenges that continue to cause great concerns in the medical community and colossal burden to public health. Among these challenges are the rampant asymptomatic infections, especially in women, the ineffectiveness of mass screening programs, the continuing spread of chlamydial infections among at-risk groups and locations around the world, and the apprehension that resistant variants may emerge from the excessive use of antibiotics. First, the established clinical experience is that early detection of chlamydial infections can result in successful treatment with antibacterial agents, such as tetracycline deri-vatives (e.g. doxycycline) and the macrolides or azalides (e.g. erythromycin and azithromycin) [17]; however, the high proportion of asymptomatic infections (over 60% in women) often result in severe and sometimes irreversible complications as the first symptoms of an infection [22,23]. Second, up to 40% of untreated chlamydial genital infections in women lead to sequelae such as PID and TFI [12,24,25], and the frequent asymptomatic infections in

women contributes to these complications and the associated enormous morbidity and socioeconomic burden [12,25–27]. Third, screening and treatment programs have not been very effective, but actually causing what has been described as ′arrested immunity′ whereby premature antibiotics treatments prevent host natural immunity against infection and contributing to the rising cases of chlamydial infections worldwide [24,28–35]. In fact, it has been suggested that a significant proportion of treated genital or ocular infections may lead to persistence [36–39], and the recognition that persistence plays a role in the pathogenesis of the *Chlamydia* disease, *makes* the long-term value of certain chemotherapies questionable [36–38,40–44]. Besides, genital chlamydial infection could predispose to HIV-related AIDS either due to the ulcerative presentation of some of the infections, inflammation or by other yet unknown microbial interactions mechanism [45–48]; and *importantly,* genital chlamydial infection is an established co-factor for human papilloma virus-associated cervical carcinoma [49], which have combined to heighten these concerns and the urgency to control chlamydial infections. Furthermore, according to the CDC, the United States is spending over $3 billion annually on an estimated 4 million reported clinical cases of human genital chlamydial infections [9,10].

Thus, considering these morbidity and socioeconomic issues, and the inadequacy of the different prevention and control strategies so far developed against *Chlamydia,* the current medical opinion is that a vaccine strategy is likely to be the most reliable and cost effective to make the greatest impact in controlling rising infections, global prevalence of chlamydial infections and the associated complications [17,30,31,50,51]. This medical opinion is supported by a computer modeling and prediction analysis of the impact of a protective prophylactic chlamydial vaccine which revealed that even a partially protective vaccine that prevents certain severe sequelae in a sub-optimal vaccination program would constitute an acceptable short-term goal to reduce chlamydial infections, morbidity, and associated costs [52]. Unfortunately, even after over three decades of active research, there is no acceptable human chlamydial vaccine to date due to a number of challenges ranging from safety considerations, suboptimal or inadequate immunogenicity of vaccine candidates, lack of effective delivery systems and potent adjuvants, and knowledge gap on how to induce long-term immunity [14,53,54]. Furthermore, while both prophylactic and therapeutic vaccines are needed, there is a focus on the prophylactic vaccine strategy that will more likely prevent further spread of chlamydia in the population, an imperative in any vaccine-dependent anti-chlamydial strategy.

1.2. Essential features of a potentially efficacious human *chlamydia* vaccine

The lessons from historical challenges in human *Chlamydia* vaccine development, and recent advances in vaccine antigens, immunomodulation, and protective immunity correlates that constitute the key requirements for designing and evaluating a potentially efficacious human chlamydial vaccine have been recently reviewed [14,54,55]. To briefly summarize the relevant issues and requirements, the following statements can be made about the current status of human chlamydial vaccine research: First, the experience with the early trachoma vaccine efforts of the 1960′s indicated that conventional vaccinology technical approaches using inactivated or attenuated microbial agents produced results that were described as inadequate, at best inconclusive, and unacceptably exacerbated the disease in some trials;

thus no further human clinical vaccine effort or trials have been undertaken since the late 1960s [56–62]. However, conventional vaccinology led to the successful production of veterinary chlamydial vaccines [63–68]. For example, the veterinary vaccines comprising live attenuated or inactivated *C. psittaci* and feline strains successfully protected ewes from chlamydia-induced abortion and cats from feline pneumonic chlamydial disease, respectively [65,68,69]. It is important to note that these veterinary vaccines did not prevent infectivity and their veterinary standards may not meet human use standards; but their efficacy would suggest that a safe and efficacious human vaccine is a possibility, therefore fueling the impetus and hope for future human vaccines. Second, the correlates of protective chlamydial immunity, as described in animal models and humans, are primarily CD4 + T cells that secrete IFN-γ among other Th1-associated cytokines such as TNF-α, and an accessory antibody of IgA and IgG isotype response especially in the relevant mucosal locations [14,54,55]. Third, the candidate vaccine antigens should be subunits, such as intact proteins, assembled epitope fragments, or combinations, in case the intact *Chlamydia* might contain components that can induce immunopathogenic responses and because of the earlier challenges in generating live-attenuated chlamydial variants [14,53]. Also, such subunit vaccine antigens should induce broadly genusspecific protective immune responses to cover the multiple serovars/genovars and strains of *C. trachomatis.* The several candidate subunit vaccine antigens described so far were recently reviewed [14,67,70]: briefly, they include outer membrane proteins (OMPs), such as are the 40, 60, and 15 kDa proteins encoded by the Omp-1 (omc A), Omp-2 (omp C) and Omp-3 (omp B) genes, respectively [71]; the polymorphic outer membrane proteins (pmp) and the conserved P or B family of membrane proteins [71–73], as well as an ADP/ATP translocase [74], immunogenic plasmid protein (pgp3) [75], proteasome/protease-like activity factor (CPAF) [76], a toxin mapped to the plasticity zone of several strains [77], certain members of the chlamydial type III secretory machinery [78], and a number of cloned hypothetical proteins [67,79,80] that have been evaluated in animal models of specific chlamydial diseases [66,67,70,81 –84] and showing promising results with a certain degree of protection immunity characterized by a reduction of infection burden or prevention of certain complications, including acute inflammation and infertility [82,85–87]. Fourth, while these promising pre-clinical results and outcomes of vaccine efficacy evaluations continue to inspire and accelerate the momentum toward a human vaccine, they have also brought to the fore the need to develop effective vaccine delivery systems, vehicles, vectors, and potent human-compatible adjuvants; also important are the choice of an appropriate route of vaccine administration, especially mucosal (i.e. *nasal* or sub-lingual) versus subcutaneous, as well as testing vaccine candidates for efficacy and toxicity in other animal models, including pigs and non-human primates [55]. These conditions are predicted to optimize the induction of protective immune effectors at the mucosal sites of chlamydial infection, achieve a high degree of protective, even sterilizing, long-term immunity. Fifth, effective delivery systems and adjuvants are needed for immunomodulation, especially for the subunit vaccines to induce the required immune effectors and achieve long-lasting protective immunity. Perhaps the significance of effective delivery and route of administration for an optimal chlamydial vaccine efficacy was recently underscored by the phenomenal ability of the formulation containing the poorly immunogenic UV-inactivated *C. trachomatis* EBs mixed with the charge-switching adjuvant particles (cSAP) to induce protective immunity when delivered mucosally (nasal or

intrauterine), not *parenterally* (*subcutaneous, s.c.*) [88]. The effectiveness of this delivery system was the ability of cSAP to target UV-inactivated EBs to and preferential presentation of UV-*Ct*-cSAP by immunogenic $CD103^-$ dendritic cells (DCs), while UV-Ct was primarily acquired by tolerogenic $CD103^+$ DCs, and the induction of critical tissue-resident memory T cells (T_{rm}) with genital mucosal tissue homing characteristics [55,89]. These remarkable results should prompt greater use of the cSAP-related vehicle platforms for subunit vaccine delivery against *Chlamydia*. Besides, the results further emphasized the role of the local factors, such as epithelial-DC interaction with mucosally-acquired antigens that regulate immunity at mucosal sites of infection. Importantly, the results have underscored the point, that poor delivery can compromise the efficacy of the best vaccine candidate. This review focuses on members of a class of vaccine delivery vehicles called biodegradable polymeric nanoparticles that cSAP belongs [88].

2. Biodegradable nanoparticles formulations and their potential as vaccine delivery vehicles

Vaccine delivery systems include vaccine vectors and vehicles that function primarily as carriers for targeting vaccine antigens to appropriate antigen-presenting cells and immune inductive sites [90], and secondarily to furnish the necessary immunomodulation to boost effectors [91], if the carriers possess adjuvant properties [92]. Adjuvanticity is thus a desirable property in a number of delivery systems if present [93]. Until the recent introduction of biodegradable polymeric nanoparticles [94], the vast majority of the delivery vehicles previously used for experimental chlamydial antigens had produced mixed results in various animal models, as recently reviewed [14,85,90,95,96]. Table 1 shows an updated list of common and promising delivery systems and adjuvants for chlamydial vaccine and the effectiveness of some of them in promoting the induction of protective chlamydial immunity as recently reviewed [14,54,90,97]. Unfortunately, most of the promising adjuvants for potentially efficacious chlamydial vaccines are still in their pre-clinical or initial phases of clinical trials [98,99]. However, the dawn of the era of biodegradable polymeric nanoparticles and the adjuvanted derivatives may accelerate the realization of the dream of human vaccine in the foreseeable future [53,100,101].

The use of synthesized biodegradable polymeric nanoparticles to deliver biomolecules have been explored in the past two decades [102] for potential use in biomedical applications that include *in vivo* antibiotic and drug therapies [103], as well as vaccines [94,104,105]. The cross-linking of polymer matrix allows the encapsulation of biomolecules and facilitates their release upon degradation of matrix (Figure 1) [106–108]. Biodegradable polymers offer safety, flexibility in nanoparticles sizes in fabrication, and controlled release of encapsulated biomolecules in targeted or non-targeted forms [94,108]. In addition, developing vaccines by encapsulating antigens into biodegradable polymeric nanoparticles afford safer and reliable approaches for vaccines design with or without adjuvants [104,109,110]. Biodegradable polymeric nanoparticles-based vaccine candidates against *Chlamydia* are still under pre-clinical developmental stages, as reported in recent years and are emerging as potentially efficacious vaccine candidates [88,100,109,111]. Biocompatibility and biodegradation are desirable characteristics that attract these polymers for vaccine developmental efforts against

various organisms [105]. Diverse biodegradable polymers are used to develop nano vaccines [14]: PLGA {poly (lactic-*co*-glycolic acid)} [112], PLA-PEG a copolymer of polylactic acid (PLA) and polyethylene glycol (PEG) [113] and their adjuvanted derivatives will be discussed in this review. Since adjuvanted nanoparticles are primarily delivery vehicles that possess adjuvant properties, they are not just adjuvants, and as such, they cannot and should not be described simply as adjuvants.

2.1. PLGA

PLGA is the most popular biodegradable co-polymer for the sustained release and delivery of biomolecules [107–109,114,115]. The encapsulation efficiency and drug loading are dependent on the physiochemical properties of the drug and preparation method. Its biodegradation profile is controlled by balancing the poly components (PLA and PGA) in different ratios [115] forming a solid structure of polymer matrix (Figures 2 and 4(a)) [106] which *provides high encapsulation of vaccine antigens* [114]. The safety and fabrication flexibility of PLGA have been substantiated in reported biomedical applications [94]. Attempts at using peptides as vaccine candidates against *C. trachomatis* have not been entirely successful [55] perhaps due to inefficient delivery systems [111,116] and rapid degradation caused by proteases [114] at the site of administration, thus decreasing their cellular uptake and immunogenicity [117,118]. The immunogenic major outer membrane protein (MOMP) of *Chlamydia* combined with biodegradable polymeric nanoparticles for vaccine delivery has been investigated in recent years in pursuit of an efficacious vaccine [109,119–121]. A study by Taha et al. [114], revealed that encapsulation of a recombinant peptide of MOMP (termed rMOMP-187) within PLGA (85/15) by the double emulsion process, when used to pulse the mouse J774 macrophage cell line resulted in enhanced Th1 cytokines (IL-6, IL-12p40) and nitric oxide production at low peptide concentrations. The physico-structural characterizations of PLGA-encapsulated rMOMP-187 nanoparticles disclosed that protecting the peptide′s integrity and facilitating its sustained release has the potential to trigger robust immune responses. Thus, in the study by Fairley et al. [109], the encapsulation of full-length recombinant MOMP (rMOMP) into PLGA (50/50) nanoparticles showed that T cells from subcutaneously immunized BALB/c mice secreted elevated levels of IFN-γ as well as antigen-specific serum IgG2a (Th1) antibodies. Of significance was the finding that PLGA-encapsulated rMOMP nanoparticles triggered a 64-fold higher level of Th1 versus Th2 antibody titers in immunized mice; whereas rMOMP mixed with Freund′s adjuvant only provided a four-fold increase of Th1 over Th2 antibody titer, thus validating the self-adjuvanting property of the PLGA polymeric nanoparticles.

2.2. PLA-PEG

PLA-PEG is a copolymer of polylactic acid (PLA) and polyethylene glycol (PEG). PLA is a synthetic biodegradable polymer that possesses low stimulating potential and high mechanical strength [113]. However, PLA alone has a limitation of low hydrophilicity, long degradation time, and low drug loading of *hydrophilic* compounds [113]. Also, PLA polymers show inadequate interaction with cells and can even form aggregates after displaced by serum proteins [122]. On the other hand, PEG shows high hydrophilicity, phagocytic escape, resistance to immunological recognition, *lack of binding* with serum proteins [113,123], low cytotoxicity, and high cell permeability [124]. These properties

make PEG an efficient modifier in polymer synthesis [124,125]. Polymerization of PLA and PEG to obtain PLA-PEG as block copolymer forms a solid PLA core surrounded by PEG attachment (Figures 2 and 4(b)) provides the advantage of improved hydrophilicity, increased drug-loading capacity, and a *reduced burst effect* [113]. Therefore, providing prolonged *in vivo* released time for encapsulated biomolecules [113] along with an extended biodegradation profile makes PLA-PEG an improved delivery system for vaccine candidates. The potential of PLA-PEG as a desirable vaccine delivery system against *Chlamydia* was demonstrated by Dixit et.al. [111], by encapsulating a recombinant peptide of MOMP (named M278) and demonstrating its pattern of potentiating the immune response, which corroborated the results from using PLGA as reported by Taha et al. [114], and Fairley et al., [109]. PLA-PEG-encapsulated M278 further potentiated adaptive immune responses in subcutaneously immunized mice by triggering enhanced production of T-cell specific Th1 cytokines (IFN-γ, IL-2) and serum Th1 (IgG2a) and Th2 (IgG1, IgG2b) antibodies in comparison to *non-encapsulated* M278. Furthermore, the M278-encapsulated construct induced serum anti-chlamydial neutralizing antibodies as evidenced by the reduced infectivity and expressions of TLR2 and CD80 in mouse J774 macrophages. These studies demonstrated that biodegradable polymeric nanoparticles with extended biodegradation and self-adjuvanting properties are potential alternative delivery systems to develop efficacious vaccines against *Chlamydia*. The limitations of the administration routes, especially for mucosal administration of vaccine candidates can be advantageous with synthetic polymers, since they protect the encapsulated biomolecules [88,121]. Ongoing studies are investigating the protective efficacy of PLGA- and PLA-PEG-containing recombinant proteins of *Chlamydia* in mice infected intra-vaginally with *C. trachomatis*.

2.3. Charge-switching adjuvant particle

cSAPs surface charge-switching biodegradable nanoparticles consisting of poly (D, L-lactic-co-glycolic acid)-b-poly(L-histidine)-b-poly(ethylene glycol) (PLGA-PLH-PEG) recently have been developed to deliver encapsulated antibiotics to bacterial surfaces for treating bacterial infections [126]. This triblock copolymer was formulated using a polymer end grafting strategy where PLH consisting of 20 or 30 repeats of L-histidine with an N-terminal lysine and a C-terminal cysteine was synthesized to facilitate the conjugation reactions. The developed PLH-SH and orthopyridyl disulfide (OPSS) modified PEG blocks were reacted to form a diblock copolymer followed by PLGA conjugation to the NH_2-PLH-PEG diblock copolymer resulting in formation of charge-switching synthetic particles having a hydrophobic core (PLGA) and a bilayered hydrophilic surface of PLH (inner) and PEG (outer) polymers [126]. These cSAPs carry a moderate negative charge at a pH of 7.4 but convert to a cationic charge due to protonation of the PLH imidazole group when exposed below pH 6.5, thus facilitating their attachment to the surfaces of cells. Stary et al. [88], constructed a conjugate structure where UV-*Ct* was surrounded with cSAPs (Figure 3) with a ring opening reaction for charge switching and for releasing the UV-*Ct*. A slight modification in the formulation, by adding PLA coupled with a potent TLR7/8 agonist (resiquimod), enhanced the efficacy of mucosal immunization of mice with long-term protective immunity. Production of IFN-γ, robust antibody responses and CD4 + T cell responses strongly suggested Th1 specificity. However, activation of CD8 + T and CD4 + T cells induced by intrauterine immunization suggested that clearance of *Ct* infection requires

mixed immune responses not just CD4 + T cells memory. As previously mentioned, the effectiveness of this delivery system was the ability of cSAP to target UV-inactivated EBs to and preferential presentation of UV-*Ct*-cSAP by immunogenic $CD103^-$ DCs, while UV-Ct was primarily acquired by tolerogenic $CD103^+$ DCs, and the induction of critical tissue-resident memory T cells (T_{rm}) with genital mucosal tissue homing characteristics. These remarkable results underscored the significance of effective delivery vehicles, the role of the local factors, such as epithelial-DC interaction with mucosally-acquired antigens in the regulation of mucosal immunity at mucosal sites of infection, and should prompt greater use of the cSAP-related vehicle platforms for subunit vaccine delivery against *Chlamydia*.

3. Conclusion

Overall, developing an efficacious subunit vaccine [53,127] against *Chlamydia* will require several prerequisites [55]: the selective routes of administration, antigens to cover serotypes, an efficient delivery vehicle such as the biodegradable polymeric nanoparticles providing sustained release and possibly the inclusion of TLR agonists [92,128,129]. Recently, nanoparticle adjuvants, e.g. lipid nanoparticles [130], montanide-based nanoparticle (IMS 3012, IMS 1313 N VG PR) [131–133], CpG-Ficoll [134], and KALA modified lipid nanoparticle (KALA-MEND) [135] have gained considerable interest in nano vaccinology due to their immunomodulatory effects. Hence, these nanoparticle adjuvants can also be incorporated in polymeric nano vaccine formulations and tested for their efficacy against *C. trachomatis*. In addition, a long-lasting protective immunity against chlamydial infections may require more than one route of administration, suggesting the simultaneous administration of vaccines via the mucosal and systemic routes [88,136–138]. The polymer having prolonged biodegradation properties and can bind especially to mucosal surfaces will be more desirable. Even though, PLGA seems to be the likely selection currently due to being biocompatible and efficient in delivery [107,108,115,139], the inclusion of PEG in the synthesis of polymer formulations may provide an advantage in the extended release of biomolecules [123]. Therefore, polymers with PEG [123,125] as a component like PLA-PEG or PEG-coated PLGA may be recommended in the future to develop nanoparticles for vaccine delivery. Another possibility for consideration is co-administration or simultaneous administration of more than one polymeric formulation encapsulated with the same or different biomolecules to generate robust immune responses. Consequently, biodegradable nanoparticles are highly recommended delivery systems for chlamydial antigens to obtain a robust and desired efficacious protective immune responses.

4. Expert commentary

Considering the morbidity and socioeconomic issues, and the inadequacy of the different prevention and control strategies so far developed against *Chlamydia*, the current medical opinion is that a vaccine strategy is likely to be the most reliable and cost effective to make the greatest impact in controlling rising infections, global prevalence of chlamydial infections and the associated complications. Biodegradable polymeric nanoparticles offer safety, flexibility in nanoparticles sizes in fabrication, and controlled release of encapsulated biomolecules in *passively or actively* targeted forms. More detailed basic immunobiological analysis of the mechanism of immunostimulation by adjuvanted nanoparticles that involves

targeting immunogenic DCs will contribute to our knowledge of the cellular interactions and role of the mucosal microenvironment in mucosal immunity. This will greatly impact vaccine design strategies against mucosally-acquired microbial pathogens.

5. Five-year view

An efficacious human chlamydial vaccine is a public health imperative. The safety of biodegradable polymeric nanoparticles-based experimental vaccines with or without adjuvants and the array of available chlamydial vaccine antigen candidates would suggest that clinical trials in humans may be imminent in the next 2 years. It is possible that a trachoma vaccine based on biodegradable polymeric nanoparticles may be realized simultaneously with the reproductive disease targeted vaccine. In either case, the biodegradable polymeric nano vaccines against human *C. trachomatis* infections may be in the horizons as a potent weapon to control chlamydial diseases in the next 5 years.

Acknowledgments

Funding

This work was supported by PHS grants (AI41231, GM 08248, RR03034, R21AI111159, CREST HRD-1241701) from the National Institutes of Health, National Science foundation and the Centers for Disease Control and Prevention (CDC).

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Key issues

- Infections and complications of *C. trachomatis* in the human population continue to cause considerable morbidity and economic stress on the public healthcare system of several countries.
- The failure of the screening programs has led to the medical opinion that an efficacious vaccine will be the best approach to control the myriad of ocular, genital and respiratory infections and diseases caused by *Chlamydia.*
- The research imperatives to develop effective vaccine delivery systems, vehicles, vectors, and potent human-compatible adjuvants, are crystallizing the biodegradable polymeric nanoparticles as safe and effective methods to develop nanovaccines against *Chlamydia*.

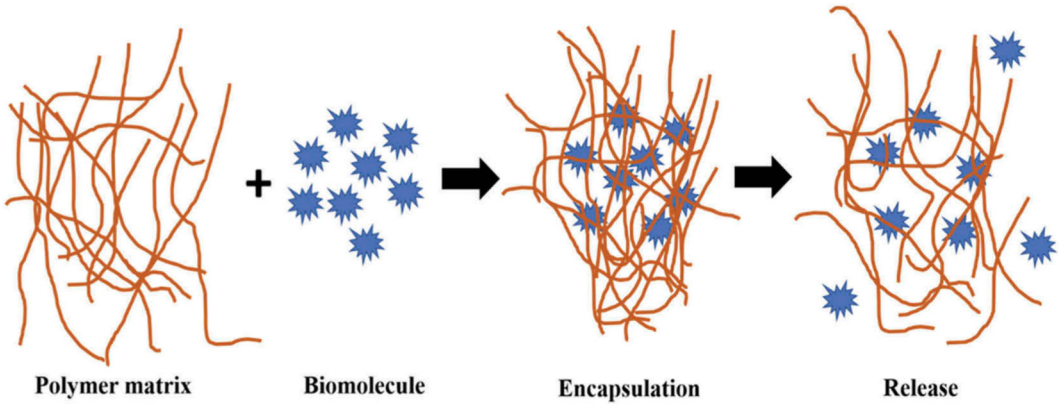

Figure 1.

Schematic representation of biomolecules packaging in polymer matrix. The cross-linking of polymer matrix allows the encapsulation of biomolecules and facilitates their release upon degradation of matrix.

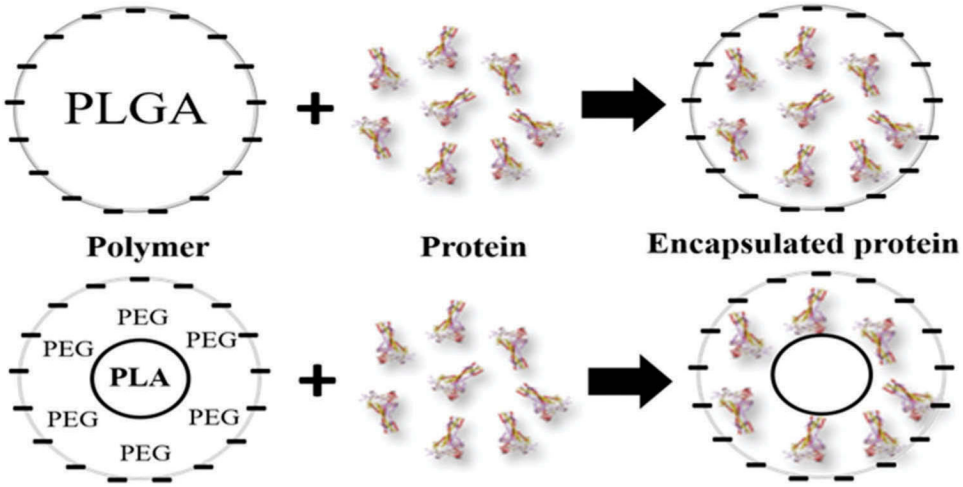

Figure 2.

Schematic representation of PLGA and PLA-PEG nanoparticles, and encapsulated protein. PLGA is the most popular biodegradable co-polymer for the sustained release and delivery of biomolecules. Polymerization of PLA and PEG to obtain PLA-PEG as block copolymer forms a solid PLA core surrounded by PEG attachment provides the advantage of improved hydrophilicity, increased drug-loading capacity and providing prolonged *in vivo* released time for encapsulated biomolecules as well an extended biodegradation profile, making PLA-PEG an improved delivery system for vaccines.

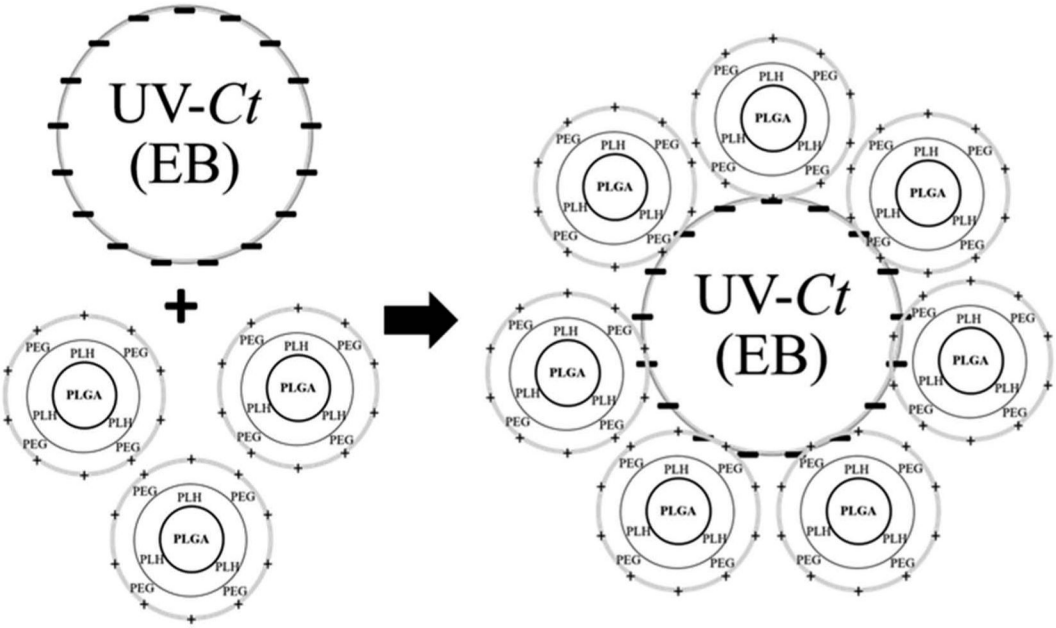

Figure 3.

Schematic representation of cSAP and UV-Ct conjugate. The constructed conjugate has UV-Ct was surrounded with cSAPs with a ring opening reaction for charge switching and for releasing the UV-Ct.

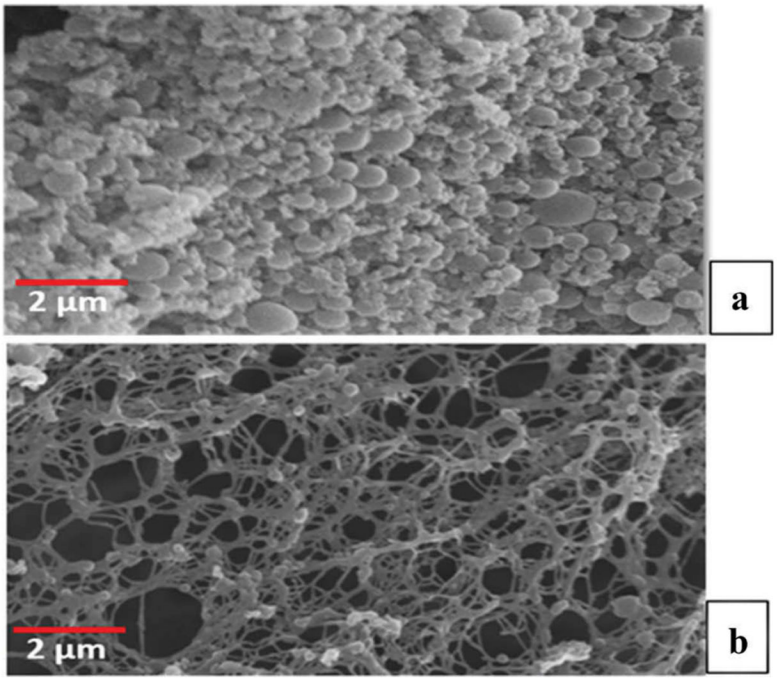

Figure 4.

Scanning electron microscopy (SEM) of (a) PLGA-encapsulated rMOMP and (b) PLA-PEG-encapsulated M278. Targeted antigens were encapsulated in biodegradable polymeric nanoparticles using the water/oil/water double emulsion-evaporation technique, and samples were analyzed using high resolution SEM.

Table 1.

common delivery systems and adjuvants used in experimental and pre-clinical vaccines.

Delivery system	Role/Function	Degree of protective immunity *	References (*Chlamydia* vaccine)
Viral/Bacterial vectors:	Vector	Partial	[81,84,90,140]
* **Live**:	Carrier/adjuvant	Partial	
Poliovirus, vaccinia, adenovirus,	Carrier/adjuvant	Yet to be tested	
Salmonella, Listeria, Canary poxvirus.	Carrier/adjuvant	Yet to be tested	
* **Non-living:** -Bacterialghosts; -Halobacteria gas vesicles -Virus-like particles (VLPs)			
Cellular delivery:	Carrier/adjuvant	Partial (WT)	[84,90]
Antigen presenting cells (APCs), Dendritic cells(wild-type, WT/IL-10KO DCs)		Sterilizing (IL-10KO)	
Immunomodulation:	Adjuvant	Partial	[81,84,90]
•Cytokines & costimulatory molecules • Antibodies •Heat shock proteins	Carrier/adjuvant	Possibly sterilizing	
* **Detergents-based**:	Adjuvant	Partial	[81,84,90]
ISCOMS, QS21			
* **Microbial-related components**: CpG-rich oligos, ospA, Cholera toxin, CFA, RIBI adjuvants; MPL-A, muramyl-dipeptides, mutant toxins (labile toxins)	Adjuvant	Partial	[81,84,141]
DNA/RNA:	Carrier/adjuvant	Partial	[81,84]
Expression plasmids/RNAs			
Biodegradable nanoparticles	Carrier/adjuvant	Yet to be tested	[88,109–111,114,142]
PLGA, DNA vaccine in chitosan, PLA-PEG, Surface charge-switching nanoparticles	Carrier/adjuvant		
Other chemical adjuvants: Alum, montanides, lipopetides, mineral oils, water-in-oil emulsions, liposomes, & particulate delivery in Ca-phosphate, & vault nanoparticles, Adjuvant systems.	Carrier/adjuvant	Yet to be tested	[81,90,143]

* Partial protective immunity is described as either the shortening the course of the infection or a significant reduction in the intensity of infection and pathology indices, as compared to non- or sham-vaccinated control animals.