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Research Article

In-vivo Immune Analysis of Capsular Type Ia Antigen of *Streptococcus Agalactiae* Encapsulated with PLGA

Faranak Mirtalebi[1], Reza Shapouri[2]*, Parivash Ghaderinia[3] and Javad Naserian[4]

[1]Department of Microbiology, Faculty of Basic Science, Zanjan Branch, Islamic Azad University, Zanjan, Iran

[2]Department of Microbiology, Assistant Professor, Zanjan Branch, Islamic Azad University, Zanjan, Iran

[3]Department of Microbiology, College of Science agriculture and modern technology, Shiraz Branch, Islamic Azad University, Shiraz, Iran

[4]Department of Mathematics and Statistics, Assistant Professor, Zanjan Branch, Islamic Azad University, Zanjan, Iran

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***Corresponding author:** Reza Shapouri, Department of Microbiology, Assistant Professor, Zanjan Branch, Islamic Azad University, Zanjan, Iran,
E-mail: rezashapoury@yahoo.com

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Abstract

Background: *Streptococcus agalactiae* (*S. agalactiae*) due to the polysaccharide capsule and causing invasive infections, bacteremia, pneumonia, and meningitis in neonatal with 5% mortality considered the most critical pathogen. In order to increase the immunity level by the capsular type Ia antigen of *S. agalactiae,* conjugated polydlyl lactide-co-glycolide nanoparticles (PLGA_NP), we proposed a more effective vaccine against infections caused by *S. agalactiae*. In addition, the cytotoxicity of the NPs as a carrier in the fabrication of the nano-vaccine in the animal model of rats was investigated.

Materials & methods: Isolation of capsules from *S. agalactiae,* and preparation of PLGA nanoparticles containing type Ia capsules done. After conjugating the PLGA_NP with type Ia capsules, it was characterized using spectroscopy techniques. Rat vaccination and immunization process, rat survival model, spleen culture method on rats and neonatal rats, and opsonophagocytosis test were performed.

Results: The zeta potential of PLGA_NP was -19 mv, and for the conjugated capsule (CPS)-PLGA was -10.34 mv. The size of PLGA-CPS is 256.5 nm. The mean of the pathogenic strain of *S. agalactiae*after exposure to the immune system of the CPS-PLGA group was lower than in other experimental groups. Reduced growth of *S. agalactiae* colonies in the vaccine candidate group compared to the other groups reported ($p < 0.05$), and in rat opsonophagocytosis test, the CPS-PLGA vaccine candidate group has the lowest growth value in the percentage of grown colonies compared to other tested groups ($p < 0.05$).

Conclusion: It can be concluded that the potential performance of the CPS-PLGA vaccine candidate group is ineffective stimulation and enhancement of infection for diagnosis. On the other hand, according to data analysis, this vaccine group has a significant difference from other vaccine groups ($p < 0.05$). The prepared antigen can be used as a candidate vaccine to treat congenital infections and premature invasive neonatal infections in the animal model of rats due to its high efficacy in infection.

Introduction

S. agalactiae, a gram-positive bacteria, is commonly found in the gastrointestinal and urogenital tract as part of the normal flora in the healthy adult population and is currently an opportunistic pathogen causing neonatal infections pneumonia meningitis in industrialized countries [1,2].

S. agalactiae due to the type II polysaccharide capsule, causes invasive infections, bacteremia, pneumonia, and meningitis in neonatal with 5% mortality considered the most critical pathogen [3,4]. *S. agalactiae* adheres to the epithelial surfaces of the host cell through the capsule and leads to the onset of the disease [5]. The presence of a capsule is essential in protecting *S. agalactiae* against phagocytosis, complement

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system, and antibodies to remove the microorganism [6]. In the natural flora of the vagina in females, during the first weeks of a neonate's life [7], the infection can manifest as sepsis, meningitis, or respiratory distress syndrome [8-10]. Treatment strategies containing several antibiotics such as Erythromycin and Clindamycin have been suggested [11]. In recent years, a resistance of 3% - 21% against clindamycin and 5% - 29% against Erythromycin has been reported [12]. Therefore, Penicillin and Ampicillin have been suggested for treatment but lead to anaphylactic shock, so the use of mentioned antibiotics has been limited, and today, Cefazolin is used instead [13,14].

Life-threatening infections in neonates led to the Centers for Disease Control and Prevention (CDC) decision in 1996 to use a vaccine to treat and prevent infections caused by *S. agalactiae* [15]. NP technology has received more attention with the development of nanoscience in various fields, especially in vaccine design. Design and production of an effective vaccine for *S. agalactiae* are essential.

PLGA (polydlyl lactide-co-glycolide) NPs with unique properties (lowest systematic biodegradability toxicity) are known as carriers for antigen delivery in the design of conjugated vaccines [16-18]. PLGA_NPs, with the ability to interact and integrate into immune cells, are effective in immunogenetics for producing a vaccine, have many binding regions, and therefore have high compatibility with the antigen and target cells [19].

Streptococcus agalactia is divided into 9 serotypes based on those sera reacting with the polysaccharide capsule. Only serotypes Ia, Ib, II, III, and V are associated with human agents. In this study, our research team chose human pathogenic *Streptococcus agalactiae* serotype Ia. In order to increase the production efficiency of the mentioned vaccine, to increase the immunity level by the capsular type Ia Antigen of *S. agalactiae*, conjugated PLGA NPs with capsular type Ia Antigen of *S. agalactiae*, we proposed a more effective vaccine against infections caused by *S. agalactiae*. In addition, the cytotoxicity of the NPs as a carrier in the fabrication of the nano-vaccine in the animal model of rats was investigated.

Material and methods

Animal model

The animals were adequately fed and kept away from stress one week before the clinical trial. Notably, the animals were preserved in the laboratory within a 12-hour light/dark cycle at a temperature of 24 °C and ambient humidity (55% - 60%). In this research, the user instructions on the animal model were followed based on the Unit 12 Declaration of Helsinki.

BALB/c mice are albino with pink eyes and white hair. BALB/c mice are inbred and now more than 230 generations have passed since their origin in 1920. BALB/c mouse is one of the most used inbred mouse models. It is used in immunology and infectious diseases. In BALB/c mice, Th2 cells are readily activated by immunogenicity, giving the strain an excellent response to immunogenicity. Therefore, BALB/c mice are used as a model to identify genes with susceptibility to infectious and neoplastic diseases. Due to their ability to produce plasma cell tumors in the tissue, they are used in the production of monoclonal antibodies. BALB/cs has attracted the attention of researchers as a research model for testing chemotherapy drugs and various vaccines. In this research, female mice were used to carry out research on pregnant mice for neonatal mice.

All animal experiments were performed using 30 female (6-8 weeks of age) rats purchased from Zanjan University of Medical Sciences, School of Medicine. Zanjan University of Medical Sciences, School of Medicine, approved animal studies by ethical code IR.IAU.Z.REC.1396.87.

Bacterial strain and culture method

S. agalactia serotype Ia with the code QA/F/723/06/06 and PTCC 1768 (ATCC13813) was used by the Iranian Research Organization for Science and Technology (IROST). *S. agalactiae* powder was mixed with distilled water, and the suspension on Brucella Broth culture medium containing sheep was cultured. Plates were incubated for 24 hours at 37 °C. After 24 hours of incubation.

Isolation of capsules from *S. agalactiae*

The synthetic culture medium was placed in a shaker incubator for 3 days at 37 °C with medium speed. After 3 days, the microbial culture was removed from the incubator, and 10 mL of Formaldehyde was added and centrifuged, then the supernatant was added to 96% ethanol and refrigerated. The supernatant was removed from the precipitate, and distilled water and alcoholic calcium chloride were added. 96% Ethanol with Sodium Chloride was added to the supernatant and refrigerated for 24 hours. After 1.5 hours of centrifugation, the supernatant was discarded, and sediments were collected. The precipitate was Capsule Ia from Group B *Streptococcus*, which was stored at -4 °C.

Preparation of PLGA_NPs containing type Ia capsules

The micelle was made using the solvent evaporation method. This method is preferred because it is simple, cost-effective, and results in monodisperse and more stable particle sizes.

PLGA_NPs were obtained from Sigma-Aldrich (Dorset, England). 1.9 mL of the sodium dihydrogen phosphate was mixed with 1.8 ml of sodium phosphate with 10 ml of distilled water. The pH of the solution was measured and adjusted to 7.4. Then sucrose was added to Phosphate-1X Buffered Saline (PBS) solution, and 2% sucrose was made. 0.05 g of antigen was dissolved with PBS + 2% sucrose solution. Dichloromethane was added to PLGA and sonicated 3 times for 20 seconds. Polyvinyl alcohol (PVA) was mixed with 6 mL of distilled water, added to the previous solution, and placed on a magnetized heater for 30 minutes. Then the solution was sonicated 3 times again for 20 seconds. After sonication, the solutions were placed in a centrifuge and kept for injection [20].

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Characterization of nanoparticles

After conjugating the PLGA_NP with type Ia capsules, it was characterized using spectroscopy techniques, including Zetasizer (Malvern, UK) [21].

Mice vaccination and immunization process

Groups of 4 rats were immunized with 0.5 mL capsule (CPS), PLGA, or CPS_PLGA as group 1: rats injected with CPS_PLGA conjugate, group 2: rats injected with the CPS, group 3: rats injected with PLGA, and group 4: rats injected with normal saline were the same as the control group.

The injections were given intramuscularly. The presence of the carbohydrate was performed at a wavelength of 210 nm, and the concentration of CPS in PLGA was adjusted to be 0.1 µg CPS per 0.5 ml of NPs. Vaccination of the neonatal rats: One month after the birth, neonatal rats were divided into two groups (with vaccinated and non-vaccinated mothers), and the first dose of the vaccine without anesthesia intramuscularly were injected, and two weeks later, the booster dose of the rats with anesthesia was injected intramuscularly by 0.3 mL CPS, PLGA or CPS_PLGA. Groups of 4 neonatal rats were immunized as neonatal rats injected with CPS_PLGA (group 1), neonatal rats injected with capsules (group 2), neonatal rats injected with PLGA (group 3), and neonatal rats injected with normal saline (group 4).

Rat survival model and spleen culture method

In order to perform a challenge test, the *S. agalactiae*, according to the 10 McFarland standard protocol, were prepared. Groups of 4 and neonatal rats were anesthetized with ether, and 0.5 ml of the suspension 3×10^9 CFU was administered by vaginal gavage. After two weeks, the rats were anesthetized with ether, sampling from the vagina was done, and cultured on a plate containing nutrient agar medium (Merck, Germany), and the plate was incubated at 37 °C for 24 h, after this time, the colonies were counted. Blood sampling was done from the hearts of rats, centrifuged, and the serum stored at -4 °C. This test is the same for rats and neonatal rats.

Under sterile conditions, the spleen was removed by autopsy and placed on a sterile plate. In order to homogenize the spleens, 10 mL of normal saline was added to the spleen tissue. Then the suspension was centrifuged and diluted. Each dilution was cultured on a nutrient agar medium by the standard loop and incubated for 24 hours at 37 °C. Then, the colonies were counted (It should be noted that this test is similar in rats and neonatal rats. Also, each group included 6 rats).

Opsonophagocytosis test

First, the serum samples were incubated at 56 °C, and serial dilutions prepared with Hanks' Balanced Salt Solution in 96-well microplates were done. Then bacterial suspension (0.5 McFarland bacterial suspension (200 CFU/well), 1 mL rabbit complement, and PMNs $(17.7 \pm 0.6) \times 10^2$ CFU/well were added and placed in a shaker incubator. A colony count was performed. PMNs isolated from healthy mice were done with ficol. The solution was taken from mice and placed on a shaker at 37 °C, and phagocytosis occurred. Dilution in cold NACL was done to each well and incubated at 37 °C. Culture in agar nutrient medium and incubating at 37 °C for 48 hours and counting colonies on the plate was performed. This test was the same in mice and neonatal mice [22].

Statistical analysis

The mean and standard deviation was calculated and a one–way ANOVA test in SPSS 21.0 software, ($p < 0.05$), (SPSS Inc., Chicago, Illinois, USA) was used to conduct the statistical analyses.

Results

Nanoparticle characterization

The results related to zeta potential measurements showed that the zeta potential of PLGA nanoparticles was -19 and for the conjugated CPS-PLGA was -10.34 (Figure 1,2). Table 1 shows the PLGA size, which is 176 nm. The size of antigens conjugates and nanoparticles containing type Ia antigen of *S. agalactiae* capsule (PLGA-CPS) is 256.3 nm.

In our study, the size of PLGA nanoparticles increased

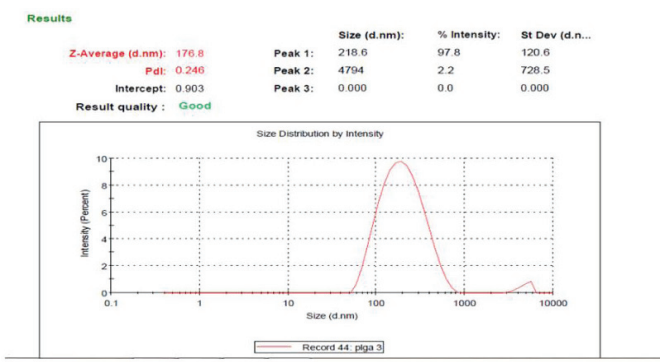

Figure 1: Graph of PLGA nanoparticle size.

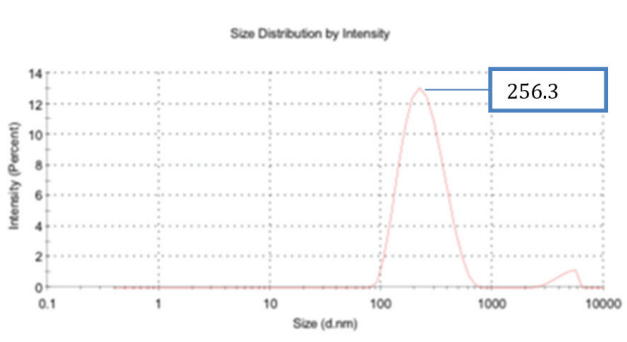

Figure 2: Size chart of PLGA_NPs containing type Ia antigen of *S. agalactiae* capsule.

Table 1: The size and electrical charge of NPs and NPs containing type Ia antigen of *S. agalactiae* capsule.

Groups	SIZE (d nm)	PDI	ZETA (mV)
PLGA	176	0.246	-19
CPS-PLGA	256.3	0.162	-10.34

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from 176.6 nm (prior to drug loading) to 265 nm after the encapsulation of the drug. This size augmentation can be attributed to multiple factors. Firstly, the adsorption of drug molecules on the surface of nanoparticles likely contributed to an additional outer layer. Secondly, the incorporation of the drug within the PLGA matrix increased the internal density and volume of the particles. Finally, possible interactions between the drug and polymer may have resulted in structural changes in the nanoparticle matrix. These findings are consistent with previously reported studies, which also observed increased particle size upon drug loading due to similar mechanisms.

1. Considering that our antigen has a negative charge, the decrease in the zeta potential after loading it onto PLGA nanoparticles could be due to the following reasons**Interference of Negative Charges:** PLGA nanoparticles usually have a negative or neutral charge. When a negatively charged antigen is loaded onto the surface of the nanoparticles, interference between the negative charges of the antigen and the surface of the nanoparticles can occur, leading to a reduction in the overall surface charge and, consequently, a decrease in the zeta potential.

2. **Reduced potential difference:** If the negatively charged antigen directly attaches to the surface of the PLGA nanoparticles, it may reduce the potential difference between the surface of the nanoparticles and the surrounding medium. This happens because the negative charge of the antigen may partially neutralize the negative charge on the surface of the nanoparticles, resulting in a decrease in the zeta potential.

3. **Effect of cationic attraction:** Some antigens may interact with cationic ions present in the environment, leading to the formation of a weak cationic coating on the surface of the particles. This coating could affect the surface charge and zeta potential.

4. **Increased particle aggregation:** The negatively charged antigen could create repulsive forces between the nanoparticles and the antigens. If the negative charge is not effectively distributed on the nanoparticles and antigens, this may lead to particle aggregation, which could reduce the zeta potential.

Results of mice and neonatal mice spleen culture

According to the results, the mean of the pathogenic strain of *S. agalactiae* after exposure to the immune system of the CPS-PLGA group was lower than in other experimental groups. This indicates the correct and potential performance of the vaccine candidate group in harvesting and removing the mentioned strain by this group of vaccine candidates and less growth of colonies in spleen culture. In addition, the mentioned group has a significant difference between the nanoparticle and the control group ($p < 0.05$). Table 2 shows the results of mice spleen culture in experimental groups.

According to the results, there was a significant difference between the results of all 8 groups of neonatal mice from the experimental groups ($p < 0.05$), which indicates the success of the vaccine candidate group and less growth of colonies as a result of the spleen culture of neonatal mice.

Results of swab culture in rats and neonatal rats

According to the results seen after the swab culture, the vaccine candidate group reduced the growth of *S. agalactiae* colonies compared to the other groups reported, which is the reason for the vaccine's effectiveness in removing the mentioned strain as a candidate for the vaccine group. This group has a significant difference between the two NPs and the control group ($p < 0.05$).

According to the results shown in Table 3, a significant difference was seen between all 8 groups of neonatal rats from the experimental groups ($p < 0.05$), which indicates the success of the vaccine candidate group and less colony growth.

Results of rat opsonophagocytosis test

The results presented in Table 4 indicate that the CPS-PLGA vaccine candidate group has the lowest growth value in the percentage of grown colonies compared to other tested groups. This is the reason for the suitable removal of the pathogenic strain. It should be noted that the candidate group of the vaccine with NPs and the control group has a significant

Table 2: The results of rat and neonatal rat spleen culture in experimental groups.

Groups	Two weeks after culture of mice Spleen	Two weeks after culture of neonatal rat Spleen	
	(Mean ± SD)	(Mean ± SD)	
	CFU/ml	Vaccinated	Non-vaccinated
CPS Ia-PLGA	*CFU/ml* (37.4 ± 1.5) × 10^3	*CFU/ml* 19000/0 ± 2000/0	*CFU/ml* 49666/6 ± 3214/55
CPS Ia	*CFU/ml* (12.7 ± 2) × 10^4	*CFU/ml* 113333/3 ± 15275/2	*CFU/ml* 356666/6 ± 30550/50
PLGA	*CFU/ml* (90.4 ± 5.2) × 10^6	*CFU/ml* 9/0 ± 5/1	*CFU/ml* 1/0 ± 1/5
NS (control group)	*CFU/ml* (96.3 ± 7.3) × 10^6	*CFU/ml* 9/2 ± 1/5	*CFU/ml* 1/0 ± 6/5

Table 3: The results of mice and neonatal mice swap cultures in the experimental groups.

Groups	Two weeks after culture of mice Swap	Two weeks after culture of neonatal rat Swap	
	mean ± SD	mean ± SD	
	CFU/ml	Vaccinated	Non-vaccinated
CPS Ia-PLGA	*CFU/ml* 125.3 ± 12.8	*CFU/ml* 26/6 ± 6/1	*CFU/ml* 79/3 ± 3/0
CPS Ia	*CFU/ml* 212 ± 8	*CFU/ml* 143/3 ± 16/0	*CFU/ml* 307/0 ± 9/5
PLGA	*CFU/ml* 2013.3 ± 189	*CFU/ml* 1294/6 ± 958/6	*CFU/ml* 2089/3 ± 72/0
NS(control group)	*CFU/ml* 1994.6 ± 72.1	*CFU/ml* 1996/6 ± 32/1	*CFU/ml* 1718/6 ± 546/1

Table 4: The results of the mice and neonatal mice opsonophagocytosis test.

Groups	Mean ± SD (mice) *CFU/ml*	Mean ± SD (Neonatal mice)
CPSIa-PLGA	(17.7 ± 0.6) × 10^2 *CFU/ml*	(25.7 ± 2) × 10^2 *CFU/ml*
CPSIa	(24.7 ± 1.5) × 10^2 *CFU/ml*	(45.4 ± 2.9) × 10^2 *CFU/ml*
PLGA	(24.7 ± 1.5) × 10^4 *CFU/ml*	(45.4 ± 2.9) × 10^4 *CFU/ml*
NS	(45.3 ± 41.2) × 10^4 *CFU/ml*	(60.8 ± 31) × 10^4 *CFU/ml*

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difference ($p < 0.05$). There was no significant difference between the vaccine group and the antigen group ($p = 0/939$).

The results indicate that the CPS-PLGA vaccine candidate group has the lowest growth rate in terms of the percentage of colonies grown compared to other tested groups in neonatal rats, which is the reason for the suitable removal of the pathogenic strain. It should be noted that the candidate group of the vaccine has a significant difference between NPs and the control group ($p < 0.05$). There was no significant difference between the vaccine candidate and antigen groups ($p = 0.909$).

Discussion

In neonates with *S. agalactiae* infected mothers, in order to prevent the proliferation of bacteria, intravenous injection of the antibiotic ampicillin or penicillin was used previously, but due to the high rate of anaphylactic shock [23] due to the use of the mentioned antibiotics in neonates, use the two antibiotics Erythromycin and clindamycin during labour suggested [24-26]. Unfortunately, the usage of these antibiotics has been associated with resistance to the bacterium since 2005 [24]. In order to control the antibiotic resistance of *S. agalactiae* and replacement with capsular antigen, the use of the vaccine as a preventive agent is recommended.

For this purpose, in this study, Ia capsular antigen was used as the main factor in the pathogenicity of *S. agalactiae*. In addition, due to its polysaccharide parts, the capsule is in the group of non-dependent antigens. Subsequently, PLGA_NPs were used as a capsule antigen carrier for the production of nano-vaccine.

In a study by *Xiaoli Ke*, et al. the immune protective effects of the LrrG protein of *S. agalactiae* encapsulated by PLGA (double emulsion-solvent evaporation method) on an animal model of tilapia were assessed. This study examined the size of PLGA-LrrG protein (between 2.1 and 7.3 µm), encapsulation efficiency, drug loading, and cumulative drug-release rate. Tilapias were immunized by intraperitoneal injection or oral administration with PLGA-LrrG microparticles. Lethal concentration LD50 (2.1 × 106 CFU/mL) was applied to challenge the tilapia. The results showed that the relative percent survival of vaccinated groups (intraperitoneal injection and oral administration) was significantly higher than that of the control groups. This research group suggested that PLGA-LrrG microparticles can be used to protect tilapia against *S. agalactiae* infection [27].

Another subunit vaccine study is the *Kan Kaneko*, et al. research project. PLGA_NPs, including recombinant pneumococcal surface protein A from family 2, clade 4 (PspA4Pro) antigen as a vaccine for targeting the vast majority of pneumococcal strains, were assessed. An in vitro examination of surface marker upregulation on a dendritic cell line, particle size, and surface charge was done. Positive surface charge increased the immunogenic effects of the NPs in this study, which could be appropriate as a vaccine design [28].

M. W. Hasan, et al. conducted a study in which the immunogenic potential of entrapped recombinant HcARF1(H. contortus ADP- ribosylation factor 1) in PLGA-chitosan nanoparticles was evaluated. The mice were vaccinated subcutaneously, and after 14 days, they were euthanized, and the spleen and blood were analyzed for lymphocyte proliferation, cytokine probing, and antigen-specific antibody production. They suggested that compared to the vaccine alone, the NP-encapsulated vaccine could stimulate a much stronger immune response in mice against H. contortus infection [29].

Further study by *Ansaya*, et al. Looked at diseases associated with different strains of *Streptococcus* in the Nile tilapia. One of the infectious strains of this species is *Streptococcus agalactiae*, which despite the possibility of vaccination, is still likely to be infected with other species. To this end, Ansaya and his colleagues, based on immunoproteomics studies and with the help of in-silico analysis, designed and built a chimeric multi-epitope vaccine using a flavodoxin backbone. The results on the control of Streptococcus in tilapia were promising and showed the effectiveness of the new flavodoxin platform for the design of chimeric vaccines. These chimeric protein backbones are suitable for providing epitopes recognized by the host immune system [30].

The next study was conducted by Q. Wang, et al. The aim was to evaluate the efficacy of the HCA59 antigen (Hepatocellular carcinoma-associated antigen 59) encapsulated in chitosan-PLGA nanoparticles in enhancing the mice's immune response. Hence, the immune responses of mice with HCA59 recombinant protein with complete Freund's adjuvant, chitosan nanoparticles, PLGA nanoparticles, and the combination of PLGA and chitosan nanoparticles were observed and evaluated. To this end, Cytokine probing followed by antibody leveling and T cell and dendritic cell phenotype analysis were carried out. They found that the rHCA59-PLGA-Chitosan nanosystem elicited a greater immune response in mice than in other antigen-delivery groups, which could suggest it is an effective antigen-delivery nanosystem against Haemonchus contortus disease [31].

In the present study, a nano-vaccine based on one of the main pathogens in *S. agalactia* called la-type polysaccharide capsules compared with other antigens used. The presence of capsules in this bacterium due to its role in bacterial colonization in the mother's vagina and then transmission from the pregnant mother to the neonate and the production of aggressive premature infections on the first day of life after delivery and inhibition of deletion by the host immune system with other pathogens assessed in previous projects in vaccination is important. In addition, in this research, unlike previous research, no intermediary is used as a spacer and pairing to achieve a direct and strong connection without reversibility and observing the smallest error in nanoparticle size. The use of PLGA_NPs as a carrier of this antigen compared to other carriers and the stimulation of immune system function in opsonization indicates that the nanoparticles are biodegradable due to their biocompatibility [32,33]. Also, the ability to deliver a small amount of antigen without changing the chemical and physical structure makes the opsonization of this strain easier and faster. It should be

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noted that the mentioned NP with no decrease in inflammation and accumulation of neutrophils in vital organs of the host has no harmful effects. The immunogenicity in the present study includes spleen culture in rats and neonatal rats after two weeks of vaginal challenge test and then two weeks after spleen culture and swab culture from the maternal and neonatal vagina. In performing maternal spleen culture, the most effective function in antigen opsonization about the CPS la-PLGA vaccine group is the count of grown colonies derived from bacterial strain compared to other tested groups. In addition, this vaccine group had a significant difference with the NP group and the control group, but there was no significant difference with the type Ia capsule. (0/667 = P).In neonatal rat spleen cultures, such as the maternal group, the CPS-PLGA vaccine group has a powerful performance in opsonizing the antigen compared to other vaccine groups. In addition, this group of vaccines had a significant difference with the NPs and the control group, but in comparison with the vaccinated group by CPS, there was no significant difference ($p = 0.964$) in performing maternal and neonatal swab culture, as in the spleen culture of the group. The low colony count obtained from the CPS-PLGA strain has a stronger and more effective function in stimulating the immune system than other groups. On the other hand, this group significantly differs from other groups ($p < 0.05$).

Based on the results of maternal and neonatal spleen culture tests and maternal and neonatal swab culture, challenge test, and opsonophagocytosis tests, it can be concluded that the potential performance of the CPS-PLGA vaccine candidate group is ineffective stimulation and enhancement of infection for diagnosis. On the other hand, according to data analysis, this vaccine group has a significant difference from other vaccine groups ($p < 0.05$). It can be concluded that this vaccine can be used as a candidate vaccine in the treatment of congenital infections and premature invasive neonatal infections in the animal model of rats due to its high efficacy in infection.

Conclusion

Streptococcus agalactiae (*S. agalactiae*) is the cause of pneumonia (side chest) and meningitis in infants and sometimes bacteremia (blood infection) in the elderly. Bacteria can colonize the intestines and female reproductive organs. Premature delivery and premature rupture of membranes occur to reduce the risk of transferring bacteria to the baby. In this study, our research team chose human pathogenic *Streptococcus agalactiae* serotype Ia.

In order to increase the immunity level by the capsular type Ia antigen of *S. agalactiae*, conjugated PLGA nanoparticles (PLGA_NP), we proposed a more effective vaccine against infections caused by *S. agalactiae*. In addition, the cytotoxicity of the NPs as a carrier in the fabrication of the nano-vaccine in the animal model of rats was investigated.

The zeta potential of PLGA_NP was -19 mv, and for the conjugated capsule (CPS)-PLGA was -10.34 mv. The size of PLGA-CPS is 256.3 nm. The mean of the pathogenic strain of *S. agalactiae* after exposure to the immune system of the CPS-PLGA group was lower than in other experimental groups. Reduced growth of *S. agalactiae* colonies in the vaccine candidate group compared to the other groups was reported, and in rat opsonophagocytosis test, the CPS-PLGA vaccine candidate group has the lowest growth value in the percentage of grown colonies compared to other tested groups.

It can be concluded that the potential performance of the CPS-PLGA vaccine candidate group is ineffective stimulation and enhancement of infection for diagnosis. On the other hand, according to data analysis, this vaccine group has a significant difference from other vaccine groups. The prepared antigen can be used as a candidate vaccine to treat congenital infections and premature invasive neonatal infections in the animal model of rats due to its high efficacy in infection.

In tuning the size of Poly(lactic-co-glycolic acid) (PLGA) nanoparticles and their drug loading, this study demonstrated that while drug loading and surface modifications can cause swelling of PLGA nanoparticles, the increases in size are generally minor and predictable. The research emphasized the importance of controlling synthesis methods and operational parameters to achieve desired particle sizes.

Regarding controllable microfluidic production of drug-loaded PLGA nanoparticles, researchers developed a microfluidic approach to produce drug-loaded PLGA nanoparticles with precise control over size. They found that by adjusting flow rate ratios, polymer concentration, and solvent volume ratios, the nanoparticle size could be finely tuned. This method allowed for consistent production of nanoparticles with specific sizes suitable for various drug delivery applications.

On evaluating the effect of synthesis, isolation, and characterisation variables on reported particle size and dispersity of drug-loaded PLGA nanoparticles, This investigation highlighted how changes in energy input during emulsification (e.g., using a sonic probe versus a homogenizer) had significant effects on particle size and polydispersity index. The study provided recommendations for fabricating PLGA-based nanoparticles with controlled sizes and dispersity.

In our study, the polydispersity index (PDI) of PLGA nanoparticles decreased from 0.246 to 0.162 after drug loading. This reduction in PDI indicates a more uniform size distribution of nanoparticles upon drug encapsulation. Several factors may contribute to this observation. First, the incorporation of the drug into the nanoparticle matrix likely enhanced the structural stability, preventing aggregation and ensuring consistent particle sizes. Additionally, possible interactions between the drug and PLGA polymer could have led to better cohesion of the nanoparticle matrix.

Furthermore, the drug might have acted as a stabilizing agent during the formulation process, promoting the formation of uniformly sized nanoparticles. This improved homogeneity is crucial for achieving reproducible drug release profiles and enhancing the overall efficacy of the nanoparticle formulation.

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