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(RESEARCH ARTICLE)

CHITOSAN AS DELIVERY SYSTEM FOR HBCAG GENE EXPRESSION WITH PEGFP-C1 ON HELA CELLS

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ABSTRACT

Hepatitis B is a viral liver infection caused by hepatitis B virus (HBV), which may lead to chronic liver disease, cirrhosis, and liver cancer. Recombinant DNA technology offers a promising approach for developing innovative hepatitis B vaccines. In this approach, the gene encoding hepatitis B core antigen (HBcAg) can be introduced into *Escherichia coli* to produce recombinant antigenic proteins. These proteins then formulated with nanoparticles as vaccine delivery systems. The aim of this study was to determine the effectiveness of chitosan nanoparticles as a delivery system for HBcAg VHB gene expression using pEGFP-C1 in HeLa cells as eukaryotic host cells. The methods used in this study included reculturing transformant bacteria obtained from previous research, isolating recombinant DNA plasmids, performing plasmid restriction analysis and PCR, sequencing, transfecting HeLa cell cultures, and observing EGFP-HBcAg protein expression through fluorescence microscopy. PCR analysis confirmed that the transformant E. coli DH5α successfully harbored the pEGFP-C1-HBcAg plasmid. Restriction analysis of the recombinant plasmid produced two distinct DNA bands, indicating successful plasmid digestion. Following transfection of HeLa cells, green fluorescence was observed in cells treated with chitosan nanoparticles, suggesting expression of the EGFP-HBcAg protein. These findings indicate that chitosan nanoparticles have potential as a delivery system for hepatitis B DNA vaccines using the pEGFP-C1 vector

Keywords: chitosan, HeLa, Hepatitis, pEGFP-C1, Vaccine

1 INTRODUCTION

Hepatitis B (HB) is a communicable viral infection caused by the Hepatitis B Virus (HBV), which primarily targets liver cells (hepatocytes) (1). In addition to being a global health concern, HB remains a significant public health issue in developing countries, including Indonesia. According to the 2018 Basic Health Research conducted by the Indonesian Ministry of Health, the prevalence of hepatitis was approximately 0.39%, corresponding to nearly one million affected

individuals, as reported by the Ministry of Health's Center for Data and Information (Pusdatin). Chronic HBV infection can result in serious complications, including liver cirrhosis and hepatocellular carcinoma, contributing substantially to morbidity and mortality. Prevention and management strategies include health promotion, specific protection, immunization, risk-factor control, surveillance, early detection, and appropriate treatment of cases.

Immunization is widely recognized as an effective and cost-efficient strategy for preventing infectious diseases, highlighting the importance of developing vaccines with both preventive and therapeutic potential. Hepatitis B vaccines have been commercially available since 1981, initially using inactivated viral preparations such as Heptavax, which demonstrated satisfactory safety and effectiveness. Subsequently, recombinant DNA technology enabled the development of improved hepatitis B vaccines (2). In 1986, Recombivax HB became the first hepatitis B vaccine produced using recombinant DNA technology (3). Current research on next-generation HBV vaccines focuses on recombinant viral antigens combined with novel adjuvants, as well as DNA-based vaccine approaches (3,4). DNA vaccines are generally developed by incorporating a target gene sequence into a eukaryotic expression vector, allowing the encoded antigenic protein to be produced and stimulate an immune response in the host (5). Ongoing studies aim to improve DNA vaccine efficacy through plasmid optimization, enhanced delivery systems, appropriate antigen selection, and the incorporation of immunological adjuvants.

In addition to the choice of DNA vaccine vector and target antigen, the delivery system plays a crucial role in determining vaccine effectiveness. The relatively low expression of DNA vaccines and their limited ability to cross cellular membranes can restrict the amount of genetic material that reaches antigen-presenting cells, thereby reducing the resulting immune response. Natural and synthetic polymers have attracted attention as potential vaccine delivery materials because of their biodegradability, biocompatibility, low toxicity, and ability to be modified for transporting genetic material (6). Among these materials, chitosan has considerable potential as a DNA vaccine delivery agent. Its positively charged structure enables it to interact electrostatically with negatively charged DNA and helps protect the genetic material from nuclease degradation (7). Therefore, this study aims to evaluate the potential of chitosan nanoparticles as a delivery system for HBcAg gene expression using the pEGFP-C1 vector in HeLa cells.

2 MATERIAL AND METHODS

This research is a laboratory experimental study with a Posttest Only Control Group research design, because data collection was carried out after treatment and compared with experimental controls. This research is divided into 3 research stages in general. The first stage was the isolation of the recombinant DNA plasmid (pEGFP-N1-HBcAg) which had been constructed by previous researchers and confirmed by plasmid restriction analysis and confirmation of the gene sequence by sequencing using the pEGFP-N1 sequencing primer. The second stage is making a complex with a chitosan delivery system and its formulation (chitosan-pEGFP-N1-HBcAg). The third stage is transfection of the HeLa cell line and expression testing. The ability of the delivery system was observed qualitatively based on fluorescence observations of EGFP-HBcAg protein expression using an inverted microscope. This research has Ethical Clearance with number KE/FK/0929/EC/2022 issued by the Medical and Health Research Ethics Commission, Faculty of Medicine, Public Health and Nursing, Gadjah Mada University.

This study employed a laboratory-based experimental approach using a Posttest-Only Control Group design, in which observations were conducted following treatment and compared with appropriate control groups. The research consisted of three main phases. The first phase involved isolating the recombinant DNA plasmid (pEGFP-C1-HBcAg), which had been constructed in a previous study. The recombinant plasmid was subsequently verified through restriction analysis and gene sequencing using a pEGFP-C1 sequencing primer. The second stage involved preparing the chitosan-based delivery system and forming the chitosan–pEGFP-C1-HBcAg complex. The final stage consisted of transfecting the complex into HeLa cells and evaluating gene expression. The effectiveness of the delivery system was assessed qualitatively by observing EGFP-HBcAg protein fluorescence using an inverted microscope. This study received ethical approval under Ethical Clearance No. KE/FK/0929/EC/2022, issued by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada in Yogyakarta, Indonesia.

2.1 Recombinant DNA plasmid preparation

Bacterial colonies were first propagated in liquid culture, followed by plasmid isolation using the Plasmid DNA Extraction Maxi Kit (Favorgen Biotech Corp) according to the manufacturer's instructions. The concentration and purity of the extracted plasmid DNA were subsequently determined using a NanoDrop spectrophotometer. For double-restriction digestion, 1 µL of the isolated plasmid was combined with 1 µL of *Bgl*II enzyme (10 U/µL), 1 µL of *Eco*RI enzyme (20 U/µL), 2 µL of restriction enzyme buffer, and 15 µL of nuclease-free water (NFW). The reaction mixture was incubated at 37°C for 15 minutes. The digested plasmid was then subjected to agarose gel electrophoresis to verify the success of the restriction process.

Agarose gel electrophoresis was conducted using a 1% agarose gel, prepared by dissolving 0.5 g of agarose in 50 mL of 1× TBE buffer. The mixture was heated in a microwave until the agarose was completely dissolved, followed by the addition of 5 µL of SYBR Safe DNA Gel Stain (Invitrogen). The resulting solution was poured into a gel casting tray and allowed to solidify for approximately 20 minutes. Once the gel had set, it was transferred to an electrophoresis chamber containing 1× TBE buffer. For sample loading, 5 µL of the isolated plasmid DNA was mixed with 5× Gel Pilot DNA Loading Dye (Qiagen) on parafilm before being introduced into the gel wells. Electrophoresis was performed at 100 V for 60 minutes, after which the DNA bands were visualized using a UV illuminator.

The isolated plasmid DNA was further evaluated by polymerase chain reaction (PCR). The reaction mixture consisted of 30 µL of GoTaq® 2X Master Mix, 4.8 µL of 10 µM pEGFP-C1 forward primer (5′-CATGGTCCTGCTGGAGTTCGTG-3′), 4.8 µL of 10 µM pEGFP-C1 reverse primer (5′-GAAATTTGTGATGCTATTGC-3′), and 17.4 µL of nuclease-free water. PCR amplification was performed using an annealing temperature of 56.9°C. The resulting PCR products were subsequently subjected to DNA sequencing, and the sequencing data were analyzed using appropriate computer software.

2.2 Chitosan-DNA complex preparation

Chitosan stock solutions for the preparation of the chitosan–DNA complexes were prepared using a modified complex coacervation method (7). Briefly, 20 mg of medium-molecular-weight chitosan (MMWC; 150–700 kDa) was dissolved in 100 mL of 1% acetic acid adjusted to pH 5.0. The resulting solution was sterilized by filtration through a 0.2 µm membrane and stored in a sterile conical tube. For complex formation, 1 µg of pEGFP-C1-HBcAg was combined with different concentrations of chitosan at DNA-to-chitosan weight ratios of 1:0.5, 1:1.0, 1:2.0, and 1:3.0. Each solution was separately heated at 50°C for 10 minutes. The pEGFP-C1-HBcAg preparation was then mixed with the respective chitosan solutions by vortexing at 2,500 rpm for 30 seconds at 4°C. Since the initial 1:0.5 ratio still showed positive DNA entrapment, a subsequent experiment was conducted using lower DNA-to-chitosan ratios of 1:0.1, 1:0.2, 1:0.3, and 1:0.4.

The formation of the chitosan–plasmid DNA complexes were further evaluated using agarose gel electrophoresis. A 1% agarose gel was prepared in 50 mL of 1× TBE buffer by heating the solution until the agarose was completely dissolved. Subsequently, 5 µL of SYBR Safe DNA Gel Stain (Invitrogen) was added, and the solution was poured into a gel casting mold. Electrophoresis was performed at 100 V for 30 minutes, followed by visualization under UV illumination. Samples containing chitosan/DNA nanoparticles were compared with free plasmid DNA. Uncomplexed plasmid DNA was expected to migrate through the agarose gel, whereas plasmid DNA that was effectively encapsulated or formed larger complexes with chitosan nanoparticles would exhibit limited migration and remain near the loading wells.

The particle size and zeta potential of the chitosan–DNA nanoparticles were determined through analyses conducted at the Integrated Research Laboratory, Faculty of Dentistry, Universitas Gadjah Mada. Cytotoxicity evaluation was performed at the Parasitology Laboratory, Faculty of Medicine, Universitas Gadjah Mada, using different concentrations of the chitosan nanoparticles.

2.3 In vitro transfection assay and EGFP-HBcAg protein expression

HeLa cells were cultured in 24-well Nunc™ Cell-Culture Treated Multidishes at an initial density of $1x10^5$ cells per well, with a final culture volume of 0.5 mL per well. The cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 for 72 hours, with the culture medium replaced daily. The culture medium was prepared by combining 10 mL of fetal bovine serum (FBS), 2 mL of Pen-Strep, 500 µL of Fungizone, and 1 mL of MEM-NEAA, followed by the addition of DMEM to a final volume of 100 mL. Once the HeLa cells reached approximately 70–80% confluence, corresponding to a density of ($3.2x10^6$) cells per Petri dish, transfection was performed using five experimental conditions: 1. Untreated cells, as the negative control; 2. Chitosan-only treatment, as the negative control; 3. Recombinant DNA plasmid-only treatment, as the negative control; 4. Chitosan nanoparticle–DNA plasmid complex, as the treatment group; 5. Commercial transfection reagent combined with recombinant DNA plasmid, as the positive control. Each transfection condition received 8 µg of recombinant DNA plasmid. Following transfection, the cells were incubated for 4 hours, after which the medium was replaced with fresh culture medium. The cells were then incubated for an additional 24 hours. EGFP fluorescence expression was subsequently examined using an inverted microscope (Nikon Eclipse T2s) at 200x magnification.

3 RESULT AND DISCUSSIONS

3.1 Recombinant DNA plasmid preparation

Recombinant plasmid DNA was isolated using the Plasmid DNA Extraction Maxi Kit (Favorgen Biotech Corp) in accordance with the manufacturer's instructions. Two recombinant plasmid samples were subsequently analyzed by 1% agarose gel electrophoresis to assess the quality and integrity of the isolated DNA (Figure 1). The extracted plasmids were further evaluated for DNA concentration and purity using a NanoDrop UV/Vis spectrophotometer (Table 1).

Assessment of DNA purity and integrity is an important quality-control step before subsequent molecular analyses. The evaluation was performed by measuring absorbance at 230, 260, and 280 nm, which provides information regarding DNA concentration and potential contamination by proteins, salts, or other organic compounds.

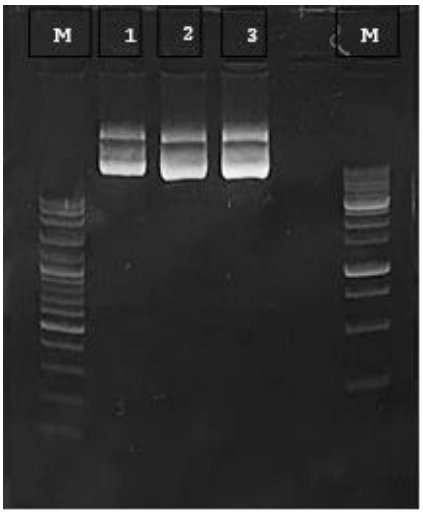

Figure 1: Isolate of pEGFP-C1-HBcAg (1, 2 and 3); Ladder 100bp (M1) and 1000 bp(M2)

Table 1: Purity and concentration of DNA plasmid recombinant

No	Sample	$A_{260/230}$	$A_{260/280}$	Concentration (ng/µL)
1	$C1_1$	2.002	1.832	99.22
2	$C1_2$	1.992	1.814	100.08

Electrophoresis is a relatively effective technique for separating DNA fragments, particularly those ranging from approximately 100 bp to 25 kb, including plasmid DNA. When an electric field is applied, the phosphate backbone of DNA and RNA carries a negative charge, causing the DNA fragments to migrate toward the positively charged anode. The electrophoresis results indicated the presence of two recombinant plasmid DNA bands, corresponding to the supercoiled and nicked circular forms. DNA conformation is an important factor affecting migration speed, even when DNA molecules have the same molecular weight.

DNA generally occurs in three main conformations: supercoiled or covalently closed circular DNA (Form I), nicked or open circular DNA (Form II), and linear DNA (Form III). Form I has a compact structure because the polynucleotide strands remain intact. When a break occurs in one strand of the DNA double helix, the molecule changes into the open circular Form II. If breaks occur in both strands, the DNA adopts the linear Form III. These different conformations migrate at different rates during electrophoresis. Supercoiled DNA (Form I) generally migrates faster than linear DNA (Form III), whereas nicked circular DNA (Form II) migrates the most slowly. In addition to DNA conformation, migration rate is also affected by factors such as DNA fragment size, agarose concentration and type, applied voltage, ethidium bromide concentration, and the composition of the electrophoresis buffer (8).

The spectrophotometric analysis of DNA purity produced a ratio within the expected range. DNA purity is commonly evaluated using the absorbance ratio at 260 and 280 nm (A260/A280). A ratio above 2.0 may indicate RNA contamination, whereas a ratio below 1.8 can suggest contamination by proteins, phenol, or other organic compounds (9). Plasmid restriction is an approach used to ensure the presence of target gene inserts by utilizing restriction enzymes. General restrictions are carried out before sequencing because the sequencing process is quite time consuming and expensive. The enzymes used in this research were the *Bgl*II and *EcoR*I enzymes to cut the recombinant plasmids pEGFP-C1-HBcAg at the end of the insert (MCS). These two enzymes are included in restriction endonucleases which can cut DNA at certain sequences, namely the AGACTC (*Bgl*II) sequence and the GAATTC (EcoRI) sequence. Visualization of restriction results using 1% agarose gel electrophoresis (Figure 2).

Plasmids labeled UC and UN represent plasmid isolation samples that were not subjected to restriction enzyme digestion (uncut). These samples produced results consistent with the 1% agarose gel electrophoresis shown in Figure

1, where a DNA band was observed within the 2000–5000 bp range. Following restriction digestion, two distinct DNA bands were observed at positions 1 and 2, as presented in Figure 2. The first band was located within the 2000–5000 bp range and was estimated to have a size of approximately 4731 bp. Meanwhile, the second band, which appeared slightly above the 500 bp, was estimated to be approximately 564 bp. The appearance of these two bands can be attributed to the double-digestion process using the restriction enzymes BglII and EcoRI. The approximately 4731 bp fragment corresponds to the complete pEGFP-C1 vector, whereas the approximately 564 bp fragment represents the HBcAg target gene insert. Therefore, the presence of both expected DNA fragments indicates that the target gene was successfully inserted into the vector plasmid.

The subsequent step involved further confirmation using DNA sequencing. In general, sequencing is performed to determine the nucleotide sequence of a DNA molecule by identifying its individual bases and comparing the resulting sequence with a previously established reference sequence. In this study, the HBcAg nucleotide sequence reported in previous research was used as the reference for comparison (5).

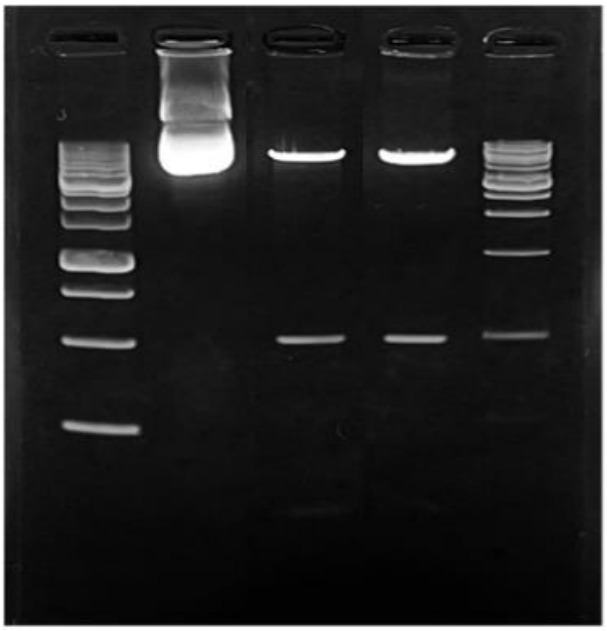

Figure 2: Restriction using *Bgl*II and *Eco*RI enzymes. From left to right: 1000 bp marker; Unrestricted isolated plasmid; restricted isolated plasmids (1 and 2); 100 bp marker.

The sequencing procedure employed the Sanger method, which is based on the termination of DNA synthesis through the incorporation of chain-terminating dideoxynucleotides. These nucleotides lack the 3′-hydroxyl group required for further DNA strand elongation during the DNA polymerase reaction. DNA synthesis begins when a primer binds to a specific complementary region of the DNA template, followed by strand extension mediated by DNA polymerase. The reaction consists of four separate but simultaneous reactions, with each reaction containing one type of deoxynucleotide corresponding to A, C, G, or T (10).

In this study, sequencing analysis was performed by Genetica Science Corp. The sequencing results showed no substantial differences among the samples obtained from the recombinant plasmid isolation. Therefore, the recombinant plasmid can be considered to possess a nucleotide sequence consistent with the HBcAg target gene sequence (Appendix 2). Before sequencing, PCR amplification was conducted using the plasmid isolation products to increase the amount of the target DNA fragment and facilitate detection of the inserted gene. PCR was performed using a universal sequencing primer for pEGFP-C1 to verify that the isolated plasmid corresponded to the pEGFP-C1-HBcAg construct. The PCR amplification produced a DNA fragment within the 700–800 bp range, with an estimated size of approximately 775 bp. The larger fragment size is attributed to the additional base pairs contributed by the EGFP gene, which is positioned at the N-terminal region of the core protein.

Electrophoresis is a separation technique based on differences in the migration rates of charged molecules when subjected to an electric field. The ability of chitosan to interact with and form complexes with plasmid DNA was evaluated using SYBR Safe DNA Gel Stain (Invitrogen). This fluorescent stain interacts with double-stranded DNA and produces luminescence when exposed to UV light, allowing the DNA bands to be visualized during electrophoresis (Figure 3).

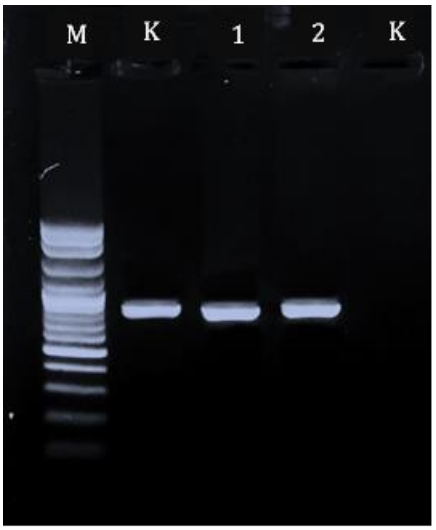

Figure 3: PCR isolation product of pEGFP-C1-HBcAg (K+: positive control; 1 & 2: Sample; K-: negative control; M: ladder 100 bp)

3.2 Chitosan-DNA complex preparation

The chitosan–DNA nanoparticle complexes were prepared using the complex coacervation method. Complex formation at ratios ranging from 1:0.1 to 1:0.4 was initially evaluated through a complex migration assay using 1% agarose gel electrophoresis, also referred to as the gel retardation assay (Figure 4). In this experiment, chitosan alone and recombinant DNA plasmid alone were included as control samples.

After visualization under a UV transilluminator, the chitosan–pEGFP-C1-HBcAg complex at a ratio of 1:0.3 showed that the DNA remained within the agarose gel wells. This indicates that complex formation prevented the DNA from migrating through the gel. The interaction within the nanoparticle complex is primarily driven by electrostatic forces between the positively charged chitosan molecules and the negatively charged DNA. Larger molecular complexes have greater difficulty passing through the pores of the agarose matrix and therefore migrate more slowly than smaller DNA molecules (8). In the P sample, which did not contain chitosan, the DNA was able to migrate through the agarose gel. As the proportion of chitosan increased, the DNA bands became progressively less visible or thinner, indicating stronger complex formation.

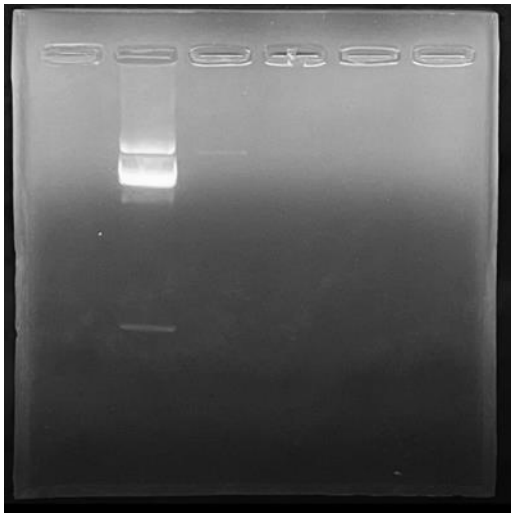

Figure 4: Agarose gel retaradation assay of chitosan-DNA complexes. From left to right; Chitosan only; Plasmid only; chitosan–pEGFP-C1-HBcAg complex at a ratio of 1:0.1; 1:0.2; 1:0.3; 1:0.4

The subsequent analysis involved a cytotoxicity assay of the chitosan–DNA complex to determine whether the transfection agent could cause toxic effects on cells. Based on the experimental results, the chitosan–pEGFP-C1-HBcAg complex was found to have low cytotoxicity toward HeLa cells. This was demonstrated by the high cell viability observed after treatment, with 86.7% viability in cells treated with the chitosan–pEGFP-C1-HBcAg complex, 94.6% in cells treated with the PBS negative control, and 100% in cells treated with chitosan alone as the negative control (Figure 5).

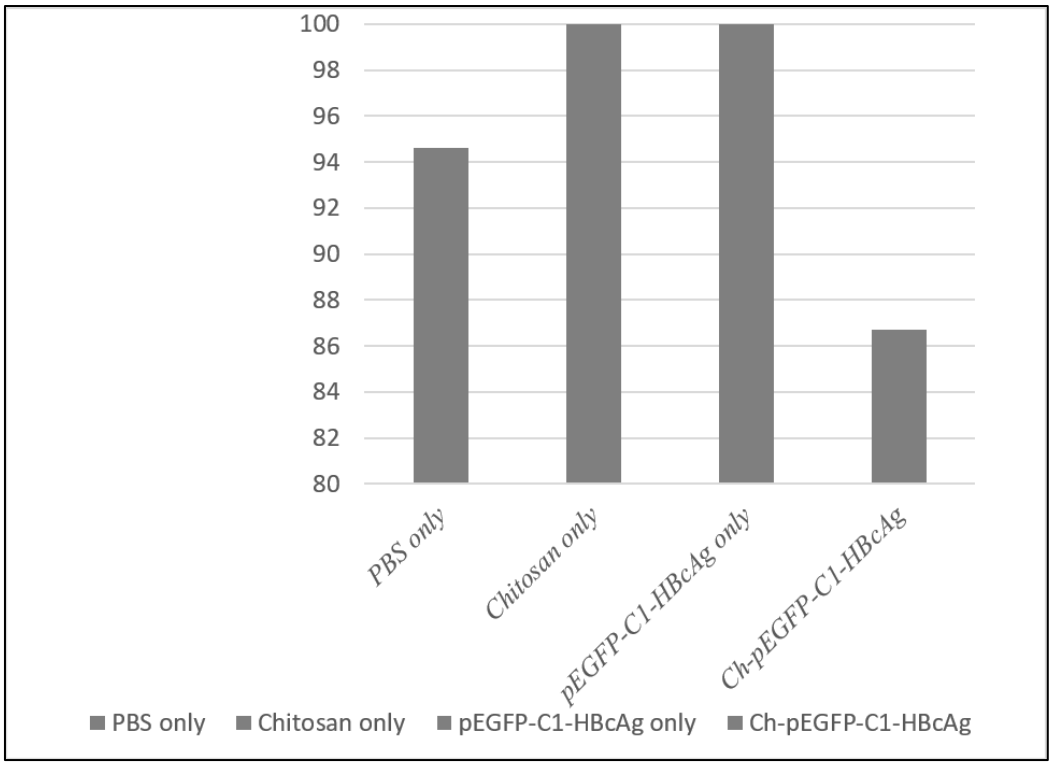

Figure 5: Cytotoxic assay

3.3 In vitro transfection assay and EGFP-HBcAg protein expression

Transfection refers to a series of processes used to introduce foreign nucleic acids into eukaryotic cells. This technique is widely applied to transfer genetic material into mammalian cells, such as the introduction of plasmid DNA into HeLa cells. The structural components of plasmids, including a promoter, origin of replication, multiple cloning sites, gene of interest, and selectable marker, facilitate the delivery and expression of genetic material within eukaryotic cells.

Plasmid DNA can exist in either linear or supercoiled forms during the transfection process. Previous studies have reported that supercoiled plasmid DNA generally provides higher transfection efficiency because its compact structure makes it less susceptible to degradation by exonucleases. In contrast, linear DNA has the advantage of being more readily recombined and may exhibit greater stability under certain transfection conditions. Another benefit of plasmid-based DNA delivery is its relatively low immunogenicity compared with viral DNA, which may reduce the potential risk of unwanted or pathological integration into host cells. Nevertheless, plasmid DNA transfection also has limitations, particularly its relatively low transfection efficiency and reduced recombinant protein production compared with some alternative delivery systems.

In this study, the pEGFP-C1 vector containing the HBcAg gene inserted at the *Bgl*II and *EcoR*I restriction sites was used. Consequently, expression of the HBcAg gene results in the production of HBcAg protein fused with the EGFP protein encoded by the *egfp* gene. Following transfection, HeLa cells were examined for fluorescence using a confocal microscope. The fluorescence analysis showed no detectable signal in cells treated with the negative control. In contrast, cells treated with the chitosan–DNA complex and the positive control exhibited positive fluorescence signals (Figure 6).

The use of appropriate controls is essential in transfection experiments to evaluate the effectiveness and efficiency of the transfection reagents and nucleic acids. Commonly used controls include positive, negative, untransfected, and mock controls. A positive control consists of DNA or RNA that has previously been demonstrated to successfully undergo transfection and produce the expected expression of the target gene. These controls provide a basis for determining whether the observed gene expression is attributable to the transfection procedure and the nucleic acid construct used.

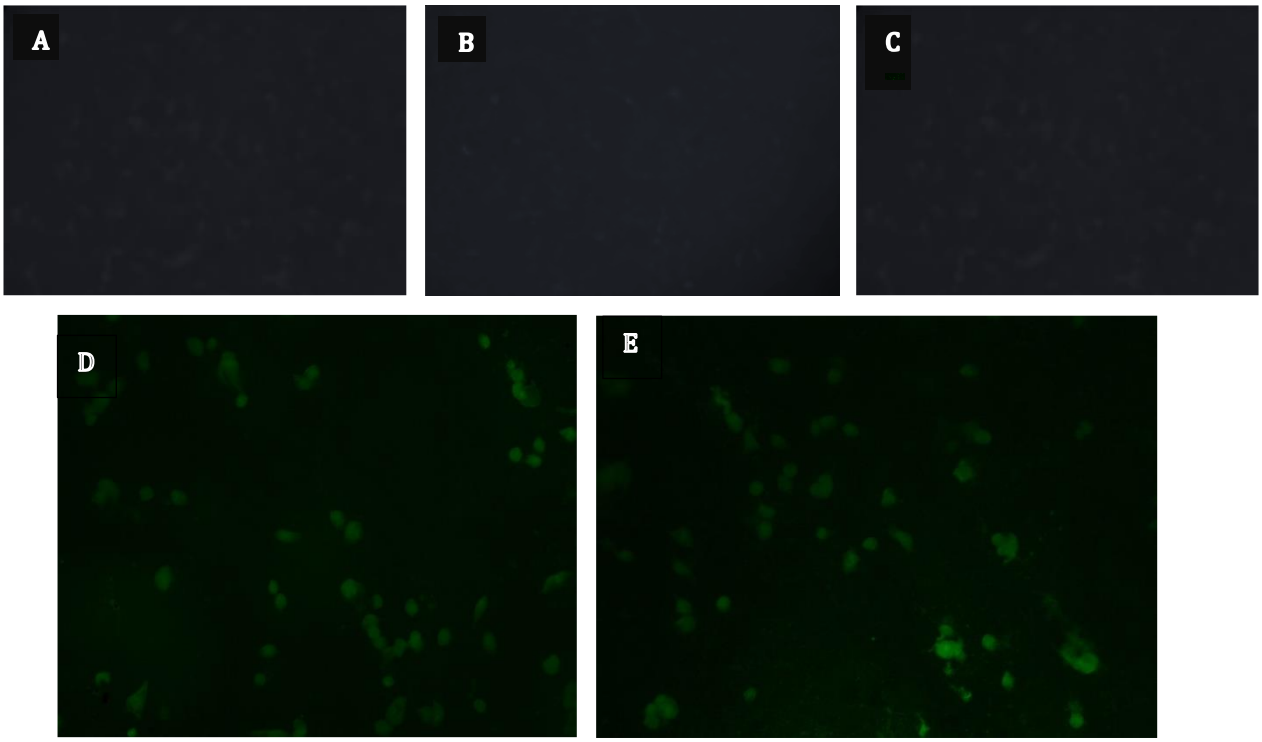

Figure 6: Fluorescence observation. A). Control untransfected HeLa cells only; B). Negative control HeLa cells + chitosan; C). Negative control HeLa + pEGFP-C1-HBcAg cells; D). Chitosan-pEGFP-C1-HBcAg Treatment; E). HighGene-pEGFP-C1-HBcAg Treatment

Conversely, negative controls are important for confirming that the observed expression of the target gene is specifically associated with the transfection process rather than being caused by other factors. These controls may consist of host cells cultured without the addition of the target DNA and/or delivery agent. An untransfected control, on the other hand, consists solely of host cells cultured without any transfection reagent or nucleic acid. This control provides baseline information about the condition of the host cells and the normal level of target gene expression in the absence of transfection.

A mock control involves performing the transfection procedure without introducing the target gene or nucleic acid. This type of control allows researchers to evaluate potential effects caused by the transfection reagent itself, including possible background signals or autofluorescence that could interfere with fluorescence-based observations. In this study, the commercial transfection reagent HighGene was used as the positive control because it has previously been demonstrated to successfully deliver the HBcAg target gene using the pEGFP-C1 vector. The untransfected control consisted of cultured cells without the addition of chitosan-based delivery nanoparticles or recombinant DNA plasmids. Meanwhile, the negative controls included cells treated with chitosan nanoparticles alone in the absence of DNA plasmids and cells treated with DNA plasmids alone without chitosan nanoparticles (11–13).

4 CONCLUSION

Based on the findings of this study, it can be concluded that the HBcAg gene was successfully transfected into HeLa cells, as demonstrated by the green fluorescence observed following treatment with the chitosan nanoparticles. Further studies are recommended to conduct quantitative gene expression analyses and in vivo experiments to evaluate the effectiveness of the recombinant chitosan–DNA nanoparticle complex as a potential DNA vaccine candidate.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

REFERENCES

[1] Bruce MG, Bruden D, Hurlburt D, Zanis C, Thompson G, Rea L, et al. Antibody Levels and Protection After Hepatitis B Vaccine: Results of a 30-Year Follow-up Study and Response to a Booster Dose. J Infect Dis. 2016 July 1;214(1):16–22.

[2] Van Damme P. Long-term Protection After Hepatitis B Vaccine. J Infect Dis. 2016 July 1;214(1):1–3.

[3] Zhao H, Zhou X, Zhou YH. Hepatitis B vaccine development and implementation. Human Vaccines & Immunotherapeutics. 2020 July 2;16(7):1533–44.

[4] Endmann A, Klünder K, Kapp K, Riede O, Oswald D, Talman EG, et al. Cationic Lipid-Formulated DNA Vaccine against Hepatitis B Virus: Immunogenicity of MIDGE-Th1 Vectors Encoding Small and Large Surface Antigen in Comparison to a Licensed Protein Vaccine. Chemin IA, editor. PLoS ONE. 2014 July 3;9(7):e101715.

[5] Jannah R, Unsunnidhal L. KONSTRUKSI DAN KLONING PLASMID PCDNA3.1 (+) DENGAN SUBGENOTIP B3 HEPATITIS B CORE ANTIGEN (HBcAg) SEBAGAI KANDIDAT VAKSIN DNA HEPATITIS B. 2019;5(2).

[6] Unsunnidhal L, Wasito R, Nugraha Setyawan EM, Warsani Z, Kusumawati A. Potential of polylactic-co-glycolic acid (PLGA) for delivery Jembrana disease DNA vaccine Model (pEGFP-C1-tat). J Vet Sci. 2021;22(6):e76.

[7] Ishak J, Unsunnidhal L, Martien R, Kusumawati A. *In vitro* evaluation of chitosan-DNA plasmid complex encoding Jembrana disease virus Env-TM protein as a vaccine candidate. Journal of Veterinary Research. 2019 Mar 1;63(1):7–16.

[8] Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose Gel Electrophoresis for the Separation of DNA Fragments. JoVE. 2012 Apr 20;(62):3923.

[9] Bunu S, Otele D, Alade T, Dodoru R. Determination of serum DNA purity among patients undergoing antiretroviral therapy using NanoDrop-1000 spectrophotometer and polymerase chain reaction. Biomed Biotechnol Res J. 2020;4(3):214.

[10] McGinn S, Gut IG. DNA sequencing – spanning the generations. New Biotechnology. 2013 May;30(4):366–72.

[11] Chong ZX, Yeap SK, Ho WY. Transfection types, methods and strategies: a technical review. PeerJ. 2021 Apr 21;9:e11165.

[12] Haraguchi T, Koujin T, Shindo T, Bilir Ş, Osakada H, Nishimura K, et al. Transfected plasmid DNA is incorporated into the nucleus via nuclear envelope reformation at telophase. Commun Biol. 2022 Jan 20;5(1):78.

[13] Fus-Kujawa A, Prus P, Bajdak-Rusinek K, Teper P, Gawron K, Kowalczuk A, et al. An Overview of Methods and Tools for Transfection of Eukaryotic Cells in vitro. Front Bioeng Biotechnol. 2021 July 20;9:701031.