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Investigation of the Usability of Polymeric Nanoparticle-Based Transfection Agent for CRISPR/CAS9 in HUVEC Cell Line

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HUVEC Hücre Hattında CRISPR/CAS9 için Polimerik Nanopartikül Tabanlı Transfeksiyon Ajanının Kullanılabilirliğinin Araştırılması

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Abstract

Genome editing technology is a promising, popular technology for diagnosing and treating many diseases. One of the most popular techniques utilized in genome editing research nowadays is the CRISPR/Cas system. The CRISPR/Cas system holds considerable potential for diagnostic and therapeutic applications due to its ease of use, speed, and affordability. The safe and effective delivery of the CRISPR-based genome editing system to the intended human body cells is one of the system's problems. This study investigated the silencing of the COX-2 gene via the CRISPR/Cas system using a PβAE-based transfection agent in the HUVEC cell line. Western blot analysis was performed to confirm COX-2 gene silencing in the HUVEC cell line, and the effects of COX-2 gene silencing on cell migration in these cells were examined. As a result, the transfection efficiency of the PβAE-based transfection agent for the HUVEC cell line was determined as 90-95%, and the CRISPR efficiency was calculated as 57.12% based on the Western blot results. It was revealed that the PβAE-based nanoparticles used in this study can be used as a good transfection agent in CRISPR/Cas-mediated genome editing in the HUVEC cell line, especially in plasmid transfection.

Keywords: CRISPR, Genom Editing, HUVEC Cell Line, Transfection

Öz

Genom düzenleme teknolojisi birçok hastalığın teşhisi ve tedavisi için umut vadeden, popüler bir teknolojidir. Günümüzde genom düzenleme araştırmalarında kullanılan en popüler tekniklerden biri CRISPR/Cas sistemidir. CRISPR/Cas sistemi, kullanım kolaylığı, hızı ve uygun fiyatı nedeniyle teşhis ve tedavi uygulamaları için önemli bir potansiyele sahiptir. CRISPR tabanlı genom düzenleme sisteminin amaçlanan insan vücudu hücrelerine güvenli ve etkili bir şekilde iletilmesi, sistemin sorunlarından biridir. Bu çalışma, HUVEC hücre hattında PβAE tabanlı bir transfeksiyon ajanı kullanılarak CRISPR/Cas sistemi aracılığıyla COX-2 geninin susturulmasını araştırdı. HUVEC hücre hattında COX-2 geninin susturulmasını doğrulamak için Western blot analizi yapıldı ve COX-2 geninin susturulmasının bu hücrelerdeki hücre göçü üzerindeki etkileri incelendi. Sonuç olarak, HUVEC hücre hattı için PβAE bazlı transfeksiyon ajanının transfeksiyon etkinliği %90-95 olarak belirlendi ve Western blot sonuçlarına göre CRISPR etkinliği %57,12 olarak hesaplandı. Bu çalışmada kullanılan PβAE bazlı nanopartiküllerin, özellikle plazmid transfeksiyonunda, HUVEC hücre hattında CRISPR/Cas aracılı genom düzenlemede iyi bir transfeksiyon ajanı olarak kullanılabileceği ortaya konuldu.

Anahtar Kelimeler: *CRISPR, Genom Düzenleme, HUVEC Hücre Hattı, Transfeksiyon*

1. Introduction

Genome editing involves technologies that allow scientists to alter an organism's genetic material. Thanks to these technologies, target-specific changes in genetic material can be achieved. Various methods for genome editing have been developed so far. The most common of these methods are transcriptional activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated nuclease 9 (Cas9)(Gaj et al. 2013; Hosur et al. 2023). Among these, the recently discovered CRISPR/Cas-mediated genome editing method allows for more effective, straightforward, cheaper, and faster changes in genetic material (Hryhorowicz et al. 2023). The CRISPR/Cas9 system was first discovered in the bacterial immune system. Scientists have observed repetitive CRISPR sequences in the bacterial genome, where "intermediate" DNA sequences exist between the repeats that match the viral sequences exactly. Later, when reencountering the virus, it was discovered that bacteria copied these DNA elements into

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RNA. This RNA, called guide RNA, provides immunity against the virus by directing a nuclease (a protein that cuts DNA) to its target to cut the viral DNA. Nucleases used to cut DNA are called Cas proteins (Zhang et al. 2014). This study aims to characterize the Poly-β-amino ester (PβAE) based transfection agent for CRISPR applications and to investigate the transfection efficiency in the HUVEC cell line for genome editing study.

In the last five years, scientists have realized the therapeutic potential of the CRISPR/Cas9 genome editing system against diseases, and they have paved the way for intensive work on the CRISPR/Cas9 genome editing system (Khoshandam et al. 2024). The first clinical studies using CRISPR/Cas9 were carried out in 2016. These studies showed that CRISPR/Cas9 held great promise for treating diseases with genetic disorders and creating disease models (Baylis and McLeod, 2017).

Nevertheless, akin to the challenges encountered with gene therapy and other therapies based on nucleic acids, CRISPR/Cas9 must contend with issues such as unintended effects at sites other than the target, the absence of secure and effective methods for delivery, and ethical concerns, all of which pose obstacles to its therapeutic use in humans. (Liu et al. 2017).

In plasmid-based systems studies, off-target effects can arise during CRISPR/Cas9-mediated gene editing (Guo et al. 2023). These consequences may result in epigenetic alterations, genomic instability, and disruption of gene functioning. Off-target effects result from the Cas9/sgRNA complex binding to regions away from the PAM (Protospacer Adjacent Motif) region. Given that sgRNA is designed to target a specific 20-base pair (bp) sequence of DNA, there is a possibility of encountering similar sequences elsewhere in the human genome. This raises concerns about off-target effects, which can pose challenges for using sgRNA in therapeutic settings involving humans. To mitigate potential off-target effects of the CRISPR/Cas9 system, strategies such as using a paired Cas9 nickase, carefully designing sgRNAs, and selecting the targeting site correctly are being considered. Choosing the appropriate delivery system and CRISPR/Cas9 type for carrying the genetic cargo can also help reduce off-target effects. Non-viral delivery methods are typically associated with fewer off-target effects than viral delivery systems (Liu et al. 2013).

Finding secure and efficient delivery systems to successfully implement CRISPR/Cas9 in clinical settings is important. The transportation of CRISPR/Cas9 systems to specific tissues or cells within the human body is a significant challenge, and various delivery systems have been used recently to address this obstacle.(Kang et al. 2017). The methods used to deliver CRISPR/Cas9 systems are like those used for nucleic acids and proteins for the past thirty years. For example, delivering plasmid-based CRISPR/Cas9 systems is like delivering plasmid-based gene therapy. Vectors designed for gene therapy, whether viral or non-viral, can be used for plasmid-based CRISPR/Cas9 systems without needing additional adjustments. While delivering Cas9 messenger RNA (mRNA) and sgRNA may seem complex, it is similar to delivering small interfering RNA (siRNA). This means that delivery systems created for siRNA can be repurposed to transport Cas9 mRNA and sgRNA with minimal changes. (Tao et al. 2019). The Cas9 protein/sgRNA ribonucleoprotein complex's delivery method is unique due to its positive charge of the Cas9 protein, distinguishing it from current nucleic acid delivery systems. Different techniques have been developed to alter the Cas9 protein to form a stable complex with sgRNA and non-viral vectors (Kuhn et al. 2020). The obtained nanoparticles In-depth research on the CRISPR/Cas9 system continues to develop safe and efficient delivery systems for CRISPR/Cas9-based therapeutics (Nie et al. 2022; Zhou et al. 2024).

The biodegradability of PβAEs reduces the risk of long-term toxicity and accumulation in cells and tissues (Anderson et al. 2008; Mirón-Barroso et al. 2022; Wang et al. 2024). Furthermore, their structure can be easily modified using different monomers, optimizing them for specific gene delivery needs (Lynn and Langer, 2000a). PβAE-based agents show high efficiency and low toxicity in gene delivery (Anderson et al. 2005; İmamoglu et al. 2022). Furthermore, PβAEs have low immunogenicity, meaning they are unlikely to be recognized by the immune system and cause a reaction (Karlsson et al. 2020a). These features make PβAEs an attractive and safe option in gene therapy applications.

COX-2 is an enzyme which belongs to the prostaglandin G/H synthase family. It comprises 604 amino acids and has a molecular weight of 68.996 kDa. The COX-2 enzyme acts as a dioxygenase and peroxidase, catalyzing the conversion of arachidonic acid to prostaglandin H2. This conversion is the rate-limiting step in producing prostaglandins (PGs) and thromboxane A2 (TXA2).(Rouzer and Marnett, 2009). COX-2 is a potent mediator of inflammation and plays a role in activity-dependent plasticity in prostanoid signaling. COX-2 enzyme is expressed in endothelial cells (Liu et al. 2018). It is an enzyme that can be activated and has crucial roles in various pathological processes, such as inflammation, angiogenesis, and tumorigenesis. (Ricciotti and Fitzgerald, 2011). Angiogenesis is the new blood vessel formation process in existing vasculature, which occurs continuously in both health and disease. (Auerbach et al. 2003). Human umbilical cord vein endothelial cells (HUVEC) are commonly used to study vascular biology in the lab. COX-2, an enzyme,

can stimulate the production of prostaglandins, which in turn can increase the expression of VEGF in tumor cells. This suggests that COX-2 may create a positive feedback loop between tumor cells and endothelial cells during forming new blood vessels (angiogenesis). Blocking COX-2 could disrupt this feedback loop and potentially inhibit the growth of tumors that depend on angiogenesis. (Wu et al. 2006).

In this study, the efficiency and biocompatibility of polymeric nanoparticle-based transfection agents to deliver CRISPR/Cas9 system to HUVEC cell lines were evaluated. The findings showed that polymeric nanoparticles could be an alternative to traditional methods for safely and effectively delivering CRISPR/Cas9 system to endothelial cell lines. Transfection efficiency, intracellular targeting success and cytotoxicity profile were comprehensively analyzed.

2. Materials and Methods

2.1 Procurement and Preparation Plasmids

PX601-GFP plasmid (84040) was obtained from addgene. To knock out the COX-2 gene sequence, a ready-made vector named Cox-2 Double Nickase Plasmid (h) and coded sc-400072-NIC, commercially offered by Santa Cruz, was used. Plasmid PX601-GFP was transformed into *E.coli* DH5alpha cells and purified from the grown cells by using an endotoxin-free kit(Kuduğ et al. 2019; Kaplan et al. 2021).

2.2. Cell Culture

To investigate the effect of the prepared PβAE-based transfection agent on human umbilical cord endothelial cells (HUVEC) and to use it in transfection studies, the cells were grown in DMEM-High glucose medium (Dulbecco's Modified Eagle Medium) at 37°C in a humid environment containing 5% CO2. The growth of the cells was examined with an inverted microscope during the 24–48-hour incubation period (Kaplan et al. 2021).

2.3. Polymer synthesis

The transfection agent polymer used in this study is a system patented by Gök et al. (2022) For the synthesis of a PβAE-based transfection agent, diacrylate and diamine were used as monomers. The reaction was carried out in a fume hood by passing nitrogen. After synthesis, the polymer was kept in a vacuum oven for three days to remove the ether (Gök et al., 2022).

2.4. Preparation of nanoparticulate carrier systems using PβAE polymer

2 mg of the synthesized polymer was taken and dissolved in 2 mL DMSO. To prepare PβAE-based transfection agent and determine the resulting nanoparticles' size, plasmid-free nanoparticles were first prepared using the nanoprecipitation method. Various volumes of polymer solution were added dropwise into cold ultrapure water and quickly vortexed. PBAE nanoparticles were directly used for applications. The obtained nanoparticles particle size distribution, zeta potential, and particle size values were determined (Gök et al. 2022).

2.5. Zeta Potential and Particle Size Analysis

Zeta potential and particle size of the synthesized PβAE-based transfection agent were determined with a zetasizer (Horiba) device.

2.6. Transfection of PX601 plasmid with PβAE-based transfection agent synthesized into HUVEC cells

One 6-well plate was used for the transfection process. According to the ratio determined based on the agarose gel result (7:1), nanopolymer and plasmid were treated in a microcentrifuge tube for 30 minutes. Then, 45.5 µL of transfection medium, purchased from Santa Cruz (sc-108062), was added dropwise to the microcentrifuge tube, and vortexing was performed. Plasmid and nanoparticle mixture was slowly deposited from the wall of the plate onto the HUVEC cells prepared the day before and 500 µL of transfection medium was added to the plate well and incubated for 4 hours. After 4 hours, 2 ml of HUVEC medium containing FBS was added to the wells. After 24 hours, the medium was withdrawn from the medium, and 3 ml of fresh medium was added. After 48-72 hours, the medium in the wells was withdrawn and washed with PBS. Then, imaging was performed with a fluorescence microscope.

2.7. Analysis of transfection rate with the ImageJ program

Analysis of HUVEC cells co-transfected with the PβAE-based transfection agent producing different amounts of px601-GFP plasmid was performed using LEICA invert-fluorescence microscopy. The area where the cells to be examined were located was photographed using light and fluorescence filter mode. Then, using the ImageJ program and the cell counting tab, the proportions of cells counted in the same area with normal light and fluorescence light images were calculated as percentages relative to each other.

2.8. Performing the transfection process with a PβAE-based transfection agent synthesized into HUVEC cells

2 mg of the synthesized PβAE-based transfection agent was taken and dissolved in 2 mL DMSO. Then, the plasmid that would knock out the Crispr-Cox-2 gene was converted into a concentration of 1 µg/mL, and the plasmid DNA and PβAE-based transfection agent were treated in a dark environment in a microcentrifuge tube for 30 minutes. Following this, the nanoprecipitation process was started, and this process was carried out via a vortex. The processes for cell transfection were repeated as in method 2.6 repeated.

2.9. Western blot Analysis

Protein isolation was performed from HUVEC cells transfected and untransfected with the Santacruz Double Nickase plasmid. The isolation process was carried out using RIPA buffer. Primary and secondary antibodies used in Western blotting were Cox-2 (Abcam-ab52237), β-actin (Abcam-ab119716), and Goat anti-rabbit IgG H&L (HRP(Abcam-ab205718)).

2.10. Cell migration analysis

For proliferation analysis, cells were planted in two 6-well cell culture plates at 105 cells/well, with and without cox-2 gene knock-out. When the cells became confluent, a line was drawn in the middle of the wells with a 200 µL pipette tip. Thus, a standard in-vitro wound model was created. Then, the cells were incubated at 37°C in a 5% CO_2 environment, and cell migration was observed and photographed with a microscope at the 0^{th}, 24^{th}, and 48^{th} hours. Experiments were performed as two repetitions and wound closure rates were determined using the ImageJ program (Songur et al., 2020).

2.11. Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.01 software and comparable datasets. Probability values of $p<0.05$ were considered statistically significant. Differences between the groups were analyzed using one-way ANOVA and two-way ANOVA tests.

3. Results and Discussions

3.1. Zeta Potential and Particle Size Analysis

Size and zeta potential optimization studies of the PβAE-based transfection agent were carried out. Results revealed that the ratio of 200 µL PβAE to 1300 µL dH2O was the most optimum ratio for carrying out the study. The results are given in Table 1. It was determined that the zeta potential was 54.1 mV, and the particle size was 60.40 nm. Zeta potential and particle size analysis of plasmid-loaded nanoparticles (Cox-2 Double Nickase Plasmid (h)) are presented in Figure 1 and Table 1.

Table 1. Effect of PβAE on size and zeta potential of treatment with different solvent amounts" was conducted with three replicates for each condition.

PβAE-dH_2O(µL)	Particle Size (nm)	PDI	Zeta Potential (mV)
50-1450	53.9±0,1	0.264±0.018	+1±0.1
100-1400	55.0±0.3	0.204±0.001	+27.1±2.5
150-1350	57.5±1.1	0.180±0.047	+3±0.2
200-1300	60.4±2.8	0.151±0.016	+54.1±4.1
250-1250	64.5±0.3	0.189±0.006	+60.5±3.2
300-1200	70.5±1.2	0.184±0.039	+6±0.3
350-1150	72.6±1.0	0.180±0.03	+29.2±0.5
400-1100	73.9±0.3	0.176±0.015	+48.6±2.6
450-1050	77.3±0.3	0.200±0.047	+37.7±3.5
500-1000	81.0±0.6	0.186±0.02	+28.1±1.1
600-900	106.9±0.9	0.184±0.002	+16.9±3.5

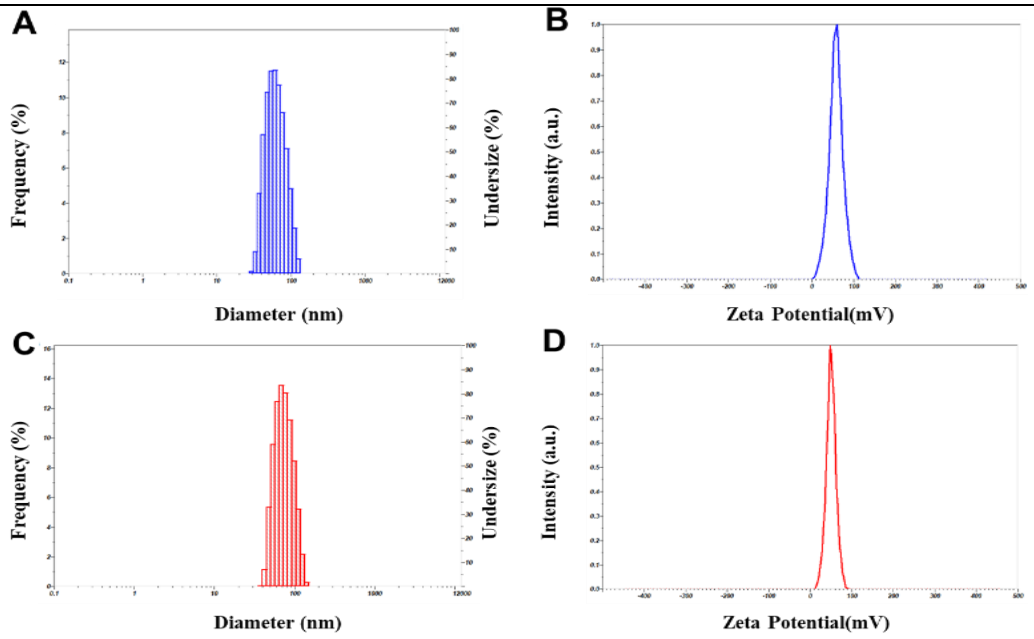

Figure 1. Particle size and zeta potential of PβAE used in the transfection process, A) Particle size of empty PβAE, B) Zeta potential of empty PβAE C) Particle size of PβAE interacted with plasmid, D) Zeta potential of PβAE interacted with plasmid

Table 2. Particle size and zeta potential of PβAE interaction with plasmid and its empty state

	No Plasmid	Plasmid Loaded
Size (nm)	60.4±2.8	71.2±3.2
PDI	0.151±0.016	0.161±0.018
Zeta (mV)	+54.4±4.1	+50.5±1.2

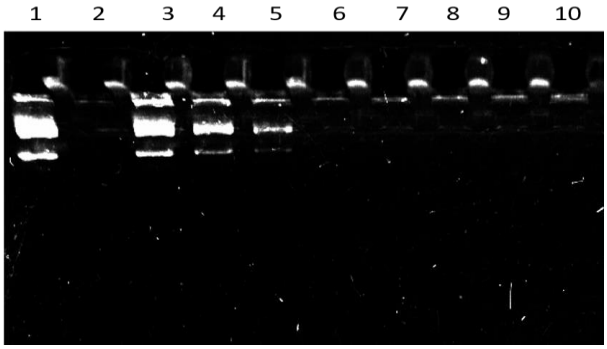

Figure 2. Retention capacity of the polymer 1st well plasmid DNA, 2nd well-synthesized polymer, 3rd well 2 µL polymer 1 µLplasmid DNA, 4th well 3 µL polymer 1 µLplasmid DNA, 5th well 5 µL polymer 1 µLplasmid DNA, 6th well 7 µL polymer 1 µLplasmid DNA, 7th well 10 µL polymer 1 µLplasmid DNA, 8th well 12 µL polymer 1 µLplasmid DNA, 9th well 15 µL polymer 1 µLplasmid DNA, 10th well 17 µL polymer 1 µLplasmid DNA

3.2. Plasmid Retention Capacity of the Synthesized Nanopolymer

The plasmid retention capacity of the nanopolymer is shown in Figure 2 as a result of agarose gel electrophoresis. According to agarose gel electrophoresis, it was determined that the nanoparticle prepared using 7 µL of polymer and 1 µL of plasmid DNA retained all of the plasmid DNA and was the most ideal ratio.

3.3. Examination of Transfection Efficiency with PX601-GFP Plasmid

The transfection efficiency of the synthesized PβAE-based transfection agent was examined with the PX601-GFP plasmid. The transfected cells are shown in Figure 3. Since the plasmid contains the gene sequence encoding the green fluorescent protein, the cells appear green under a fluorescent microscope after transfection. Figure 3 shows the images of the cells under a fluorescence microscope in Figures A and B. The cells successfully received the PX601-GFP plasmid, and transfection was carried out using the synthesized PNP. Then, using the ImageJ program, the ratios of cells counted with normal light and fluorescence light images in the same area were calculated as percentages relative to each other using the cell counting tab. As a result, the transfection efficiency achieved with the synthesized PNP was calculated as 90-95%.

3.4. Knock-out of the Cox-2 Gene with Cox-2 Double Nickase Plasmid (h)

The synthesized PNP carrier system was used to deliver the Double Nickase plasmid, which facilitated the knock-out of the COX-2 gene. The transfected cells are shown in Figure 4. Since the Plasmid contains the gene sequence encoding the GFP protein to monitor transfection, the cells appear green under a fluorescent microscope after transfection. Figure 4 shows the image of the cells under a light and fluorescence microscope. It is seen that the cells successfully received the plasmid and transfected with the synthesized PNP.

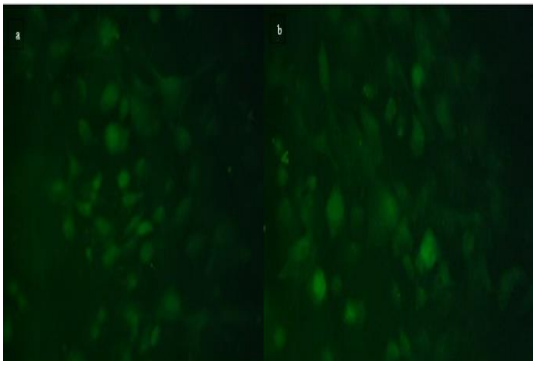

Figure 3. Results of the transfection performed with PX601-GFP Plasmid (a and b). View of transfected cells under a fluorescence microscope

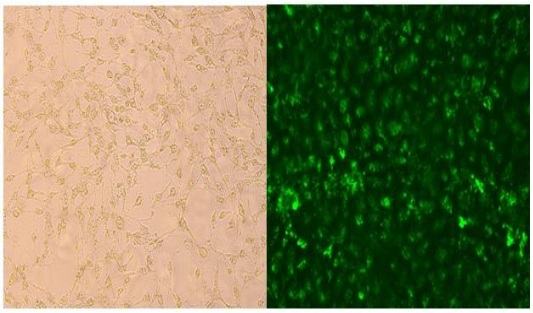

Figure 4. Results of the transfection process into HUVEC cells with *Cox-2 Double Nickase Plasmid (h)*

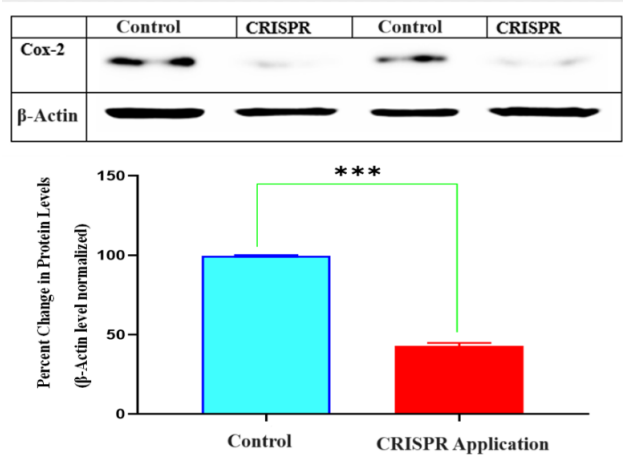

Figure 5. Western blot result (***p < 0.001 versus control group.)

3.5. Western Blot Results

Western blot analysis was performed to analyze CRISPR efficiency. The results were normalized with the β-actin antibody, and the change in protein expression level was analyzed using the image lab 6.1 program. The results are shown in Figure 5. The Western blot results showed that

when the control without CRISPR study was compared with the cells in which the COX-2 gene was silenced, the expression of COX-2 protein was calculated as 57.12% in the HUVEC cell culture in which the gene was silenced.

3.6. Cell Migration Assay in HUVEC Cells

The cell migration rate was determined using the ImageJ program. Migration results are shown in Figure 6. The figure shows that the CRISPR application's migration rate was lower than the control's. This showed that the gene silencing process was successful. The results were consistent with Western blot data.

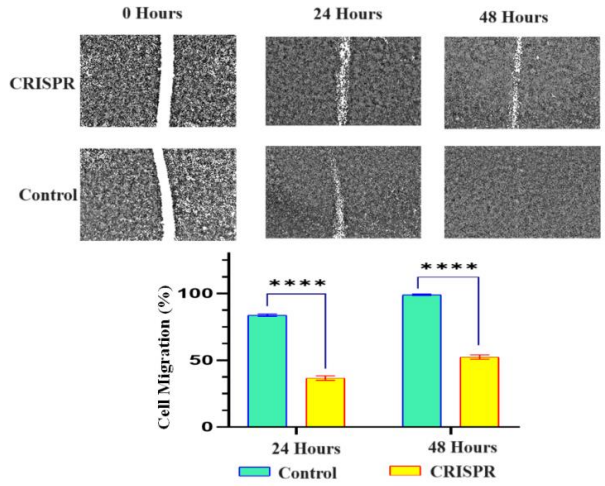

Figure 6. Cell migration result (****p < 0.0001 versus the control group.)

4. Conclusions

This study investigated the CRISPR plasmid transport and CRISPR efficiency of a PβAE-based transfection agent. The results showed that the PβAE-based transfection agent successfully carried the CRISPR plasmid, providing 90-95% transfection efficiency. In addition, CRISPR efficiency was calculated as 57.12% based on the Western blot results. While COX-2 induction may be significant in cell migration and wound healing, inhibition of COX-2 promises new approaches to prevent angiogenesis for cancer cells. Since COX-2 inhibitors and inhibiting the COX-2 gene are responsible for inhibiting cell proliferation in several cancer cell models, silencing COX-2 inhibitors and the COX-2 gene suggests that it will be one of the critical future therapeutic targets.

Future studies should optimize the PβAE-based delivery system to increase transfection and CRISPR efficiency. In addition, the therapeutic potential of this approach can be validated in vivo by evaluating its efficacy in different cancer models and investigating potential off-target effects. COX-2 inhibition is significant, especially in cancer treatment and strategies against inflammatory diseases. Increasing prostaglandin production, COX-2 is critical in essential cell proliferation, angiogenesis, and metastasis processes. Inhibition of COX-2 can prevent cancer progression by inhibiting the growth and metastasis of tumor cells. In addition, angiogenesis can be suppressed by decreasing the production of prostaglandin E2 (PGE2), which can limit the ability of tumors to form new vessels. In conclusion, targeted silencing of COX-2 with gene editing technologies such as CRISPR/Cas9 may pave the way for new and effective strategies in cancer therapies, primarily by reducing side effects. This will pave the way for the translation of findings into clinical applications and advance the field of precision medicine and targeted cancer therapy.

In the literature, many PBAE-based transfection agents have been synthesized, and the particle sizes of these agents have been reported to be generally in the range of 50-200 nm (Lynn and Langer, 2000a; Yin et al. 2011). For example, developed a large PBAE library and emphasized that the sizes of these particles ranged from 50-200 nm and that the polymer structure influenced gene delivery efficiency (Anderson et al. 2005b; Karlsson et al. 2020b). Considering these studies, the particle size of 60 nm obtained in our study is within the limits reported in the literature and may offer various advantages and disadvantages in terms of transfection efficiency.

Declaration of Ethical Standards

This study is derived from the doctorate thesis (thesis number: 672486) under the supervision of Prof. Dr. İsa Gökçe and co-supervisor Assoc. Prof. Dr. Mehmet Koray Gök on the date of May 20, 2021, titled "Investigation of the usability of polymeric nanoparticle-based transfection agent for CRISPR/CAS9-mediated editing of COX-2 gene".

Credit Authorship Contribution Statement

Author-1: Conceptualization, Methodology / Study design, Software, Validation, Investigation, Resources, Writing – original draft,
Author-2: Conceptualization, investigation and writing – review and editing.
Author-3: Conceptualization, investigation, methodology and supervision
Author-4: Conceptualization, investigation, methodology, and supervision and writing – review and editing.

Declaration of Competing Interest

The authors have no conflicts of interest to declare regarding the content of this article.

Data Availability

All data generated or analyzed during this study are included in this published article.

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5. References

Anderson, D.G., Akinc, A., Hossain, N., Langer, R., 2005a-b. Structure/property studies of polymeric gene delivery using a library of poly(β-amino esters). Molecular Therapy, **11**, 426-434. https://doi.org/10.1016/j.ymthe.2004.11.015

Anderson, D.G., Green, J.J., Langer, R., 2008. A combinatorial polymer library approach yields insight into nonviral gene delivery. Acc Chem Res, **41**, 749–759 https://doi.org/10.1021/ar7002336

Auerbach, R., Lewis, R., Shinners, B., Kubai, L., Akhtar, N., 2003. Angiogenesis assays: A critical overview. Clin Chem, **49**, 32-40 https://doi.org/10.1373/49.1.32

Baylis, F., McLeod, M., 2017. First-in-human Phase 1 CRISPR Gene Editing Cancer Trials: Are We Ready? Curr Gene Ther, **17**, 309-319. https://doi.org/10.2174/1566523217666171121165935

Gaj, T., Gersbach, C.A., G.A., Barbas, C.F., 2013. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering: Trends in Biotechnology. Trends Biotechnol, **31**, 397-405. https://doi.org/10.1016/j.tibtech.2013.04.004.

Gök, M.K., Demir, K., Cevher, E., Pabuccuoğlu, S., Özgümüş, S., 2022. Efficient polycation non-viral gene delivery system with high buffering capacity and low molecular weight for primary cells: Branched poly(β-aminoester) containing primary, secondary and tertiary amine groups. Eur Polym J **166**, 111046 https://doi.org/10.1016/j.eurpolymj.2022.111046

Guo, C., Ma, X., Gao, F., Guo, Y., 2023. Off-target effects in CRISPR/Cas9 gene editing Front Bioeng Biotechnol, **9**;11:1143157 https://doi.org/10.3389/fbioe.2023.1143157

Hosur, V., Low, B.E., Wiles, M. V., 2023. Genetic modification of mice using CRISPR-Cas9: Best practices and practical concepts explained, in: Rigor and Reproducibility in Genetics and Genomics, 425-452 https://doi.org/10.1016/B978-0-12-817218-6.00018-8

Hryhorowicz, M., Lipiński, D., Zeyland, J., 2023. Evolution of CRISPR/Cas Systems for Precise Genome Editing. Int J Mol Sci, **18**;24(18):14233. https://doi.org/10.3390/ijms241814233

İmamoğlu, R., Kaplan, Ö., Gök, M.K., Gökçe, İ., 2022. Polymer-Based Transfection Agents Used in Crispr-Cas9 System. Türk Doğa ve Fen Dergisi, **11**,151-156 https://doi.org/10.46810/tdfd.795053

Kang, Y.K., Kwon, K., Ryu, J.S., Lee, H.N., Park, C., Chung, H.J., 2017. Nonviral Genome Editing Based on a Polymer-Derivatized CRISPR Nanocomplex for Targeting Bacterial Pathogens and Antibiotic Resistance. Bioconjug Chem, **28**, 957-967 https://doi.org/10.1021/acs.bioconjchem.6b00676

Kaplan, Ö., Gökşen Tosun, N., Özgür, A., Erden Tayhan, S., Bilgin, S., Türkekul, İ., Gökce, İ., 2021. Microwave-assisted green synthesis of silver nanoparticles using crude extracts of Boletus edulis and Coriolus versicolor: Characterization, anticancer, antimicrobial and wound healing activities. J Drug Deliv Sci Technol **64**, 102641. https://doi.org/10.1016/J.JDDST.2021.102641

Kaplan, Ö., İmamoğlu, R., Şahingöz, İ., Gökçe, İ., 2021. Recombinant production of Thermus aquaticus single-strand binding protein for usage as PCR enhancer. International Advanced Researches and Engineering Journal **5**, 42–46. https://doi.org/10.35860/IAREJ.766741

Karlsson, J., Rhodes, K.R., Green, J.J., Tzeng, S.Y., 2020a-b. Poly(beta-amino ester)s as gene delivery vehicles: challenges and opportunities. Expert Opin Drug Deliv, 31;17(10):1395–1410. https://doi.org/10.1080/17425247.2020.1796628

Khoshandam, M., Soltaninejad, H., Mousazadeh, M., Hamidieh, A.A., Hosseinkhani, S., 2024. Clinical applications of the CRISPR/Cas9 genome-editing system: Delivery options and challenges in precision medicine. Genes Dis, **11**, 268-282. https://doi.org/10.1016/j.gendis.2023.02.027

Kuduğ, H., Ataman, B., İmamoğlu, R., Düzgün, D., Gökçe, İ., 2019. Production of red fluorescent protein (mCherry) in an inducible E. coli expression system in a bioreactor, purification and characterization. International Advanced Researches and Engineering Journal **3**, 20–25.

Kuhn, J., Lin, Y., Krhac Levacic, A., Al Danaf, N., Peng, L., Höhn, M., Lamb, D.C., Wagner, E., Lächelt, U., 2020. Delivery of Cas9/sgRNA Ribonucleoprotein Complexes via Hydroxystearyl Oligoamino Amides. Bioconjug Chem, **31**, 29–742. https://doi.org/10.1021/acs.bioconjchem.9b00853

Liu, B.Y., He, X.Y., Xu, C., Xu, L., Ai, S.L., Cheng, S.X., Zhuo, R.X., 2018. A Dual-Targeting Delivery System for Effective Genome Editing and in Situ Detecting Related Protein Expression in Edited Cells. Biomacromolecules, **19**, 2957-2968. https://doi.org/10.1021/acs.biomac.8b00511

Liu, C., Zhang, L., Liu, H., Cheng, K., 2017. Delivery strategies of the CRISPR-Cas9 gene-editing system for

therapeutic applications. Journal of Controlled Release, **28**, 17-26. https://doi.org/10.1016/j.jconrel.2017.09.012

Liu, D., Ji, L., Wang, Y., Zheng, L., 2013. Cyclooxygenase-2 Expression, Prostacyclin Production and Endothelial Protection of High-density Lipoprotein. Cardiovascular & Hematological Disorders-Drug Targets, **12**, 98-105. https://doi.org/10.2174/1871529x11202020098

Lynn, D.M., Langer, R., 2000a-b. Degradable poly(β-amino esters): Synthesis, characterization, and self-assembly with plasmid DNA. J Am Chem Soc **122**, 0761–10768. https://doi.org/10.1021/ja0015388

Mirón-Barroso, S., Correia, J.S., Frampton, A.E., Lythgoe, M.P., Clark, J., Tookman, L., Ottaviani, S., Castellano, L., Porter, A.E., Georgiou, T.K., Krell, J., 2022. Polymeric Carriers for Delivery of RNA Cancer Therapeutics. Noncoding RNA, **8**, 58. https://doi.org/10.3390/ncrna8040058

Nie, D., Guo, T., Yue, M., Li, W., Zong, X., Zhu, Y., Huang, J., Lin, M., 2022. Research Progress on Nanoparticles-Based CRISPR/Cas9 System for Targeted Therapy of Tumors. Biomolecules, **5**, 1239. https://doi.org/10.3390/biom12091239

Ricciotti, E., Fitzgerald, G.A., 2011. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol, **31**, 986-1000. https://doi.org/10.1161/ATVBAHA.110.207449

Rouzer, C.A., Marnett, L.J., 2009. Cyclooxygenases: Structural and functional insights. J Lipid Res, **50**, 29-34 https://doi.org/10.1194/jlr.R800042-JLR200

Songur, M., Bilgin, S., Erden Tayhan, S., Gökçe, İ., 2020. Investigation of Anti-proliferative and Anti-migratory Properties of Recombinant J2-C2 Against Tumor Cells. Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi **10**, 2943-2951 https://doi.org/10.21597/jist.730267

Tao, Y., Hou, X., Zuo, F., Li, X., Pang, Y., Jiang, G., 2019. Application of nanoparticle-based siRNA and CRISPR/Cas9 delivery systems in gene-targeted therapy. Nanomedicine, **14**, 511-514. https://doi.org/10.2217/nnm-2018-0522

Wang, Xianqing, Li, Y., A, S., Lyu, J., Wang, Xi, He, Z., Lara-Sáez, I., Li, M., Wang, W., 2024. Cyclization-enhanced poly(β-amino ester)s vectors for efficient CRISPR gene editing therapy. Journal of Controlled Release, **368**, 444-452. https://doi.org/10.1016/j.jconrel.2024.02.032

Wu, G.F., Luo, J., Rana, J.S., Laham, R., Sellke, F.W., Li, J., 2006. Involvement of COX-2 in VEGF-induced angiogenesis via P38 and JNK pathways in vascular endothelial cells. Cardiovasc Res, **69**, 512–519. https://doi.org/10.1016/j.cardiores.2005.09.019

Yin, Q., Gao, Y., Zhang, Z., Zhang, P., Li, Y., 2011. Bioreducible poly (β-amino esters)/shRNA complex nanoparticles for efficient RNA delivery. Journal of Controlled Release, **151**, 35-44. https://doi.org/10.1016/j.jconrel.2010.12.014

Zhang, F., Wen, Y., Guo, X., 2014. CRISPR/Cas9 for genome editing: Progress, implications and challenges. Hum Mol Genet **23**, 40-46. https://doi.org/10.1093/hmg/ddu125

Zhou, S., Li, Y., Wu, Q., Gong, C., 2024. Nanotechnology-based CRISPR/Cas9 delivery system for genome editing in cancer treatment. MedComm- Biomaterials and Applications, **176**, 113891. https://doi.org/10.1002/mba2.70