RESEARCH ARTICLE
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Synthesis and Characterization of Oleic Acid-Coated Calcium Phosphate Nanoparticles for Gene Delivery Applications

ABSTRACT

Background: Efficient gene delivery remains a major challenge in gene therapy, necessitating the development of biocompatible and effective carrier systems. This study aimed to synthesize and evaluate oleic acid-modified calcium phosphate (OA-CaP) nanoparticles for their potential as a non-viral gene delivery vector.

Methods: Oleic acid-modified calcium phosphate nanoparticles were synthesized and characterized using dynamic light scattering (DLS), zeta potential analysis, scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR). Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and transfection efficiency was evaluated in L929 cells using fluorescence microscopy. Phase-contrast microscopy was used to observe cell morphology and proliferation.

Results: Oleic acid-modified calcium phosphate nanoparticles exhibited a smaller size (193.1 ± 12.7 nm) and a higher surface charge (+22.6 ± 2.7 mV) compared to unmodified CaP, resulting in improved colloidal stability and cellular interactions. Cytotoxicity analysis indicated high cell viability at lower concentrations, while a dose-dependent decrease in viability was observed at higher concentrations. Transfection studies demonstrated significantly higher gene expression in OA-CaP/pDNA-transfected cells compared to naked pDNA. Transfection efficiencies increased from 22.24% ± 20.13% at 24 hours to 72.43% ± 36.57% at 72 hours. Additionally, phase-contrast microscopy confirmed maintained cell morphology and enhanced proliferation.

Conclusion: Oleic acid-modified calcium phosphate nanoparticles exhibit high transfection efficiency and minimal cytotoxicity at optimized concentrations, highlighting their potential as a promising gene delivery system. Future studies should focus on in vivo evaluations to further establish their therapeutic applicability in gene therapy.

Keywords: Cytotoxicity, gene delivery, L929 cells, OA-CaP nanoparticles, transfection efficiency

What is already known on this topic?

- Calcium phosphate (CaP) nanoparticles are promising carriers due to their biocompatibility and ability to condense DNA. To improve their physicochemical properties like size, surface charge, and stability, CaP nanoparticles are often modified, with oleic acid being a common modification. In vitro studies suggest that these modifications can enhance transfection efficiency, though in vivo efficacy still needs further investigation.

What this study adds on this topic?

- This study demonstrates that oleic acid-modified calcium phosphate (OA-CaP) nanoparticles exhibit improved physicochemical properties, such as smaller size and higher surface charge, enhancing gene delivery efficiency. The findings highlight OA-CaP as a promising non-viral gene delivery system with minimal cytotoxicity and high transfection efficiency, particularly at optimized concentrations.

INTRODUCTION

Gene transfer is a process that enables the delivery of genetic material to target cells.[1] In this process, genetic material is transferred into cells using carrier vectors.[2] Achieving high transfection efficiency is one of the most critical factors for successful gene transfer. Both viral and non-viral vectors are widely used in gene delivery.[3] Although viral vectors exhibit high transfection efficiency, they have several disadvantages, including immunogenic responses, cytotoxic effects, the risk of insertional mutagenesis, and limited cargo capacity.[4] Non-viral vectors, on the other hand, have been developed as alternatives to viral vectors due to their minimized biosafety risks, low immunogenicity, cost-effectiveness, and broader application potential.[5]

Calcium phosphate nanoparticles are advanced carrier systems developed as alternatives to conventional delivery systems such as polymeric nanoparticles, liposomes, and emulsions.[6] These nanoparticles, ranging in size from 10 to 200 nm, exist as colloidal suspensions in aqueous solutions.[7] Due to their biocompatible and biodegradable nature, they are widely used in biomedical applications such as gene delivery, drug delivery, and tissue engineering.[8] Negatively charged DNA molecules bind to positively charged regions on the surface of

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calcium phosphate nanoparticles through electrostatic interactions. The resulting complex binds to receptors on the cell membrane and enters the cell via endocytosis. The acidic environment of endosomes facilitates the dissolution of the nanoparticles and the release of DNA. The subsequent transport of DNA into the cell nucleus is a critical step for gene expression.[9] However, the nuclear migration mechanism of DNA is not yet fully understood, and it is believed that nuclear transport carriers and signaling pathways play a crucial role in this process.[10]

Oleic acid is a monounsaturated fatty acid commonly found in both plant and animal fats. It plays a significant role in cell membrane structure and can enhance membrane fluidity. Due to its amphiphilic nature, oleic acid (OA) improves the interaction capacity of carrier vectors with cellular membranes, facilitating the passage of nanoparticles through the cell membrane.[11] Additionally, OA coating prevents nanoparticle aggregation, enhances stability, and increases transfection efficiency.[12] Therefore, coating calcium phosphate nanoparticles with OA plays a critical role in improving their effectiveness as gene carriers.

In this study, oleic acid-coated calcium phosphate (OA-CaP) nanoparticles were synthesized and evaluated for their gene delivery potential. Comprehensive physicochemical characterizations (FTIR, SEM, DLS, and zeta potential) were performed to assess their structural and surface properties. Cytotoxicity and transfection efficiency were examined in L929 fibroblast cells. The findings highlight the potential of OA-CaP nanoparticles as biocompatible gene delivery carriers.

MATERIAL AND METHODS

Synthesis of Calcium Phosphate Nanoparticles

Calcium phosphate nanoparticles were synthesized using a 2-step microemulsion method.[13] In the first step, Microemulsion A was prepared by dissolving calcium chloride (CaCl2) at 0.05 M concentration, sodium dodecyl sulfate (SDS) at 1% (w/v), and Tween80 at 1% (w/v) in a continuous stirring process for 24 hours. Then, Microemulsion B was formed by dissolving sodium citrate at 0.025 M, disodium hydrogen phosphate at 0.025 M, SDS at 1% (w/v), and Tween80 at 1% (w/v), also under continuous stirring for 24 hours. After 24 hours of incubation, Microemulsion B was slowly added to Microemulsion A. Centrifugation was performed at 10 000 rpm, and the supernatant was removed. The mixture was then stirred for 72 hours at 600 rpm using a magnetic stirrer. After 72 hours, washing was carried out 4 times at 10 000 rpm for 30 minutes at +4°C with ethanol. After washing, the pellet was dissolved in CTAB (Cetyldimethylethyl Ammonium Bromide) at 2 µM concentration. The obtained CaP nanoparticles were stored at −20°C for lyophilization.

Calcium phosphate nanoparticles were coated with OA to enhance their surface properties. The lyophilized nanoparticles were resuspended in an appropriate solvent and subjected to ultrasonication for homogeneous dispersion. A 15.85 mM OA solution was added dropwise to the nanoparticle suspension, and the mixture was stirred at 600 rpm for 24 hours at room temperature to facilitate coating. After the coating process, the suspension was centrifuged at 10 000 rpm for 30 minutes at +4°C, followed by the removal of the supernatant. Excess OA was removed by washing the precipitate twice with absolute ethanol. After the washing steps, the nanoparticles were redispersed and ultrasonicated for 5 minutes to ensure homogeneity. The OA-coated CaP nanoparticles were stored at −20°C for further characterization and applications.

The oleic OA-CaP nanoparticles' particle size and surface charge were measured using the Zeta Sizer (Malvern 3000 HSA, UK). Scanning electron microscopy (SEM, Zeiss EVO® LS 10) and ZetaSizer (Malvern, NanoZS) were employed to analyze their physicochemical properties. Stock nanoparticle solutions were prepared at a concentration of 100 µg/mL in distilled water for the measurements of zeta potential and particle size. These measurements were performed at 25°C using Dynamic Light Scattering (DLS) by the ZetaSizer (Malvern, NanoZS).[14] For morphological analysis, a thin gold/palladium (Au/Pd) coating was applied to the CaP nanoparticles, and imaging was conducted at varying magnifications using the SEM (Zeiss EVO® LS 10).[15] Additionally, the functional groups and chemical bonds present in the nanoparticles were identified through Fourier Transform Infrared (FT-IR) Spectroscopy.[16]

In Vitro Cytotoxicity Evaluation

The in vitro cytotoxicity of the synthesized and characterized calcium phosphate nanoparticles was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.[17] The MTT assay aimed to evaluate the cytotoxicity of the synthesized OA-coated calcium phosphate nanoparticles on L929 mouse fibroblast cells. The nanoparticle suspensions were sterilized by filtration through 0.22 µm syringe filters under aseptic conditions prior to biological assays. L929 fibroblast cells were seeded into a 96-well plate at a density of 1 × 106 cells/well for the analysis.The plate was incubated at 37°C under 5% CO2 for 24 hours. After incubation, different concentrations of OA-CaP nanoparticles were added to the wells. A 50% DMSO solution was used as a positive control, and DMEM-F12 medium was used as a negative control. L929 cells treated with different concentrations of OA-CaP nanoparticles were incubated at 37°C in a 5% CO_2 environment for 24 hours. Following incubation, the culture medium was removed, and 5 mg/mL MTT solution (diluted 1/10 with DMEM) was added to each well and incubated for 4 hours. At the end of the incubation period, the MTT solution was removed, and the formed formazan crystals were dissolved using DMSO(Dimethyl sulfoxide). Cell viability was determined by measuring the absorbance at 570 nm using an ELISA reader (Thermo Scientific Multiskan Go, USA).

Plasmid DNA / Nanoparticle Conjugate Preparation and Characterization

Conjugates of plasmid DNA (pDNA) and OA-coated calcium phosphate (OA-CaP) nanoparticles were prepared by adding 1 µg of pDNA solution to 40 µL of OA-CaP solution (1 µg/µL) and incubating for 30 minutes. The DNA purity was evaluated based on the 260/280 nm absorbance ratio and determined to be 1.87. The size and zeta potential of pDNA-OA-CaP conjugates were determined using a ZetaSizer (Malvern, NanoZS).[18]

Transfection of L929 Fibroblast Cells

The day before transfection, 5×10^5 cells were seeded into each well of a 6-well culture plate, and 2 mL of DMEM-F12 medium containing 10% FBS was added to each well. One hour before transfection, the medium was replaced with serum-free DMEM-F12. The OA coated calcium phosphate nanoparticle-pDNA conjugate was prepared and incubated for 1 hour at room temperature. The conjugates were prepared in separate Eppendorf tubes for each well. The predetermined amount of plasmid DNA was dissolved in 100 µL serum-free DMEM-F12 medium and added to the tube. The appropriate amount of nanoparticles was then added, and the tube was sealed and incubated at room temperature. After incubation, the conjugate was used for transfection. Transfected cells were incubated for 4 hours at 37°C and 5% CO2. After 4 hours, the medium was replaced with DMEM-F12 containing serum. Transfection efficiency was evaluated by GFP (Green Fluorescent Protein) expression. Luminescence in the transfected cells was observed at 24, 48, and 72 hours using an inverted microscope with a fluorescent attachment. Transfection efficiency was determined by counting GFP-expressing cells in 3 different regions of the plate using both phase contrast and fluorescence microscopy, with 3 repetitions. The ratio of GFP-positive cells to the total cell number was calculated.[19]

Ethics Committee Statement

This study did not require approval from an Ethics Committee as it did not involve human participants, human data, or animal subjects.

RESULTS

Synthesis of Calcium Phosphate Nanoparticles and Characterization

The mean particle size, zeta potential, and polydispersity index (PDI) of the CaP and OA-CaP nanoparticles were determined using the Zetasizer device with the DLS method. Polydispersity index indicates size distribution heterogeneity and is dimensionless. For lipid-based carriers, PDI should range between 0.05 and 0.7; values above 0.7 denote broad size distribution, making DLS analysis unsuitable. In gene transfer studies, PDI must be below 0.7.[20] The particle size and PDI of CaP were measured as 234.5 ± 5.2 nm and 0.1, respectively, while those of OA-CaP were recorded as 193.1 ± 12.7 nm and 0.3. The zeta potential values were determined as +12.8 ± 1.3 mV for CaP and +22.6 ± 2.7 mV for OA-CaP.

The particle size of 234.5 (±5.2) nm for CaP and 193.1 (±12.7) nm for OA-CaP falls within an appropriate range for gene delivery applications. Nanoparticles of this size can enhance cellular uptake and facilitate endosomal escape, crucial for efficient gene transfer. The zeta potential values of +12.8 (±1.3) mV for CaP and +22.6 (±2.7) mV for OA-CaP contribute to nanoparticle stability and interaction with cell membranes. The positive surface charge promotes electrostatic interactions with negatively charged cellular surfaces, improving internalization efficiency.[21] The colloidal stability of calcium phosphate nanoparticles depends on their surface properties, and a zeta potential above +30 mV or below −30 mV is considered to ensure a stable dispersion.[7] Positively charged calcium phosphate nanoparticles as nonviral vectors for DNA transfection, highlighting their potential in cancer gene therapy due to improved cellular uptake and transfection efficiency.[22]

The SEM images of CaP and OA-CaP nanoparticles reveal distinct morphological characteristics. The CaP nanoparticles (left image) exhibit an aggregated structure with irregularly shaped particles, suggesting a tendency for agglomeration (Figure 1). The high magnification provides a detailed view of the nanoscale features, indicating a relatively small particle size. In contrast, the OA-CaP nanoparticles (right image) display a more dispersed and porous morphology with smaller, well-distributed particles. Morphological analysis revealed that the nanoparticles were spherical, well-separated, and well-dispersed. Similarly, other research reported that calcium phosphate nanoparticles exhibited comparable morphological characteristics.[23] The increased dispersion observed in OA-CaP could be attributed to the OA modification, which likely enhances stability and reduces particle aggregation. These structural differences play a crucial role in nanoparticle behavior, influencing their colloidal stability, cellular interactions, and potential applications in gene delivery.

Fourier Transform Infrared Spectroscopy (FTIR) spectra of CaP and OA-CaP nanoparticles are presented in the figure, demonstrating characteristic functional groups (Figure 2). In the CaP spectrum, the presence of phosphate (PO_4^{-3}) stretching vibrations is observed around 1000 cm^{-1}, confirming the phosphate structure of the nanoparticles. Additionally, the peaks corresponding to C=O and C-H stretching vibrations indicate the presence of organic components. In the OA-CaP spectrum, a more pronounced C=O stretching band is detected, suggesting successful surface modification with OA. The disappearance or shift of certain peaks in OA-CaP compared to CaP further supports the interaction between the calcium phosphate structure and OA. These spectral differences confirm the successful functionalization of CaP nanoparticles with OA, which may influence their physicochemical properties and biological interactions. Morejón et al[24] have used FTIR analysis to understand the surface modifications of calcium phosphate nanoparticles, providing valuable information on their potential in

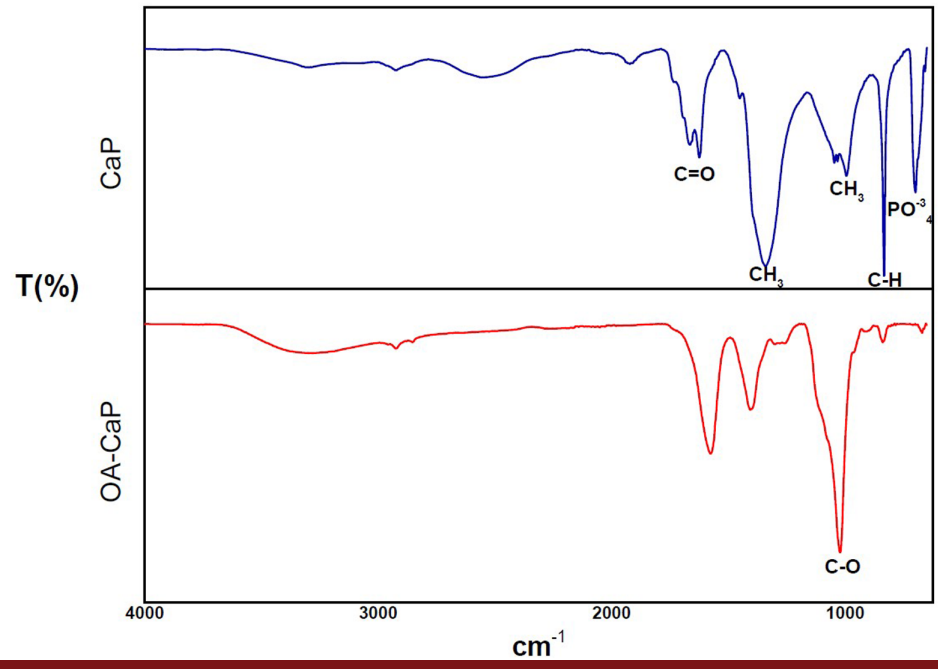

Figure 1. Comparative scanning electron microscopy images of uncoated calcium phosphate (CaP) and oleic acid-coated CaP (OA-CaP) nanoparticles.

scaffolds. According to the literature, the FT-IR spectrum of OA typically exhibits a C=O (carbonyl) stretching band around 1700 cm^{-1}, C-H stretching vibrations in the 2800-3000 cm^{-1} range, and C-O stretching vibrations between 1000 and 1300 cm^{-1}.[25] These characteristic bands are evaluated to verify the chemical structure of OA in the conducted analyses.

The physicochemical properties of the nanoparticles were evaluated, including size, zeta potential, and PDI, and the results reveal significant differences between the various formulations (Table 1). Literature reports suggest that pure calcium phosphate (CaP) nanoparticles typically have sizes ranging from 100 nm to 300 nm with positive zeta potentials due to the surface charge, which helps in preventing aggregation and promoting stability in aqueous environments.[26] The CaP nanoparticles exhibited a size of 234.5 ± 5.2 nm, a positive zeta potential of +12.8 ± 1.3 mV, and a low PDI of 0.1, indicating a well-dispersed and stable nanoparticle suspension. Studies have shown that coating with OA decreases the zeta potential to more moderate positive values, which is consistent with the observed +22.6 ± 2.7 mV for OA-CaP nanoparticles in this study.[27,28] This increase in zeta potential is typically due to the OA's hydrophobic interactions and enhanced surface charge stabilization. In comparison, the OA-CaP nanoparticles had a smaller size of 193.1 ± 12.7 nm, a higher positive zeta potential of +22.6 ± 2.7 mV, and a slightly higher PDI of 0.3, suggesting improved surface charge and stability compared to the CaP nanoparticles. Upon incorporation with pDNA, the size of the nanoparticles increased significantly. For example, 10 µL OA-CaP/1 µL pDNA formulation resulted in a size of 321.9 ± 20.7 nm with a negative zeta potential of –11.3 ± 1.8 mV and a PDI of 0.4, indicating successful complex formation but with increased size and more heterogeneity. Further increasing the amount of

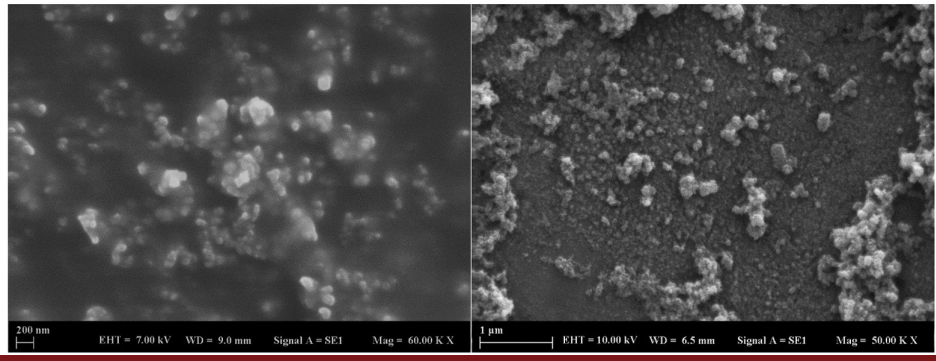

Figure 2. Identification of functional groups in oleic acid-modified calcium phosphate nanoparticles by Fourier transform infrared spectroscopy.

Table 1. Zeta Potentials of Calcium Phosphate and Oleic Acid-Modified Calcium Phosphate Nanoparticles and pDNA/Calcium Phosphate Conjugates

Nanoparticles	Size (nm)	Zeta Potentials (mV)	Polydispersity Index
CaP	234.5 ± 5.2	+12.8 ± 1.3	0.1
OA-CaP	193.1 ± 12.7	+22.6 ± 2.7	0.3
10 µL OA-CaP/ 1 µL pDNA	321.9 ± 20.7	–11.3 ± 1.8	0.4
20 µL OA-CaP/ 1 µL pDNA	339.5 ± 63.1	–8.6 ± 1.4	0.3
30 µL OA-CaP/ 1 µL pDNA	429.7 ± 11.4	–1.2 ± 0.3	0.4
40 µL OA-CaP/ 1 µL pDNA	463.7 ± 32.7	+13.1 ± 1.6	0.2

OA-CaP in the formulations (20 µL, 30 µL, and 40 µL) led to even larger sizes: 339.5 ± 63.1 nm, 429.7 ± 11.4 nm, and 463.7 ± 32.7 nm, respectively. The zeta potential for these formulations became more negative (ranging from –8.6 ± 1.4 mV to –1.2 ± 0.3 mV), with a trend toward increased heterogeneity in the PDI values (ranging from 0.2 to 0.4). The increase in size and the shift in surface charge indicate that the complexation of pDNA with OA-CaP significantly alters the particle properties, which may influence the efficiency of gene delivery and stability of the formulation. Oleic acid, a monounsaturated fatty acid, possesses a carboxyl group that can interact with the surface of calcium phosphate, thereby increasing the surface negative charge due to its deprotonated state at physiological pH. The results indicate that the conjugation with pDNA increases the nanoparticle size while changes in the zeta potential and the increase in PDI provide important insight into optimizing the carrier system's effectiveness for gene transfer applications, demonstrating that pDNA conjugates can serve as suitable carrier systems for successful gene therapy.

The effect of OA-CaP concentrations on cell viability was evaluated based on the results of treatment groups at different concentrations (Figure 3). According to the results, the group treated with 10 µg/µL OA-CaP showed a cell viability of 97.33% (±2.39), while 20 µg/µL and 30 µg/µL OA-CaP concentrations resulted in cell viabilities of 94.79% (±3.57) and 92.44% (±1.55), respectively. These results indicate that OA-CaP treatment has a minimal effect on cell viability at low concentrations. However, in the group treated with 40 µg/µL OA-CaP, cell viability decreased to 86.64% (±1.35), and in the 50 µg/µL OA-CaP-treated group, it decreased to 78.25% (±2.32), suggesting that higher concentrations negatively impact cell health. The negative control group showed a cell viability of 100.24% (±1.76), while the positive control group recorded a viability of 4.92% (±0.33). These data suggest that OA-CaP may affect cell viability at certain concentrations, and toxic effects are more pronounced at higher concentrations.

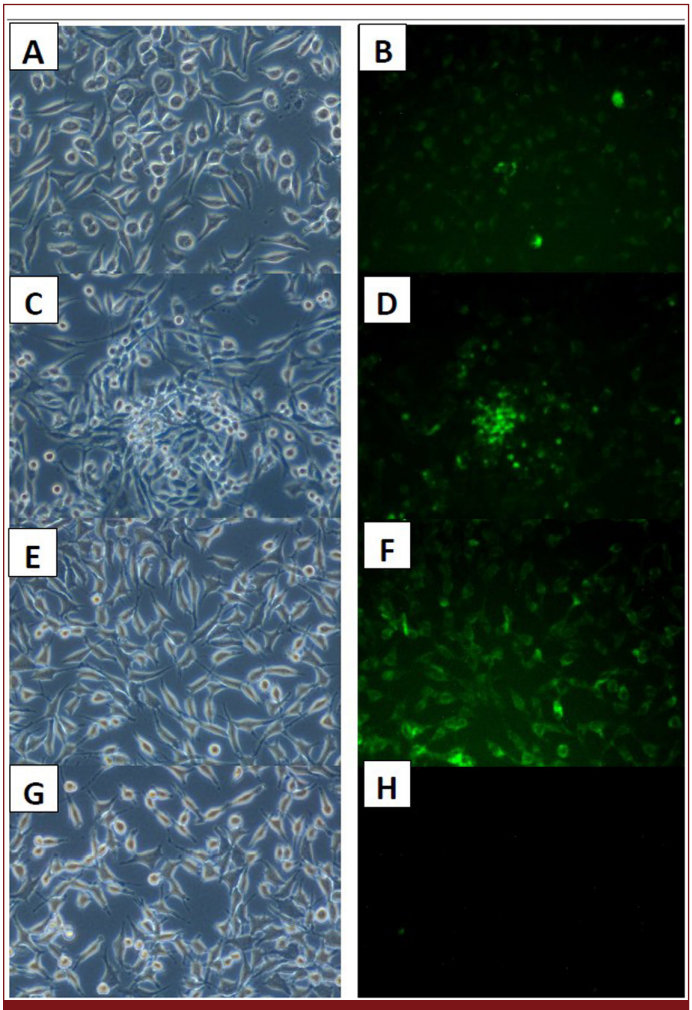

Figure 3. Effect of oleic acid-modified calcium phosphate concentration on L929 fibroblast viability determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

In the literature, several studies have reported that calcium phosphate nanoparticles (CaP NPs) are generally considered non-toxic to cells or exhibit only low cytotoxicity at certain concentrations.[29] These nanoparticles have shown excellent biocompatibility and are widely used in biomedical applications such as drug delivery and tissue engineering. However, some studies suggest that when calcium phosphate nanoparticles are modified or combined with certain additives, their toxicity can increase. For instance, CaP nanoparticles prepared with silver or ammonium additives have been shown to exhibit significantly higher cytotoxicity compared to pure CaP nanoparticles. This increase in toxicity is often attributed to the additional properties introduced by these additives, such as the antimicrobial activity of silver or the increased surface reactivity of ammonium-modified nanoparticles, which can lead to greater cellular interaction and potentially harmful effects on cell viability.[30] Thus, while CaP nanoparticles are typically well-tolerated by cells, their toxicity can vary depending on their preparation methods and surface modifications,

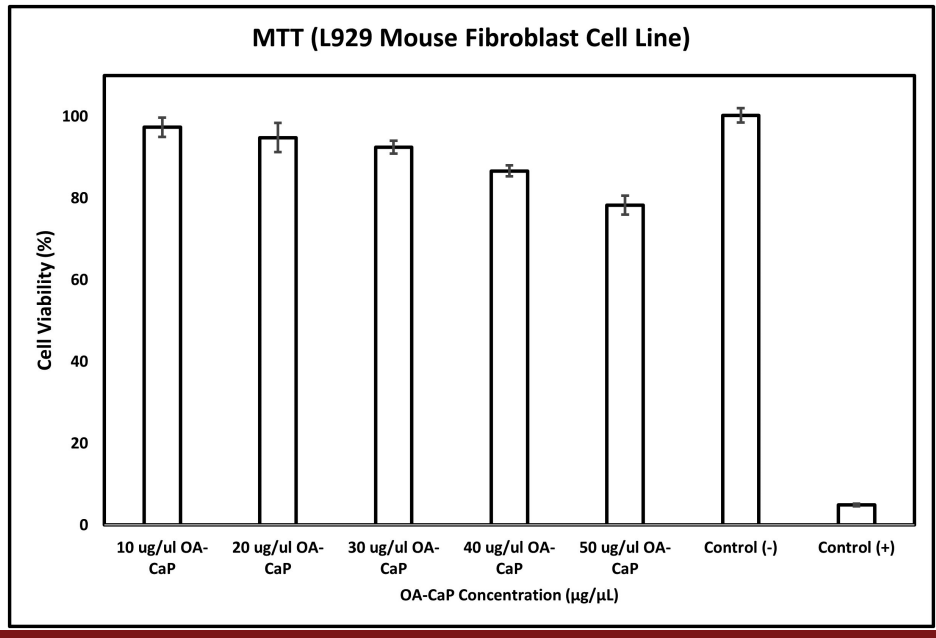

Figure 4. Transfection images of L929 Cells with OA-CaP/pDNA and naked pDNA. Transfected cells with OA-CaP/pDNA. (A): 24th hour, 10x Phase Contrast. (B): 24th hour, 10x Fluorescent. (C): 48th hour, 10x Phase Contrast. (D): 48th hour, 10x Fluorescent. (E): 72nd hour, 10x Phase Contrast. (F): 72nd hour, 10x Fluorescent. Transfected cells with naked pDNA. (G): 24th hour, 10x Phase Contrast. (H): 24th hour, 10x Fluorescent.

making it crucial to carefully control their composition and concentration for safe use in biomedical applications.

This study evaluated the condition of L929 cells after transfection with OA-CaP/pDNA and naked pDNA at 24, 48, and 72-hour intervals using phase-contrast and fluorescence microscopy (Figure 4). A distinct green fluorescence signal was observed in cells transfected with OA-CaP/pDNA (B, D, F), indicating successful gene expression. The fluorescence signal, which began at 24 hours with a transfection efficiency of 22.24% ± 20.13%, intensified at 48 hours (54.86% ± 32.86%) and reached its highest level at 72 hours (72.43% ± 36.57%). In contrast, no significant fluorescence signal was detected in cells transfected with naked pDNA (H), demonstrating the crucial role of the OA-CaP carrier in gene transfer. Phase-contrast images (A, C, E, G) revealed that cell morphology was preserved after transfection, but increased cellular proliferation was observed in cells transfected with OA-CaP/pDNA. These findings support that OA-CaP is an effective carrier system that enhances pDNA transfection efficiency.

The results of this study align with previous research demonstrating the effectiveness of calcium phosphate-based nanoparticle carriers in enhancing gene delivery. Several studies have shown that calcium phosphate nanoparticles, when used as carriers, significantly improve the transfection efficiency of plasmid DNA (pDNA) compared to naked pDNA.[31,32] The green fluorescence signal observed in cells transfected with OA-CaP/pDNA in this study is consistent with previous findings, where calcium phosphate nanoparticles were reported to facilitate successful gene expression and increased transfection efficiency over time.[33] The gradual increase in transfection efficiency, from 22.24% at 24 hours to 72.43% at 72 hours, mirrors the typical behavior of nanoparticle-based systems, where enhanced gene expression is observed as the particles facilitate the release and uptake of the pDNA into cells.[18] Furthermore, the preservation of cell morphology and the observed increase in cellular proliferation in the OA-CaP/pDNA group supports the biocompatibility of the system, which has been similarly reported for other calcium phosphate nanoparticle systems.[34,35] Overall, these findings highlight that OA-CaP nanoparticles serve as an efficient and biocompatible carrier system for gene delivery, enhancing transfection efficiency without compromising cell viability, as indicated in the existing literature.

DISCUSSION

This study demonstrated that OA-CaP nanoparticles significantly enhance pDNA transfection efficiency in L929 cells compared to naked pDNA. Physicochemical characterization of OA-CaP revealed favorable particle size, surface charge, and stability properties, making it a suitable carrier for gene delivery applications. The DLS and zeta potential analyses confirmed the successful functionalization of CaP with OA, which contributed to improved dispersion and reduced aggregation. The SEM images further supported these findings by showing a more homogeneous and porous morphology in OA-CaP compared to CaP.

The MTT assay results demonstrated that OA-CaP maintains high cell viability at lower concentrations, but cytotoxic effects become evident at higher doses, emphasizing

the importance of optimizing nanoparticle concentration for safe and effective gene delivery. The observed trends in nanoparticle size, zeta potential, and transfection efficiency suggest that OA-CaP forms stable and effective pDNA complexes, leading to enhanced gene transfer efficiency.

Fluorescence microscopy analysis of transfected cells indicated that OA-CaP-mediated gene delivery resulted in significantly higher gene expression, with transfection efficiencies increasing from 22.24% ± 20.13% at 24 hours to 72.43% ± 36.57% at 72 hours. In contrast, naked pDNA showed minimal transfection efficiency, highlighting the crucial role of OA-CaP in facilitating cellular uptake and gene expression. Moreover, phase-contrast microscopy revealed that OA-CaP/pDNA transfection preserved cell morphology while promoting cellular proliferation.

Overall, these findings confirm that OA-CaP is a promising gene delivery vector with high transfection efficiency and minimal cytotoxicity at optimized concentrations. Future studies should focus on in vivo evaluations to further establish its potential for therapeutic applications.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: This study did not require approval from an Ethics Committee as it did not involve human participants, human data, or animal subjects.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.A.Y., M.A.Y.; Design – M.A.Y.; Supervision – M.A.Y.; Resources – M.A.Y.; Materials – M.A.Y.; Data Collection and/or Processing – M.A.Y.; Analysis and/or Interpretation – M.A.Y.; Literature Search – M.A.Y.; Writing – M.A.Y.; Critical Review – M.A.Y.

Declaration of Interests: The author have no conflicts of interest to declare.

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References

1. Manohar SK, Gowrav MP, Gangadharappa HV. Materials for gene delivery systems. In: Sheikh FA, Majeed S, Beigh MA, eds. *Interaction of Nanomaterials With Living Cells*. Berlin: Springer; 2023:411-437. **[CrossRef]**
2. Kamimura K, Suda T, Zhang G, Liu D. Advances in gene delivery systems. *Pharmaceut Med*. 2011;25(5):293-306. **[CrossRef]**
3. Nayerossadat N, Maedeh T, Ali PA. Viral and nonviral delivery systems for gene delivery. *Adv Biomed Res*. 2012;1:27. **[CrossRef]**
4. Xie L, Han Y, Liu Y, et al. Viral vector-based cancer treatment and current clinical applications. *Comm Oncol*. 2023;2(4):e55. **[CrossRef]**
5. Ramamoorth M, Narvekar A. Non viral vectors in gene therapy—an overview. *J Clin Diagn Res*. 2015;9(1):GE01-GE06. **[CrossRef]**
6. Sayed N, Allawadhi P, Khurana A, et al. Gene therapy: comprehensive overview and therapeutic applications. *Life Sci*. 2022;294:120375. **[CrossRef]**
7. Sokolova V, Epple M. Biological and medical applications of calcium phosphate nanoparticles. *Chemistry*. 2021;27(27):7471-7488. **[CrossRef]**
8. Epple M, Ganesan K, Heumann R, et al. Application of calcium phosphate nanoparticles in biomedicine. *J Mater Chem*. 2010;20(1):18-23. **[CrossRef]**
9. Maitra A. Calcium phosphate nanoparticles: second-generation nonviral vectors in gene therapy. *Expert Rev Mol Diagn*. 2005;5(6):893-905. **[CrossRef]**
10. Das A, Ghosh S, Ringu T, Pramanik N. A focus on biomaterials based on calcium phosphate nanoparticles: an indispensable tool for emerging biomedical applications. *BioNanoScience*. 2023;13(2):795-818. **[CrossRef]**
11. Santa-María C, López-Enríquez S, Montserrat-de la Paz S, et al. Update on anti-inflammatory molecular mechanisms induced by oleic acid. *Nutrients*. 2023;15(1):224. **[CrossRef]**
12. Ong HT, Suppiah DD, Julkapli NM. Fatty acid coated iron oxide nanoparticle: effect on stability, particle size and magnetic properties. *Colloids Surf A Physicochem Eng Asp*. 2020;606:125371.
13. Liu T, Tang A, Zhang G, et al. Calcium phosphate nanoparticles as a novel nonviral vector for efficient transfection of DNA in cancer gene therapy. *Cancer Biother Radiopharm*. 2005;20(2):141-149. **[CrossRef]**
14. Dolai J, Mandal K, Jana NR. Nanoparticle size effects in biomedical applications. *ACS Appl Nano Mater*. 2021;4(7):6471-6496. **[CrossRef]**
15. Lara-Ochoa S, Ortega-Lara W, Guerrero-Beltrán CE. Hydroxyapatite nanoparticles in drug delivery: physicochemistry and applications. *Pharmaceutics*. 2021;13(10):1642. **[CrossRef]**
16. Joudeh N, Linke D. Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. *J Nanobiotechnology*. 2022;20(1):262. **[CrossRef]**
17. Kavasi RM, Coelho CC, Platania V, Quadros PA, Chatzinikolaidou M. In vitro biocompatibility assessment of nanohydroxyapatite. *Nanomaterials (Basel)*. 2021;11(5):1152. **[CrossRef]**
18. Yildirim MA, Varli HS, Türkoğlu N. The transfection efficiency of newly developed calcium phosphate nanoparticles in reprogramming of fibroblast cells. *J Nanopart Res*. 2024;26(7):170. **[CrossRef]**
19. Varli HS, Alkan F, Demirbilek M, Türkoğlu N. A virus-free vector for the transfection of somatic cells to obtain IPSC. *J Nanopart Res*. 2019;21(11):1-11. **[CrossRef]**
20. Danaei M, Dehghankhold M, Ataei S, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*. 2018;10(2):57. **[CrossRef]**
21. Behzadi S, Serpooshan V, Tao W, et al. Cellular uptake of nanoparticles: journey inside the cell. *Chem Soc Rev*. 2017;46(14):4218-4244. **[CrossRef]**
22. Li J, Yang Y, Huang L. Calcium phosphate nanoparticles with an asymmetric lipid bilayer coating for siRNA delivery to the tumor. *J Control Release*. 2012;158(1):108-114. **[CrossRef]**
23. Sapino S, Chindamo G, Chirio D, et al. Calcium phosphate-coated lipid nanoparticles as a potential tool in bone diseases therapy. *Nanomaterials (Basel)*. 2021;11(11):2983. **[CrossRef]**

24. Morejón L, Delgado JA, Antunes Ribeiro A, et al. Development, characterization and in vitro biological properties of scaffolds fabricated from calcium phosphate nanoparticles. *Int J Mol Sci*. 2019;20(7):1790. **[CrossRef]**
25. Mourdikoudis S, Menelaou M, Fiuza-Maneiro N, et al. Oleic acid/oleylamine ligand pair: a versatile combination in the synthesis of colloidal nanoparticles. *Nanoscale Horiz*. 2022;7(9):941-1015. **[CrossRef]**
26. Khalifehzadeh R, Arami H. Biodegradable calcium phosphate nanoparticles for cancer therapy. *Adv Colloid Interface Sci*. 2020;279:102157. **[CrossRef]**
27. Bhaskaran S, Francis J, Ann Maria PW, Malini KA, Veena Gopalan E. A comparative study of structural properties of oleic acid coated metal nanoparticles (Co, Ni, Fe) by co-precipitation method. *Mater Today Proc*. 2023. **[CrossRef]**
28. Urian YA, Atoche-Medrano JJ, Quispe LT, Félix LL, Coaquira JAH. $^{3}O^{4}$ nanoparticlesStudy of the surface properties and particle-particle interactions in oleic acid-coated Fe. *J Magn Magn Mater*. 2021;525:167686. **[CrossRef]**
29. Masouleh MP, Hosseini V, Pourhaghgouy M, Bakht MK. `osphate nanoparticles cytocompatibility versus cytotoxicity: a serendipitous paradox. *Curr Pharm Des*. 2017;23(20):2930-2951. **[CrossRef]**
30. Peetsch A, Greulich C, Braun D, et al. Silver-doped calcium phosphate nanoparticles: synthesis, characterization, and toxic effects toward mammalian and prokaryotic cells. *Colloids Surf B Biointerfaces*. 2013;102:724-729. **[CrossRef]**
31. Sokolova VV, Radtke I, Heumann R, Epple M. Effective transfection of cells with multi-shell calcium phosphate-DNA nanoparticles. *Biomaterials*. 2006;27(16):3147-3153. **[CrossRef]**
32. Cao X, Deng W, Wei Y, et al. Encapsulation of plasmid DNA in calcium phosphate nanoparticles: stem cell uptake and gene transfer efficiency. *Int J Nanomedicine*. 2011;6:3335-3349. **[CrossRef]**
33. Welzel T, Radtke I, Meyer-Zaika W, Heumann R, Epple M. Transfection of cells with custom-made calcium phosphate nanoparticles coated with DNA. *J Mater Chem*. 2004;14(14):2213-2217. **[CrossRef]**
34. Chernousova S, Epple M. Live-cell imaging to compare the transfection and gene silencing efficiency of calcium phosphate nanoparticles and a liposomal transfection agent. *Gene Ther*. 2017;24(5):282-289. **[CrossRef]**
35. Sokolova V, Rojas-Sánchez L, Białas N, Schulze N, Epple M. Calcium phosphate nanoparticle-mediated transfection in 2D and 3D mono- and co-culture cell models. *Acta Biomater*. 2019;84:391-401. **[CrossRef]**