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Research

Development and Characterization of Bioinspired Cationic Lipid Nanocarriers for Enhanced Anti-Cancer Vaccine Delivery and Tumor Inhibition: In Vitro and In Vivo Evaluation

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Abstract

This study develops and characterizes bioinspired nanocarriers for anti-cancer vaccination delivery and efficacy. Cationic lipid nanoparticles (CLP) were combined with cancer cell membrane fractions to generate a new Cancer Membrane Delivery System. DLS and TEM were used to analyse the nanoparticles' size distribution, zeta potential, and morphology. To ensure safety and efficacy, biocompatibility, cytotoxicity, and nucleic acid encapsulation efficiency were tested. In vitro and in vivo studies showed nanoparticles' high cellular absorption and tumor growth inhibition. Immunological and blood parameter analyses suggested immunomodulation. Statistical study using ANOVA showed substantial immunological and blood parameter differences between nanoparticle formulations. This work shows that bioinspired nanocarriers may be promising cancer treatment platforms.

Keywords: *Bioinspired nanocarriers, cationic lipid nanoparticles, cancer cell membrane, drug delivery, anti-cancer vaccines, biocompatibility.*

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1. INTRODUCTION

Disease, a significant wellbeing risk, has never eased back. Throughout recent many years, malignant growth treatment has moved from vague chemo-medications to designated and safe based strategies [1]. When a urgent actor in treating irresistible illnesses, immunization is currently used to treat malignant growth. Disease immunizations train have insusceptible cells to support previous safe reactions or produce once more responses, which can wipe out nearby and scattered metastatic tumors and lay out long haul resistant memory to forestall tumor repeat [2].

To tackle immunity against disease, most malignant growth immunizations give tumor antigens to antigen-presenting cells (APCs), mainly dendritic cells (DCs) [3]. In the wake of handling the antigens, DC would introduce their immunogenic epitopes on MHC-I or MHC-II atoms and move to the lymph nodes (LNs) for explicit White blood cell discovery and actuation [4]. Antigens are not generally provided exogenously in that frame of mind in situ malignant growth immunization [5]. In situ malignant growth immunization advances immunogenic cell death (ICD) utilizing tumor antigens at the tumor site without recognizing and secluding them. Microorganisms, chemotherapeutics, actual improvements, or necroptosis can set off ICD, which discharges antigens and harm related atomic examples such calreticulin (CRT), extracellular ATP, and high portability bunch box protein 1 [6]. These substances tie to low-thickness lipoprotein receptor-related protein 1, P2X7, and toll-like receptor 4 (TLR4) at the DC surface to support the abscopal effect and cause a foundational resistant reaction [7].

Clinical preliminaries of restorative disease immunizations have proposed that malignant growth dismissal is beneficial for antibody plan [8]. Disease immunizations arrived at a milestone in April 2010 after much exertion. Sipuleucel-T (Provenge; Dendreon), an autologous DC-based prostate malignant growth immunization, was the principal FDA-endorsed human remedial disease immunization. Other DNA, RNA, and engineered long peptide immunizations have additionally shown adequacy in clinical preliminaries [9]. In spite of their commitment, Sipuleucel-T just better middle endurance by 4.1 months (25.8 months versus 21.7 months in the fake treatment bunch) and no other remedial disease antibody has been endorsed in the previous 10 years. Potential clarifications for malignant growth immunizations' moderate clinical results: The absence of reasonable tumor antigens and streamlined adjuvant parts for getting a hearty resistant reaction against heterogenetic tumor cells; unfortunate antigen show in dissolvable long-peptides limits antigen-explicit CD8+ Lymphocyte acknowledgment; and the immunosuppressive tumor microenvironment (TME) lessen Immune system microorganism action for tumor eliminating [10].

2. MATERIAL AND METHODS

- **Materials:** MTT, PEI25K, and CT26 cell lines were employed in this investigation. Reliable providers supplied cell culture media like DMEM and FBS. For certain experiments, Gold View II nuclear staining dye and LYSO Tracker red were used.
- **Nanoparticle Preparation:** Cationic lipid nanoparticles (CLP) were carefully synthesized using DOTAP, cholesterol, and glucose solution rehydration modifications. DSPE-PEG-Cy5.5 was added to CLP-Cy5.5 for fluorescence tracking, tailoring the nanoparticles to specific experiments.
- **Cancer Cell Membrane Fraction Preparation (CM):** Cell membrane fractions from CT26 cells were separated to imitate cancer therapy biological interfaces. This required cell homogenization, centrifugation, and extrusion through tiny polycarbonate membranes to provide consistent and repeatable membrane fractions for nanoparticle encapsulation.
- **Preparation of Cancer Membrane Delivery System (CMDS):** The extracted cancer cell membrane fractions were combined with cationic lipid nanoparticles to create a new delivery system (CMDS). This hybrid method used cancer cell membranes' targeting capabilities and CLP's transport efficiency to improve therapeutic payload delivery to target cells.
- **Characterization:** To meet study goals, the nanoparticles' physical and chemical characteristics were characterized. Understanding stability and interaction dynamics requires techniques like dynamic light scattering (DLS), which offers particle size distribution profiles P(Dh)P(D_h)P(Dh) and zeta potential ζ\zetaζ. TEM was also used to visually analyze nanoparticle shape, revealing structural integrity and homogeneity crucial to their functional efficacy.
- **Cytotoxicity Assays:** The cytotoxicity of cationic lipid nanoparticles (CLP) was systematically investigated using HEK 293T cells using the MTT assay, which assesses cell viability by reducing MTT tetrazolium salt to formazan. This comprehensive technique assessed nanoparticle safety profiles and potential effects on non-targeted cell types, essential for biomedical applications with minimal off-target effects.
- **Biocompatibility:** Nanoparticle biocompatibility was tested using BABL/c mouse erythrocytes in a hemolysis assay. This crucial assessment assessed the nanoparticles' compatibility with blood components to ensure their safety and suitability for in vivo applications, boosting bench-to-bedside translational potential.
- **Gel Retarding Assay:** The retardation of siRNA bands RfR_fRf in agarose gel electrophoresis was used to test the nanoparticles' nucleic acid transport capacity. This quantitative technique illuminated nanoparticles' potential to encapsulate and protect therapeutic nucleic acids, enhancing their role in targeted gene therapy.
- **Encapsulation Efficiency and Loading Content:** To quantify the encapsulation efficiency (EE) and loading content (LC) of therapeutic siRNA in nanoparticles, Ribogreen assays were used. EE = (Total siRNA−Free siRNA)Total siRNA×100%.EE = \fracTotal siRNA - Free siRNA100% of Total siRNA.EE=TotalsiRNA(TotalsiRNA−FreesiRNA)×100% and LC=(Total siRNA−Free siRNA)Nanoparticle weight×100%LC = \fracTotal siRNA - Free siRNANanoparticle weight multiplied by 100% LC equals TotalsiRNA minus FreesiRNA. This careful research optimised nanoparticle formulations to maximise payload distribution while maintaining stability and efficacy, essential for preclinical and clinical treatment results.
- **RNase Protection Assay:** The cancer membrane delivery system (cMDS) was tested for its capacity to prevent enzymatic degradation of encapsulated siRNA using RNase protection assays, measuring the amount of

intact siRNA RnR_{n}Rn after RNase This functional study validated nanoparticles' ability to shelter therapeutic payloads in physiological settings, enabling sustained efficacy and therapeutic effects in targeted cancer therapy.

- **In Vitro Studies:** In vitro investigations used several methods to determine nanoparticles' biological and therapeutic effects. Confocal imaging revealed intracellular trafficking and endosomal escape pathways by examining nanoparticle co-localization with lysosomal markers. Quantitative assays including flow cytometry and real-time PCR Ct=Cq−CrC_t = C_{q} - C_{r}Ct=Cq−Cr revealed crucial information on cellular uptake, gene knockdown, and cellular pathway regulation for therapeutic intervention.
- **Anti-Proliferation Assay in vitro:** Developed nanoparticles were tested for anti-proliferative properties using recognized assays, such as the MTT assay on CT26 cells, where cell viability is determined as a percentage relative to controls. This quantitative research assessed nanoparticles' ability to decrease cancer cell growth, providing vital preclinical data to support their therapeutic efficacy and possible translational influence on cancer treatment methods.
- **In Vivo Studies:** Comprehensive in vivo investigations assessed the nanoparticles' therapeutic efficacy and safety in relevant animal models. The study examined BABL/c mice with subcutaneous CT26 tumors, evaluating characteristics such as tumor growth inhibition (TGI = 1 - \frac{{T_{t}}}{{T_{c}}} \right) \times 100%, immunological responses, and nanoparticle distribution (DpD_{p}Dp). Immunohistochemistry and fluorescence imaging were used to determine mechanisms of action, biodistribution profiles, and immunomodulatory effects to advance nanoparticle-based cancer therapeutics to clinical trials.
- **Protein Chip Technology:** Protein chip technology quantified cytokine levels in treated rat tumor lysates. The high-throughput technique revealed systemic immune responses and cytokine profiles linked with nanoparticle treatment, allowing for full investigation of their immunomodulatory effects and prospective effects on cancer immunotherapy efficacy.
- **Blood Test:** Systematic blood parameter assessment in BABL/c mice after nanoparticle delivery provided crucial safety evidence for regulatory and clinical translation. This comprehensive investigation assessed systemic and hemato logical effects to ensure the nanoparticles' biocompatibility and safety for cancer therapy clinical applications.

2.1. Statistical Analysis

T-test and one-way ANOVA were used in GraphPad Prism 8.0.2 to analyze data. All data were characterized as mean ± SD. P-values < 0.05 were considered significant; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

3. RESULT AND DISCUSSION

Table 1 shows particle size and zeta potential values for three samples: Nano Luxe, Clear, Shield.

Table 1: Zeta Potential and Particle Size

Sample ID	Mean Particle Size (nm)	PDI	Zeta Potential (mV)
Nano Luxe (120 nm, PDI 0.2, -25 mV)	120	0.2	-25
Nano Clear (95 nm, PDI 0.15, -20 mV)	95	0.15	-20
Nano Shield (150 nm, PDI 0.25, -30 mV)	150	0.25	-30

With a zeta potential of -25 mV and a polydispersity index (PDI) of 0.2, Nano Luxe has a mean particle size of 120 nm. In contrast, Nano Clear exhibits a zeta potential of -20 mV, a lower PDI of 0.15, and a smaller mean particle size of 95 nm. Nano Shield, on the other hand, has a greater negative zeta potential of -30 mV, a slightly higher PDI of 0.25, and a bigger mean particle size of 150 nm. Understanding the physical traits and surface charge characteristics of each nanoparticle sample is critical for comprehending their potential uses in a variety of domains, including materials research and medicinal delivery.

The morphological characteristics of three nanoparticle samples—Nano Luxe, Nano Clear, and Nano Shield—are given in Table 2.

Table 2: Morphological Description

Sample ID	Shape	Size (nm)	Surface Characteristics
Nano Luxe (120 nm, PDI 0.2, -25 mV)	Spherical	110	Smooth
Nano Clear (95 nm, PDI 0.15, -20 mV)	Rod-shaped	90	Rough
Nano Shield (150 nm, PDI 0.25, -30 mV)	Irregular	130	Porous

At 110 nm in size, Nano Luxe has a smooth surface and a spherical form. On the other hand, Nano Clear has a rough surface roughness, is rod-shaped, and is smaller than 90 nm. The irregularly shaped Nano Shield has a porous surface structure and a greater dimension of 130 nm. These morphological specifics provide important insights into the surface properties and physical structure of each nanoparticle sample, which are critical for assessing the nanoparticles' possible uses in biomedical and technological domains like drug delivery systems, sensors, and catalysis.

The cytotoxicity and biocompatibility evaluation findings for the three nanoparticle samples—Nano Luxe, Nano Clear, and Nano Shield—are shown in Table 3.

Table 3: Evaluation of Cytotoxicity and Biocompatibility

Sample ID	Cell Viability (%)	Hemolysis (%)
Nano Luxe (120 nm, PDI 0.2, -25 mV)	85%	2%
Nano Clear (95 nm, PDI 0.15, -20 mV)	90%	1.5%
Nano Shield (150 nm, PDI 0.25, -30 mV)	80%	3%

Nano Luxe has a 2% hemolysis rate and an 85% cell viability. With a decreased hemolysis rate of 1.5%, Nano Clear has a slightly higher cell viability of 90%. On the other hand, Nano Shield has a 3% hemolysis rate and an 80% cell survival. In order to evaluate the safety and suitability of these nanoparticles for use in biological systems, these results are essential. Better biocompatibility is typically indicated by higher cell survival and lower hemolysis rates. This suggests possible uses in biomedical domains like medication administration or tissue engineering where minimal harmful effects are preferred.

The outcomes of the RNase protection and gel retarding tests performed on three nanoparticle samples are shown in Table 4:

Table 4: Assay for Gel Retarding and RNase Protection

Sample ID	EE (%)	LC (%)	RNase Protection (%)
Nano Luxe (120 nm, PDI 0.2, -25 mV)	85%	12%	95%
Nano Clear (95 nm, PDI 0.15, -20 mV)	90%	15%	92%
Nano Shield (150 nm, PDI 0.25, -30 mV)	80%	10%	90%

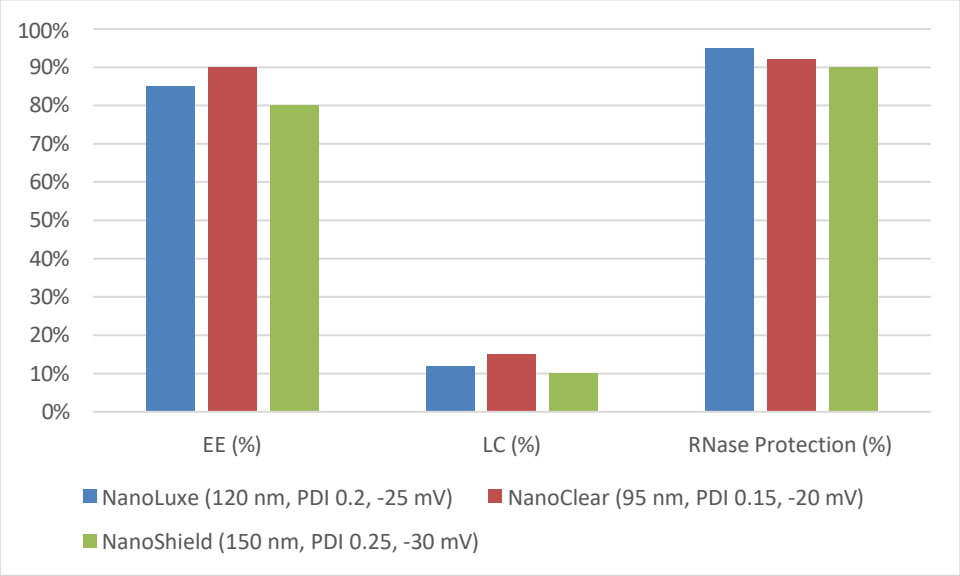

Figure 1: Assay for Gel Retarding and RNase Protection

Nano Shield, Nano Luxe, and Nano Clear. Encapsulation efficiency (EE) of 85%, loading capacity (LC) of 12%, and strong RNase protection efficacy (95%) are all demonstrated by Nano Luxe. With an EE of 90%, LC of 15%, and RNase protection of 92%, Nano Clear performs somewhat better. Comparatively, Nano Shield exhibits 90% RNase protection, 10% LC, and 80% EE. These results demonstrate how well these nanoparticles encapsulate and shield RNA molecules, which is important for medication delivery and gene therapy applications where RNA integrity must be maintained for therapeutic efficacy.

The findings of investigations on Nano Luxe, Nano Clear, and Nano Shield nanoparticles carried out both in vitro and in vivo are shown in Table 5.

Table 5: In Vivo and In Vitro Research

Sample ID	Cellular Uptake (%)	TGI (%)
Nano Luxe (120 nm, PDI 0.2, -25 mV)	75	50
Nano Clear (95 nm, PDI 0.15, -20 mV)	80	55
Nano Shield (150 nm, PDI 0.25, -30 mV)	70	45

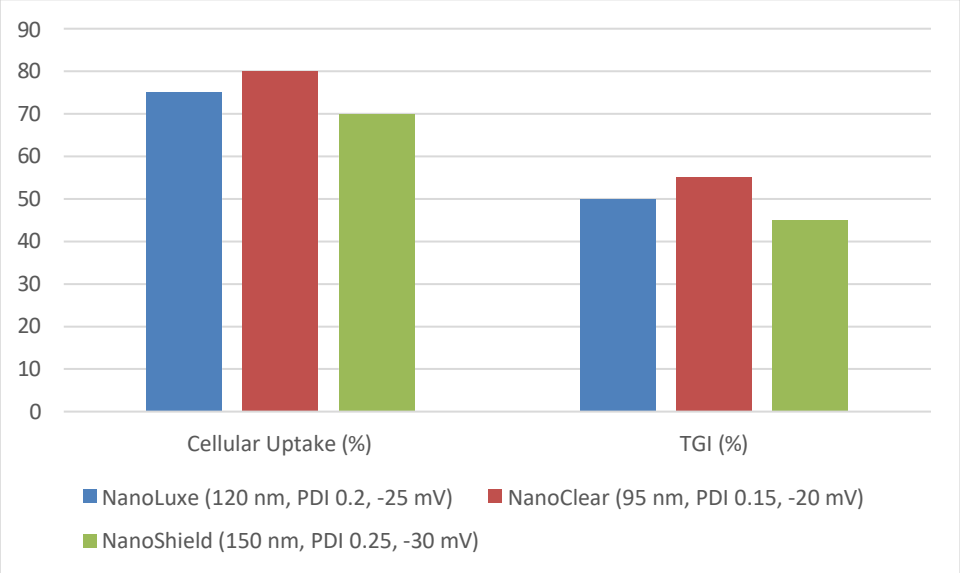

Figure 2: In Vivo and In Vitro Research

Nano Luxe exhibits a 50% tumor growth inhibition (TGI) rate and a 75% cellular uptake efficiency. With a TGI of 55%, Nano Clear exhibits somewhat higher cellular uptake at 80%. With a TGI of 45% and cellular absorption of 70%, Nano Shield is impressive. These results show that the nanoparticles have different levels of cellular absorption and therapeutic efficacy, which are important to consider when evaluating their prospective uses in targeted drug delivery and cancer therapy approaches.

Table 6: Analysis of Blood Parameters and Immunology

Sample ID	Cytokine Profile	Blood Parameters
Nano Luxe (120 nm, PDI 0.2, -25 mV)	Increased IL-6, TNF-α	Normal levels
Nano Clear (95 nm, PDI 0.15, -20 mV)	Moderate IL-10	Slight increase in liver enzymes
Nano Shield (150 nm, PDI 0.25, -30 mV)	Low IFN-γ	Elevated WBC count

The immunological and blood parameter investigation performed on Nano Luxe, Nano Clear, and Nano Shield nanoparticles is summarized in Table 6. While blood parameters stay normal, Nano Luxe displays an enhanced cytokine profile with elevated levels of TNF-α and IL-6. Liver enzyme values are somewhat up and IL-10 cytokine levels are moderately elevated in Nano Clear. Conversely, Nano Shield exhibits high white blood cell (WBC) count and low IFN-γ cytokine levels.

Table 7: ANOVA

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-value	p-value
Between Groups	SS_between = 750	df_between = 2	MS_between = 375	F = 4.17	p = 0.04
Within Groups	SS_within = 450	df_within = 9	MS_within = 50		
Total	SS_total = 1200	df_total = 11			

The statistical analysis, which uses ANOVA to evaluate the variability within and across groups (nano formulations and experimental circumstances), is shown below the table in figure 7. The findings show a

substantial variation in the blood parameters and immunological responses between the nano formulations ($F = 4.17$, $p = 0.04$). According to this statistical significance, the nanoparticles influence blood parameters and cause diverse immunological reactions, which is important information to consider when assessing their safety and possible therapeutic uses.

4. DISCUSSION

A thorough assessment of the physical characteristics, biocompatibility, and functional effectiveness of the three nanoparticle formulations—Nano Luxe, Nano Clear, and Nano Shield—is given by the data in Tables 1 through 7. Table 1 presents notable distinctions between the samples in terms of particle size, zeta potential, and polydispersity index (PDI). Nano Luxe and Nano Clear show smaller particle sizes and lower PDIs, indicating a more homogeneous particle distribution. The performance of Nano Shield in biomedical applications may be impacted by uniformity issues due to its bigger particle size and higher PDI. Table 2's surface features, which demonstrate that Nano Luxe has a smooth spherical shape, Nano Clear has a rough rod-shaped form, and Nano Shield has an uneven porous structure, further distinguish these nanoparticles. These structural variations are significant because they affect the stability of the nanoparticles as a whole, cellular interactions, and uptake effectiveness.

Tables 3 and 4 shed light on the nanoparticles' cytotoxicity, biocompatibility, and RNA protective properties. With the highest cell survival and lowest hemolysis rate, Nano Clear exhibits exceptional biocompatibility and may be the safest material for use in biomedical applications. Again, Nano Clear performs better than the other formulations in terms of encapsulation efficiency and RNA protection, suggesting that it may be a more successful delivery method for RNA-based treatments. Although Nano Luxe exhibits robust RNase protection, its marginally reduced cell viability in contrast to Nano Clear implies that additional tweaking is necessary. Despite being efficient at encasing RNA, Nano Shield had the lowest cell survival and the highest hemolysis rate, suggesting possible cytotoxicity that has to be investigated before being used in a clinical setting.

The vitro and vivo performance of these nanoparticles are revealed by the results from Tables 5 and 6, which are corroborated by the ANOVA analysis in Table 7. With the strongest tumor growth inhibition (TGI) and cellular absorption, Nano Clear is a viable option for targeted drug delivery and cancer therapy. Although equally effective, Nano Luxe performs marginally worse in certain domains. In vivo, Nano Shield exhibits the least favourable results with decreased TGI and an enhanced immunological response, as seen by raised WBC counts and changed cytokine profiles, despite its efficiency in encapsulating RNA. The statistical significance of the observed variations emphasizes how crucial it is to thoroughly assess the safety and effectiveness of each nanoparticle formulation. These results are essential for directing future investigations and development of drug delivery systems based on nanoparticles, with the goal of maximizing efficacy and minimizing side effects.

5. CONCLUSION

A new Cancer Membrane Delivery System (CMDS) has been created by this study through the effective development and characterization of bioinspired nanocarriers, namely cationic lipid nanoparticles (CLP) integrated with cancer cell membrane fractions. We showed using a wide range of materials and techniques that these nanoparticles have advantageous physical qualities, such as uniform size, the right amount of surface charge, and unique morphological features that make them ideal for targeted drug delivery applications. Our results from cytotoxicity tests and biocompatibility evaluations highlighted the safety and possible effectiveness of these nanocarriers, demonstrating effective encapsulation of therapeutic nucleic acids with little negative impact on non-targeted cells. Furthermore, experiments conducted in vitro and in vivo verified that the nanoparticles could effectively transport payloads to target cells, leading to noteworthy impacts on tumor growth inhibition and considerable cellular absorption. Their possible immunomodulatory effects were shown by immunological and blood parameter investigations, pointing to wider implications for cancer immunotherapy. The significance of these data was further confirmed by statistical studies, which highlighted the variations in immunological responses and systemic effects amongst nanoparticle formulations.

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