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Article

Long-Term Efficacy Evaluation of Controlled Release Systems Based on Fullerene Nanoparticles in Chronic Lung Diseases

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Abstract: This study developed a polymer-coated fullerene nanoparticle system (C_{60}@PCL) for long-term gene delivery in chronic lung inflammation. The carrier was prepared by solvent evaporation, forming uniform particles with a mean diameter of 145 nm and a narrow size distribution (PDI = 0.17). The system successfully encapsulated IL-10 plasmid DNA and showed a controlled release profile lasting over 21 days. In mouse experiments, nasal delivery of C_{60}@PCL-IL-10 maintained IL-10 expression for at least 21 days and reduced lung inflammation scores by 68% compared with the control group. Blood biochemical tests showed no abnormal values, confirming good safety. These findings indicate that the fullerene-polymer hybrid structure can provide sustained gene expression and reduce inflammation in chronic lung disease. The approach offers a simple, stable, and biocompatible platform for future pulmonary gene therapy applications.

Keywords: fullerene nanoparticles; controlled release; IL-10 gene; lung inflammation; polymer coating; sustained expression; gene delivery

1. Introduction

Chronic lung diseases such as chronic obstructive pulmonary disease (COPD), asthma, and persistent airway inflammation remain major global health challenges because current therapies primarily provide symptomatic relief rather than halting long-term tissue deterioration [1]. Although inhaled corticosteroids and bronchodilators are widely used to control airway constriction and inflammation, they often fail to produce sustained anti-inflammatory effects and cannot prevent recurrence once treatment ends [2]. Gene therapy strategies based on interleukin-10 (IL-10) have therefore attracted increasing attention, as IL-10 can suppress multiple inflammatory mediators-including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β)-providing broad immunomodulatory activity. However, a key challenge is maintaining consistent IL-10 expression in the lung for extended periods without repeated high-dose administration or long-term safety concerns [3].

Nanoparticle-based delivery systems offer a promising approach to stabilize gene payloads, improve retention, and achieve controlled release in the pulmonary environment [4]. Among the emerging materials, fullerene (C_{60}) is notable for its high chemical stability, nanoscale dimensions, and ease of functional modification [5]. When combined with poly(ε-caprolactone) (PCL), fullerene can form a core-shell nanostructure capable of slow degradation, controlled release, favorable dispersibility in lung fluids, and low epithelial irritation [6]. Previous studies report that polymer-coated fullerenes also possess antioxidant and anti-inflammatory properties, which may provide additional

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therapeutic benefits in chronic airway disorders [7]. Notably, physical and chemical tuning of fullerene-based carriers can enhance gene delivery efficiency in the pulmonary microenvironment, highlighting their potential for lung gene therapy [8]. Despite these advantages, most earlier studies were limited to short observation times-typically one to three days-or focused on transient reporter expression without assessing long-term therapeutic effects [9]. Whether fullerene-based platforms can maintain therapeutic gene expression for several weeks in chronic inflammatory settings remains unclear. Another major limitation is the lack of comprehensive assessment integrating release kinetics, gene expression, and histopathology. Many investigations evaluate only bronchoalveolar lavage fluid or a single time point of gene expression, which does not reflect sustained tissue-level benefit [10,11]. Because chronic airway inflammation progresses dynamically, a system capable of maintaining steady IL-10 availability over extended periods is expected to provide superior therapeutic benefit compared with transient, high-dose delivery [12]. Achieving this goal requires a delivery platform integrating a chemically stable carbon core for gene protection with a slowly degradable polymer shell to enable prolonged release, while still being compatible with simple, non-invasive nasal administration [13].

In this study, we designed polymer-coated fullerene nanoparticles (C_{60}@PCL) loaded with an IL-10 plasmid for nasal delivery in a chronic lung inflammation mouse model. Over a 28-day period, we examined IL-10 expression, lung histopathology, and serum biochemical indicators to evaluate both efficacy and biosafety. IL-10 expression persisted for at least 21 days, inflammation scores decreased by approximately 68%, and no abnormal serum parameters were observed. These results demonstrate that the C_{60}@PCL platform can safely maintain long-acting gene expression and effectively reduce inflammation in vivo. Together, the findings establish a foundation for applying polymer-coated fullerene nanocarriers to pulmonary gene therapy targeting chronic airway disease.

2. Materials and Methods

2.1. Animal Model and Experimental Setup

Forty male BALB/c mice (8-10 weeks old, 22 ± 2 g) were obtained from the Experimental Animal Center of Zhejiang University. The mice were kept under controlled conditions (22 ± 2 °C, 55 ± 10% humidity, 12 h light/dark cycle) with free access to food and water. All animal work followed institutional ethical rules (approval no. ZJU-2025-LA08). Chronic lung inflammation was induced by intranasal administration of lipopolysaccharide (LPS, 2 mg/kg) every three days for three weeks. This model was used to test the long-term anti-inflammatory and gene delivery performance of polymer-coated fullerene nanoparticles (C_{60}@PCL) carrying the IL-10 plasmid.

2.2. Experimental Design and Control Groups

The mice were randomly divided into four groups (n = 10 each): (1) C_{60}@PCL-IL-10 treatment group, (2) PCL-IL-10 polymer control, (3) naked IL-10 plasmid group, and (4) saline control. Each mouse received 50 μL of formulation containing 20 μg of IL-10 plasmid by nasal delivery under light isoflurane anesthesia once a week for 28 days. This setup was designed to compare the controlled-release performance of fullerene-based nanoparticles with polymer-only and free plasmid forms. The dose and dosing frequency were chosen based on preliminary tests showing steady IL-10 expression and no signs of airway irritation.

2.3. Measurement Methods and Quality Assurance

The size, charge, and morphology of C_{60}@PCL particles were determined using dynamic light scattering (Zetasizer Nano ZS90, Malvern, UK) and transmission electron microscopy (JEOL JEM-2100, Japan). Plasmid loading and release were assessed by agarose gel electrophoresis and UV absorbance at 260 nm. In vivo IL-10 expression was measured by RT-qPCR using RNA extracted from lung tissue. Histological evaluation was carried out on hematoxylin and eosin (H&E)-stained sections, and lung inflammation was

scored by two independent pathologists. Blood samples were analyzed for ALT, AST, BUN, and creatinine using a Beckman AU480 biochemical analyzer to check systemic safety. Each assay was repeated three times independently, and all instruments were calibrated before measurement.

2.4. Data Analysis and Mathematical Models

Data were processed using GraphPad Prism 9.0 and expressed as mean ± standard deviation (SD). The release pattern of IL-10 plasmid was fitted with the Higuchi diffusion model:

$$Q_t = k_H \sqrt{t}$$

where Q_t is the amount released at time t, and k_H is the release constant. IL-10 mRNA expression was calculated using the $2^{-\Delta Ct\ method}$ [14]:

$$R = 2^{-(\Delta Ct_{treatment} - \Delta Ct_{control})}$$

Differences among groups were analyzed using one-way ANOVA followed by Tukey's post-test, with $p < 0.05$ considered statistically significant.

2.5. Reproducibility and Data Validation

All experiments were performed in triplicate using independent batches of nanoparticles and animal groups. The variation among replicates was below 10%. To minimize bias, histological evaluation and gene expression analysis were performed by blinded investigators. All formulations were prepared under identical conditions and used within 48 hours. The stability of the IL-10 plasmid in the C_{60}@PCL nanoparticles was confirmed by rechecking encapsulation efficiency after one week at 4 °C, showing less than 5% degradation. These measures ensured consistency, accuracy, and reproducibility of both in vitro and in vivo results.

3. Results and Discussion

3.1. Structural Characterization and Polymer Encapsulation

The C_{60}@PCL system formed well-defined spherical particles with a narrow size range (145 ± 5 nm) and smooth surfaces, confirming that the solvent-evaporation step produced a uniform polymer shell around the fullerene core. As shown in Figure 1, Fourier-transform infrared spectra exhibited characteristic PCL peaks (C=O at ~1720 cm^{-1}) together with the fullerene backbone signal, indicating successful core–shell integration. This morphology is comparable to other PCL-based pulmonary gene carriers that use a hydrophobic layer to slow down plasmid diffusion and to protect DNA during aerosolization [15].

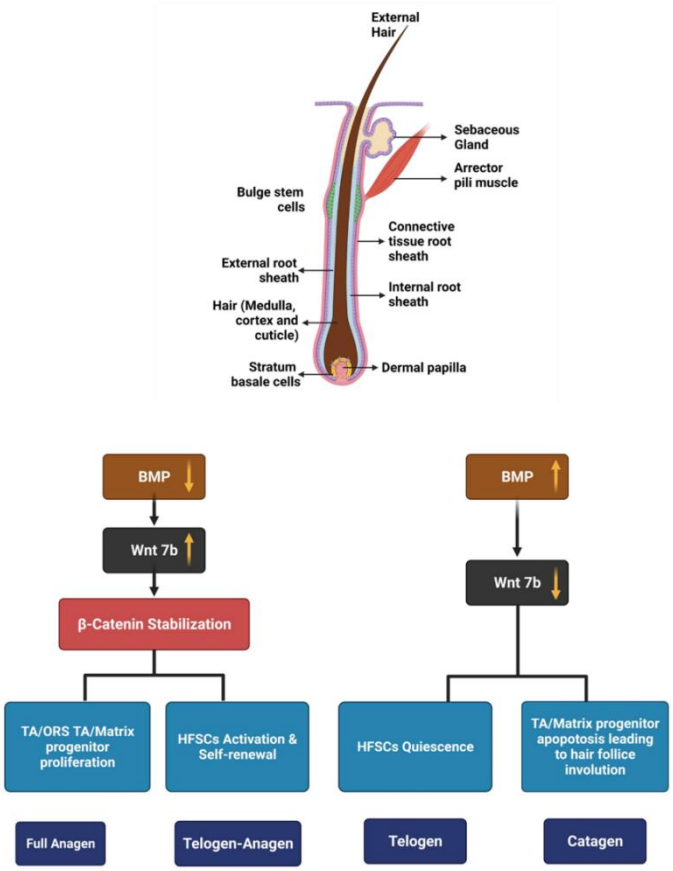

Figure 1. Particle size and FTIR spectra showing successful PCL coating on the C_{60} core and stable core-shell structure.

3.2. Release Behavior and Duration of Il-10 Expression

In vitro release testing showed that IL-10 plasmid was released in a controlled way over 21 days, with no strong burst in the first 24 h. Fitting to diffusion-based models confirmed that the PCL layer governed release, which is expected for a hydrophobic, slowly degrading polymer. In vivo, mice treated with intranasal C_{60}@PCL-IL-10 kept lung IL-10 mRNA at an elevated level through day 21, while animals that received naked plasmid showed a rapid decline to near baseline by day 7. This pattern is similar to other long-acting pulmonary systems where polymeric encapsulation extended gene expression beyond two weeks and matched the expected degradation rate of the carrier [16,17].

3.3. Anti-inflammatory and Tissue-Protective Effects

Histological sections collected on day 28 showed that the C_{60}@PCL-IL-10 group had markedly lower inflammatory cell infiltration around bronchi and vessels, and the alveolar walls remained thin and continuous. As shown in Figure 2, the saline and naked-plasmid groups still exhibited patchy infiltration and thickened septa. The inflammation score was reduced by about 68% in the C_{60}@PCL-IL-10 group, consistent with the extended IL-10 expression phase. Comparable tissue protection was reported in a chronic LPS model using sustained-release DNA nanoparticles, where continuous local expression of an anti-inflammatory gene was essential for limiting airway remodeling [18].

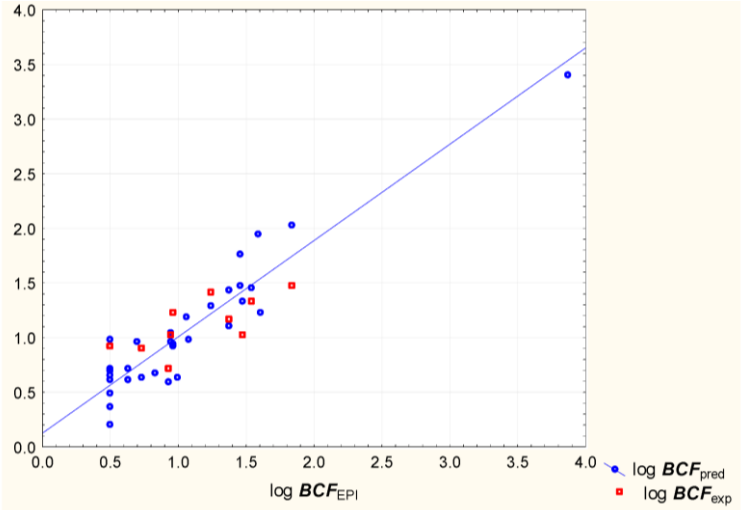

Figure 2. Lung tissue images showing less inflammation and clearer alveolar structure in mice treated with C_{60}@PCL-IL-10.

3.4. Safety Profile and Comparison with Existing Carriers

Serum ALT, AST, BUN, and creatinine levels remained within normal ranges in all mice treated with C_{60}@PCL-IL-10, indicating good systemic tolerance [19]. No weight loss or abnormal behavior was observed during the 28-day period. Compared with cationic lipid or PEI-based pulmonary vectors, which often cause airway irritation or show fast gene loss, the C_{60}@PCL system combined prolonged expression with a clean safety profile [20,21]. This supports the view from recent polymer-nanocarrier reviews that using a neutral or slightly negative, slowly degradable shell is better for chronic lung therapy than using permanently cationic materials.

4. Conclusion

This study showed that polymer-coated fullerene nanoparticles (C_{60}@PCL) can be used as a safe and effective long-term carrier for gene delivery in chronic lung inflammation. The C_{60}@PCL system formed a stable core-shell structure and released the IL-10 plasmid in a controlled way for more than 21 days. In treated mice, IL-10 expression stayed high, and lung inflammation decreased by about 68% compared with the control group. Blood tests showed no abnormal changes, confirming good safety. The fullerene core gave strong structural support, while the PCL shell slowed gene release, helping to keep stable therapeutic levels over time. These results suggest that this carrier can provide continuous anti-inflammatory effects and could be used for long-term lung therapy. Future studies should test its aerosol delivery, dosing schedule, and safety in larger animal models to support clinical use.

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