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Doxorubicin-loaded PEI-silica Nanoparticles for Cancer Therapy

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Abstract – Targeted anticancer drug delivery systems are needed to enhance therapeutic efficacy by selectively delivering drugs to tumor cells while minimizing off-target effects, improving treatment outcomes and reducing toxicity. In this study, a silica-based nanocarrier capable of targeting drug delivery to cancer cells was developed. First, silica nanoparticles were synthesized by the Stöber method using the surfactant cetyltrimethylammonium bromide (CTAB). Increasing the ratio of EtOH in the solvent produced uniformly spherical silica nanoparticles. Washing the nanoparticles removed unreacted residues, resulting in a non-toxic carrier for drug delivery in cells. Upon surface modification, the pH-responsive polymer, polyethyleneimine (PEI) exhibited slow doxorubicin release at pH 7.4 and accelerated release at pH 5.5. By exploiting this feature, we developed a system capable of targeted drug release in the acidic tumor microenvironment.

Key words: Silica nanoparticle, Drug delivery system, Doxorubicin, Cancer therapy, pH-responsive

1. Introduction

Silica nanoparticles offer several advantages in biomedical applications [1]. First, their size and shape can be controlled, allowing for the adjustment of the surface area, drug-loading capacity, and solubility [2]. Second, they are chemically stable, which ensures long-term stability under wide pH range, heat, and pressure conditions [3-5]. Additionally, their biocompatibility enables their stable presence within the body and their elimination through excretion, which ensures safe use without adverse effects [6]. Because surface modification is easily achievable, interactions with cells or tissues can be improved, enabling targeted functions [7]. Specifically, mesoporous silica nanoparticles, which have high surface area and internal payload capacity, protect loaded active substances, maintain stability and allow controlled release under specific conditions[8]. These unique characteristics have sparked significant interest and research in biomedical applications, including drug delivery, tissue engineering, diagnostics, and biosensing [9,10].

In particular, targeted anticancer drugs can reduce drug toxicity by selectively killing cancer cells while sparing healthy cells and minimizing the adverse effects of traditional chemotherapy [11]. Their precision in targeting specific molecular pathways allows for more effective treatments with reduced harm to the patient's overall health [12]. The tumor tissue's acidic environment, known as tumor acidosis, results from increased glycolytic activity, which leads to elevated production of lactic acid and low pH [13]. This acidic microenvironment is a characteristic of solid tumors, which experience inadequate blood supply and poor perfusion [14]. It promotes tumor invasion, metastasis, and resistance to therapy while adversely affecting normal tissues [15]. Exploiting the acidic nature of tumor tissues has emerged as a promising strategy for targeted drug delivery and imaging in cancer therapy [16].

Doxorubicin, an anthracycline antibiotic, is a widely used cancer chemotherapy drug [17] that functions by intercalating with DNA, inhibiting DNA and RNA synthesis, and inducing DNA double-strand breaks, leading to cell death [18]. Due to its broad spectrum of anticancer activity, doxorubicin is used to treat various cancers, including breast, lung, and ovarian cancers, as well as leukemia and lymphoma [19-21]. Despite its effectiveness, doxorubicin has significant side effects, such as cardiotoxicity and bone marrow suppression, which limit its long-term use [22,23]. Hence, there are efforts to develop targeted drug delivery systems to minimize its off-target effects and improve its overall therapeutic benefits in cancer treatment [24].

In this study, silica nanoparticles were synthesized and loaded with doxorubicin. The surface of the silica nanoparticles was coated with polyethyleneimine (PEI) to form a complete drug delivery nanocarrier (Fig. 1). PEI exhibits pH responsiveness through self-ionization and counter-ionization, leading to changes in its positive charge [25]. At low pH, PEI's amino groups are ionized into cationic forms, which increases their positive charge. At high pH, counter-ionization occurs, thereby reducing the positive charge [26]. This property can be exploited to achieve targeted drug delivery [27]. Thus, coating the nanoparticles with PEI generated a pH-responsive drug delivery system for targeted drug release [28].

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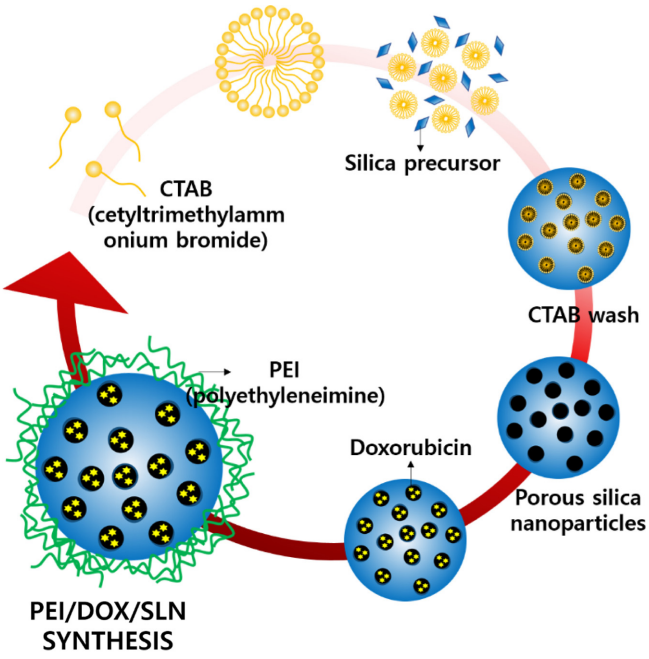

Fig. 1. Synthesis of doxorubicin-loaded PEI-silica nanoparticles (PEI/DOX/SLN).

2. Materials and Methods

2-1. Preparation of PEI/DOX/SLN

We used the Stöber process as the main method for synthesizing porous silica nanoparticles through a sol–gel reaction. First, 400 mg of cetyltrimethylammonium bromide (CTAB) (Sigma-Aldrich, USA) was dissolved in 30ml of ethanol, followed by 1.5 ml of NH_4OH addition. The solution was then reacted for 10 minutes with stirring. Next, tetraethyl orthosilicate (Sigma-Aldrich, USA) was added dropwise, and the mixture reacted in an oven at 110 ℃ overnight.

The solution was filtered and then subjected to centrifugation at 10,000 rpm and the supernatant removed. The resulting nanoparticles were rinsed with ethanol three times. To remove the surfactant, CTAB, the silica nanoparticles were incubated in a mixture of 9:1 ratio of methanol and HCl solution and reacted at 80 ℃ for two days. After the reaction, the nanoparticles were centrifuged at 10,000 rpm and the supernatant removed. They were then washed thrice with ethanol to eliminate excess HCl, yielding porous silica nanoparticles. To load the synthesized porous silica nanoparticles, they were immersed in a doxorubicin (TCI, Japan) solution and then coated with polyethyleneimine (PEI) (Sigma-Aldrich, USA) to generate doxorubicin-loaded nanoparticles.

2-2. Morphological analysis of PEI/DOX/SLN

The size of the synthesized nanoparticles was determined using dynamic light scattering (DLS) (Malvern Instruments, Worcestershire, UK). DLS measures intensity fluctuations of scattered light caused by particles' Brownian motion, which allows the determination of their hydrodynamic size in solution.

A field-emission scanning electron microscope (FE-SEM) (SIGMA; Carl Zeiss, Germany) was used to assess the morphology of the nanoparticles. First, the nanoparticles were thoroughly dried and then uniformly dispersed in ethanol using a sonicator. Before FE-SEM analysis, a platinum coating was applied with an ion putter (E-1010; Hitachi, Japan). This preparation allowed for nanoparticle morphological examination at high resolution and magnification.

2-3. Characterization of PEI/DOX/SLN

Next, we used X-ray diffraction (XRD) analysis to examine the structure of the synthesized porous silica nanoparticles. To this end, the samples were powdered through calcination and then subjected to XRD analysis on a D8-Advance instrument (Bruker-AXS, USA). The XRD analysis covered a range of 10° to 80° and provided information about the nanoparticles' crystalline structure and phase composition.

For material analysis, the powdered silica nanoparticles were analyzed using Fourier transform infrared spectroscopy (FT-IR) on a Nicolet 6700 instrument (Thermo Fisher Scientific). FT-IR analysis provided information about the silica nanoparticles' chemical bonds, functional groups, and molecular structure.

2-4. Cell culture

Cultured HeLa cells (ATCC; CCL-2) were used to evaluate the toxicity of the synthesized porous silica nanoparticles and their drug release characteristics. HeLa cells were cultured in DMEM (Gibco) supplemented with 10% FBS (Gibco) and 1% penicillin (Gibco), at 37 ℃ and 5% CO_2. The cells were passaged every two to three days.

To assess cell toxicity, a live/dead kit (Invitrogen) was used following the manufacturer's guidelines. HeLa cells were treated with doxorubicin-loaded silica nanoparticles at different intervals. After treatment, the cells were stained using a live/dead kit followed by fluorescence imaging, which distinguishes live cells (green fluorescence) from dead cells (red fluorescence), allowing the measurement of cell viability and cytotoxicity.

Cell proliferation was assessed using an MTT assay (Cell Proliferation Kit I, Roche) after 24h of treatment with silica nanoparticles. After the cells were incubated with the MTT reagent, the formazan crystals formed by metabolically active cells were dissolved, followed by absorbance reading of the resulting solution on a microplate reader at a wavelength 550-600 nm. The absorbance measurement indicates the cells' metabolic activity, which correlates with cell proliferation.

2-5. Analysis of PEI/DOX/SLN's drug release

Time-dependent release by the PEI/DOX/silica nanoparticles was evaluated by analyzing the absorbance of various doxorubicin concentrations and using standard curves. The absorbance was analyzed using a microplate reader (BioTek, SynergyTM H1) at 480 nm.

3. Results and Discussion

3-1. Analysis of the SLN based on the solvent composition

To produce spherical and uniform silica nanoparticles, experiments were conducted using 0%, 50%, and 100% EtOH as solvent. FE-SEM analysis revealed that spherical particles formed with increasing EtOH concentrations. At 0% EtOH, the nanoparticles were rod-shaped. At 50% EtOH, they exhibited a more rounded morphology. At 100% EtOH, uniformly round silica nanoparticles, approximately 100 nm in diameter, were obtained (Fig. 2(a)-(f)). Dynamic light scattering (DLS) revealed that at 0% and 50% EtOH, the silica nanoparticles' size and shape were inconsistent and polydispersed, making it difficult to obtain reliable results. However, at 100% EtOH, the nanoparticles had a diameter of about 100 nm (Fig. 2(g)). These results indicate that the proportion of EtOH during silica nanoparticles synthesis influences their uniformity and shape.

3-2. Characterization of PEI/DOX/SLN

Structural and material analyses of the synthesized silica nanoparticles involved using XRD and FT-IR. XRD analysis was done on powdered silica nanoparticles using CuKα (λ = 1.54 Å) radiation as a source. The analysis covered a range of 10° to 80° and yielded a prominent peak at 2θ=22°, which is characteristic of silica nanoparticles (Fig. 3(a)). Using FT-IR analysis, absorption peaks were observed at 800 and 1080 cm^{-1}, corresponding to the Si-O-Si bonds, which are representative absorption bands for amorphous silica particles (Fig. 3(b)). These XRD and FT–IR analyses provide valuable information on the structural and molecular characteristics of the synthesized silica nanoparticles.

3-3. PEI/DOX/SLN cytotoxicity

MTT assays were used to evaluate the cytotoxicity of the synthesized silica nanoparticles after 24h treatment, including the CTAB-washed and PEI-coated silica nanoparticles. The before CTAB-washed silica nanoparticles showed approximately 25% cell viability when compared with the control, indicating cytotoxicity of CTAB. However, after the CTAB wash, the viability increased to around 70%, indicating effective CTAB removal. Notably, the cell viability of PEI-coated silica nanoparticles was similar to that of the control, indicating a high level of biocompatibility (Fig. 4). These findings indicate that the CTAB-washing and subsequent coating, especially with PEI, enhances the silica nanoparticles' biocompatibility and reduces their cytotoxicity, making them more suitable for biomedical applications. Furthermore, because they are not toxic to non-targeted cells, the silica nanoparticles are suitable for use as drug delivery vehicles.

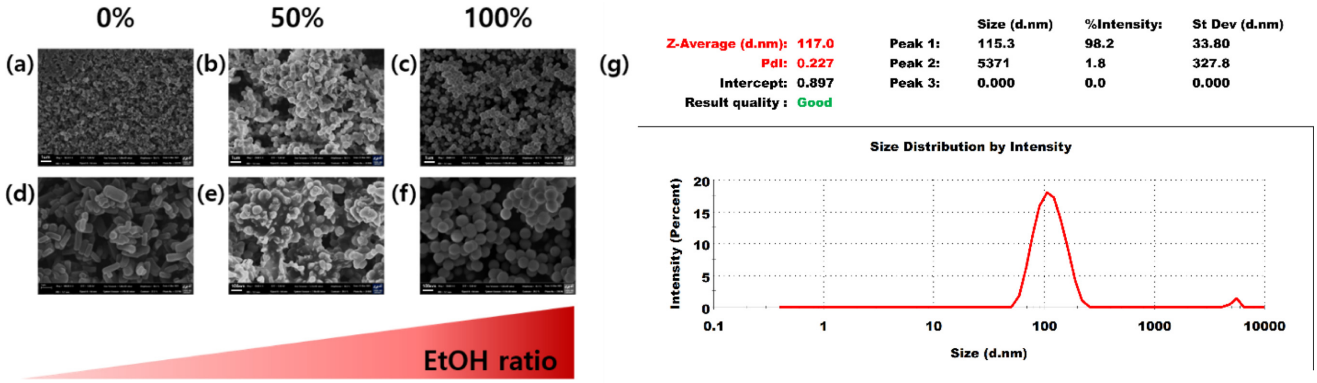

Fig. 2. An SEM image of the silica nanoparticles generated at indicated EtOH concentrations. (a) and (d) 0% EtOH, (b) and (e) 50% EtOH, (c) and (f) 100% EtOH. (g) DLS analysis results of silica nanoparticles synthesized using 100% EtOH.

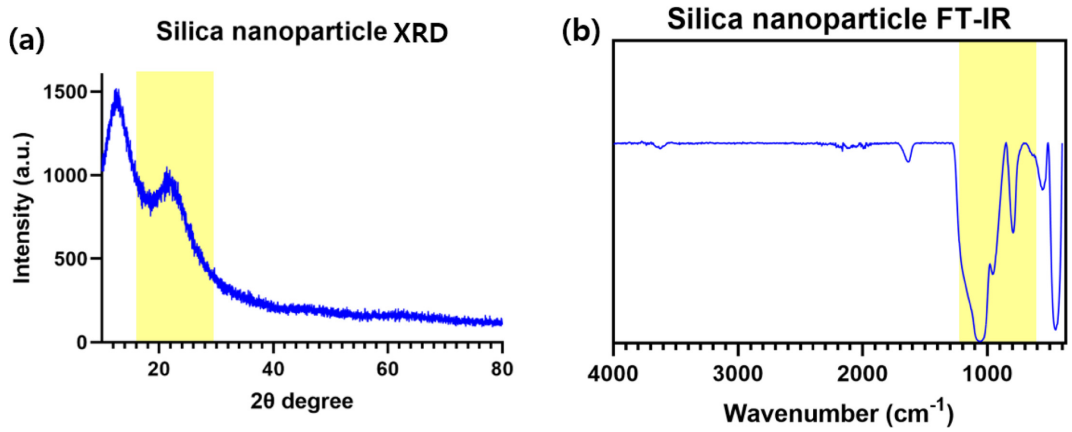

Fig. 3. (a) XRD, and (b) FT-IR analyses of the silica nanoparticles.

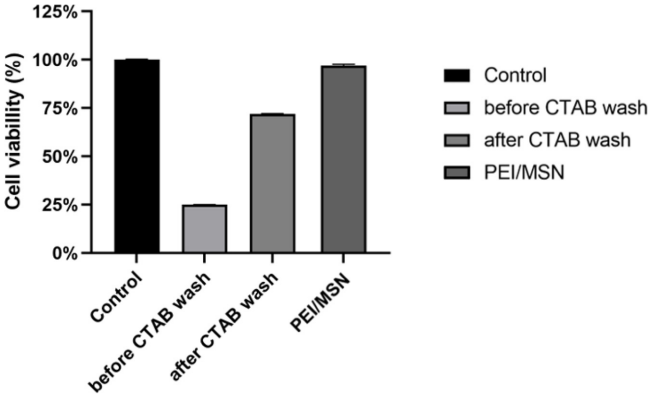

Fig. 4. Cytotoxicity in HeLa cells after 24h treatment of Silica nanoparticles.

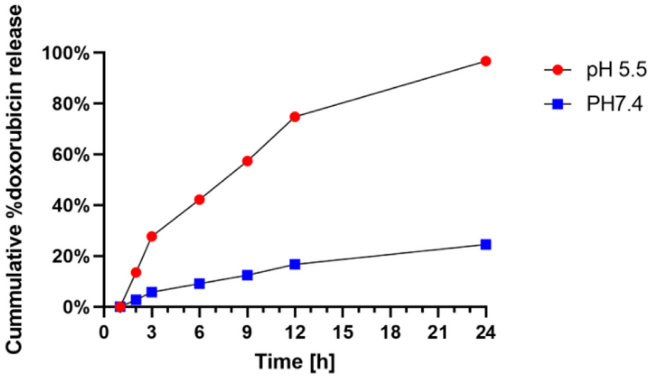

Fig. 6. Evaluation of doxorubicin release from PEI/DOX/SLN at pH 7.4 and pH 5.5.

3-4. PEI/DOX/SLN drug release characteristics

To assess drug release characteristics of the synthesized PEI/DOX-SLN, it was used to treat cultured HeLa cells, followed by live/dead fluorescent images after 0, 1, 3, 6, 12, and 24 h. ImageJ was then used to quantify cell viability and the data visualized on graphs. This analysis revealed that the number of dead cells (red) increased noticeably after 6 h, indicating the release of doxorubicin from the PEI/DOX-SLN to cells (Fig. 5).

A delivery system that targets doxorubicin to acidic tumor environments was developed by coating the pH-responsive polymer PEI with silica nanoparticles. We then analyzed the amount of doxorubicin released from PEI/DOX-SLN using a plate reader in pH 5.5 and pH 7.4 buffers. These analyses showed that in the pH 7.4 buffer, only about 20% of the doxorubicin was released over 24 h. In contrast, in the acidic buffer (pH 5.5), >20% of doxorubicin was released within three hours and nearly 100% was released over 24 h (Fig. 6), suggesting that in the neutral environment of normal tissue, doxorubicin release from PEI/DOX/SLN would be slower than in the acidic environments of tumor tissues, where it would be rapidly released. The differential release of doxorubicin from PEI/DOX/SLN in neutral, normal tissues vs acidic tumor tissues indicates that

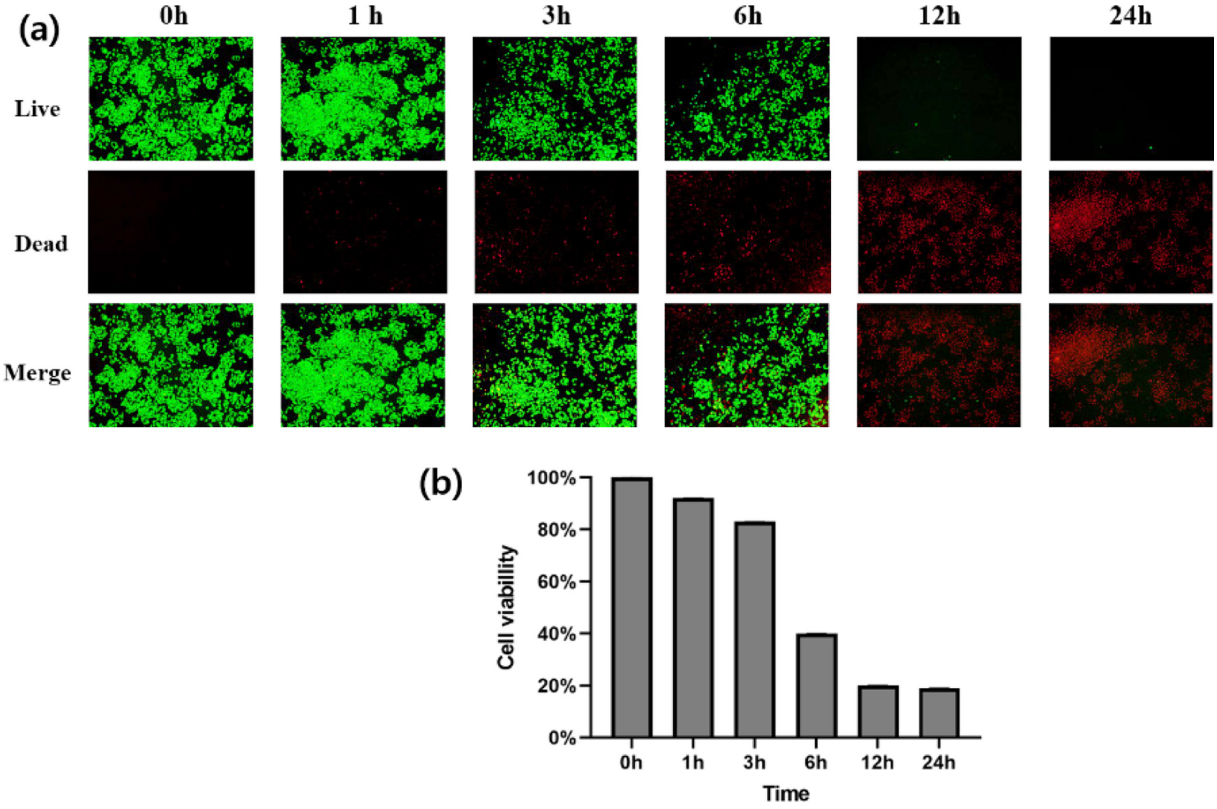

Fig. 5. (a) Doxorubicin release from PEI/DOX/SLN to cultured HeLa cell was evaluated using a live/dead assay, followed by fluorescent imaging. (b) Graph analyzed by percentage using image j program of live/dead assay.

this system can reduce drug toxicity to normal tissues while achieving targeted drug delivery to cancer cells. This inherent feature is a promising strategy for improving cancer treatment outcomes while minimizing side effects.

4. Conclusions

We successfully developed nanoparticles that exhibit pH responsiveness and that can target cancer cells for drug delivery. To synthesize the silica nanoparticles, we used the surfactant CTAB to create porous particles, which were then coated with the pH-responsive polymer, PEI.

Through this experiment, we synthesized spherical silica nanoparticles with a uniform size of approximately 100 nm. Furthermore, by efficiently loading the anti-cancer drug, doxorubicin, we induced the eradication of HeLa cells. Notably, the nanoparticles exhibited faster and more effective doxorubicin release at pH 5.5 when compared with pH 7.4, enabling targeted drug delivery to cancer cells while minimizing damage to normal cells. The PEI/DOX-SLN nanoparticles can be improved for targeted delivery by incorporating them into hydrogels or other solution-based carriers. Additionally, by exploring different drug options, these nanoparticles have the potential for diverse applications as a drug delivery system.

Overall, our findings highlight PEI/DOX-SLN nanoparticles as a potentially, highly effective targeted drug delivery system that can offer versatility for various therapeutic applications.

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