Functionalized Mesoporous Silica Nanoparticles as Potential Drug Delivery Vehicle against Colorectal Cancer

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ABSTRACT
This study explores the development of mesoporous silica nanoparticles (MSNs) as an advanced platform for the targeted delivery of doxorubicin hydrochloride (DOX), a widely used chemotherapeutic agent. Utilizing a modified sol-gel process, MSNs were synthesized and functionalized with amino (MSN-NH₂) and carboxyl (MSN-COOH) groups to enhance their physicochemical properties and drug delivery efficacy. Comprehensive characterization through scanning electron microscopy (SEM) and Powder X-ray diffraction (PXRD) confirmed the successful synthesis of spherical MSNs with a uniform internal structure and mesoporous nature. Zeta potential measurements highlighted the impact of surface functionalization on the surface charge of the nanoparticles, revealing positive and significantly negative charges for MSN-NH₂ and MSN-COOH respectively. This study further investigated the pH-responsive drug release profiles of DOX-loaded MSNs under physiological (pH 7.4) and tumor-mimicking acidic (pH 5.5) conditions. The results demonstrated a controlled release at pH 7.4, minimizing systemic toxicity, and a significantly enhanced release at pH 5.5, targeting the acidic tumor microenvironment for improved therapeutic efficacy. The findings underscore the potential of functionalized MSNs as a versatile nanocarrier system for cancer therapy, offering a promising strategy to increase the therapeutic index of DOX and reduce adverse effects. This work contributes valuable insights into the design and application of nanoparticle-based drug delivery systems, paving the way for future pre-clinical applications and advancements in targeted colorectal cancer treatments.

KEYWORDS
Mesoporous Silica Nanoparticles; Drug Delivery Vehicle; Colorectal Cancer

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1. Introduction
Cancer remains one of the leading causes of mortality worldwide, necessitating the development of more effective and targeted therapeutic strategies. Among the various cancers, colorectal cancer (CRC) is particularly challenging due to its high incidence and...
the complexity of its treatment modalities. Traditional chemotherapy, while effective to some extent, is often limited by non-specific distribution, systemic toxicity, and severe side effects. These limitations highlight the urgent need for innovative drug delivery systems that can enhance the therapeutic index of chemotherapeutic agents by improving their specificity and reducing adverse effects.

Nanotechnology offers a promising solution to these challenges, particularly using mesoporous silica nanoparticles (MSNs) as drug delivery vehicles. MSNs are characterized by their large surface area, tunable pore size, and high biocompatibility, making them ideal candidates for encapsulating a wide range of therapeutic molecules. The unique mesoporous structure allows for high drug loading and controlled release, while the surface can be easily functionalized to improve targeting specificity and drug delivery efficiency. Previous studies have demonstrated the potential of MSNs in delivering anticancer agents, with significant improvements in drug stability and bioavailability.

The functionalization of MSNs is a critical aspect that can significantly influence their interaction with biological systems. Surface modifications, such as the introduction of amino (–NH₂) and carboxyl (–COOH) groups, can alter the charge of nanoparticles, hydrophilicity, and binding affinity, thereby affecting their biodistribution and cellular uptake. For instance, amino-functionalized mesoporous silica nanoparticles (MSN–NH₂) can interact more favorably with negatively charged cell membranes, enhancing cellular uptake and drug delivery efficiency. Conversely, carboxyl-functionalized mesoporous silica nanoparticles (MSN–COOH) may offer improved colloidal stability, which is crucial for maintaining the integrity of the nanoparticles in biological fluids.

In this study, we explore the synthesis, functionalization, and characterization of MSNs for the targeted delivery of doxorubicin hydrochloride (DOX), a potent chemotherapeutic agent widely used in cancer treatment. The choice of DOX is based on its well-established efficacy against a broad spectrum of cancers, including CRC. However, its clinical use is often limited by cardiotoxicity and other systemic side effects, necessitating the development of targeted delivery systems that can enhance its accumulation at the tumor site while minimizing exposure to normal tissues.

Our approach involves the functionalization of MSNs with amino and carboxyl groups to investigate the impact of surface charge and functional groups on drug loading capacity, release kinetics, and interaction with cancer cells. The drug release behavior of DOX from the functionalized MSNs is evaluated under physiological (pH 7.4) and tumor-mimicking acidic conditions (pH 5.5), with the goal of developing a pH-responsive delivery system that releases the drug preferentially in the tumor microenvironment. This pH-sensitive release mechanism is particularly advantageous for targeting the slightly acidic extracellular milieu of solid tumors, thus enhancing the local concentration of the drug and improving its therapeutic efficacy.

In addition to the drug release studies, we employ a comprehensive suite of characterization techniques, including scanning electron microscopy (SEM), X-ray diffraction (XRD), and zeta potential measurements, to elucidate the structural and physicochemical properties of the synthesized nanoparticles. The findings from this study aim to provide a deeper understanding of the design principles governing the synthesis and functionalization of MSNs, with a focus on optimizing their performance as drug delivery vehicles for targeted cancer therapy.

This work contributes to the growing body of literature on nanoparticle-based drug delivery systems and their potential to revolutionize cancer treatment by offering more precise, effective, and safe therapeutic options. The insights gained from this study may pave the way for the development of next generation nanocarriers with enhanced targeting capabilities, animal studies, and improved patient outcomes.

2. Experimental Section
2.1 Synthesis of MSNs
The synthesis of mesoporous silica nanoparticles (MSNs) was carried out using a modified sol-gel process. The procedure involved the use of 5 mL tetraethyl orthosilicate (TEOS) as the silica precursor, 1 g cetyltrimethylammonium bromide (CTAB) as the surfactant template, and a mixture of 400 mL water as the solvent system. The reaction was initiated by adding an alkaline solution of sodium hydroxide (5 mL 2 M) to the mixture, followed by the addition of TEOS under vigorous stirring. The reaction mixture was maintained at 70°C for 2 h to facilitate the formation of silica nanoparticles. The resulting product was then filtered, washed, and calcined at 500°C to remove the CTAB template, yielding the final mesoporous silica nanoparticles.

2.2 Functionalization of MSNs
The surface of MSNs was functionalized first with amino groups by reacting to the nanoparticles with 3-aminopropyltriethoxysilane (APTES). The reaction was carried out in a toluene solution under reflux conditions for 24 h. 1 mL of APTES was added in 50 mL absolute ethanol having 0.5 g MSNs dispersed in it. The functionalized nanoparticles were then washed and dried under vacuum condition. After that the carboxyl-functionalized MSNs were prepared by further reacting the MSN–NH₂ (0.3 g) with succinic
anhydride (30 mg) in 30 mL anhydrous dichloromethane (DCM) under nitrogen atmosphere. The reaction was allowed to proceed for 12 h, followed by washing and drying to obtain the MSN-COOH nanoparticles.

### 2.3 SEM, XRD and Zeta Potential Analysis

The morphology and size distribution of the synthesized nanoparticles were analyzed using SEM. Samples were prepared by dispersing the nanoparticles in ethanol, followed by deposition on a silicon wafer. The images were obtained using a high-resolution field emission scanning electron microscope (FESEM) under appropriate acceleration voltage (2kV). The crystalline structure and mesoporosity of the nanoparticles were determined using XRD. The measurements were carried out on a powder X-ray diffractometer using Cu Kα radiation. The data were collected in the 2θ range of 2° to 5°, and the patterns were analyzed to identify characteristic peaks corresponding to the mesoporous structure. The surface charge of the nanoparticles was measured using Malvern Zetasizer instrument. The nanoparticles were dispersed in deionized water, and the zeta potential was measured at 25°C. This analysis was performed for unmodified MSNs, MSN-\( \text{NH}_2 \), and MSN-COOH to determine the effect of functionalization on surface charge.

### 2.4 Drug Loading and Release Studies

**Drug Loading:** Doxorubicin hydrochloride (DOX) was loaded into the nanoparticles by a simple adsorption method. 10 mg of MSNs were dispersed in 1 mL aqueous solution of DOX, and the mixture was stirred for 24 h at room temperature. The drug-loaded nanoparticles were then separated by centrifugation, washed to remove unbound DOX, and the drug loading was calculated from the supernatant solution by UV-Vis spectroscopy. This drug loaded nanoparticles were then coated with casein to stabilize the particles and increase the drug release efficiency. 1 mL of Blocker casein (0.5% w/w) were added in the particles and centrifuged after mixing for about 1 h in a mixer.

**Drug Release:** The release of DOX from the nanoparticles was evaluated at two different pH conditions: 7.4 (physiological) and 5.5 (tumor microenvironment). The DOX-loaded nanoparticles were dispersed in phosphate-buffered saline (PBS) at the respective pH and incubated at 37°C with gentle shaking. At predetermined time intervals, samples were withdrawn, centrifuged, and the supernatant was analyzed for DOX content using UV-Vis spectrophotometry. The cumulative drug release was calculated and plotted as a function of time.

### 3. Results and Discussion

#### 3.1 SEM Analysis

The SEM analysis of MSNs in figure 1 depicts mesoporous silica nanoparticles with a well-defined spherical morphology. The nanoparticles exhibit a range of sizes, generally clustering together, which is indicative of a uniform particle distribution. The surface of the particles appears smooth, suggesting a high-quality synthesis process with minimal aggregation. The scale bar at the bottom left of the image represents 50 nm, providing a reference for the particle dimensions, which predominantly fall within the nanoscale range. The image highlights the uniformity and consistency in size and shape, essential characteristics for applications in drug delivery systems.

**Figure 1:** Scanning Electron Microscopy image of mesoporous silica nanoparticles
3.2 Powder XRD Analysis
In figure 2, the powder X-ray diffraction (XRD) pattern displayed in the image represents the structural characterization of mesoporous silica nanoparticles. The XRD profile shows a distinct and sharp peak centered around 3° (2θ), indicating the presence of a well-ordered mesoporous structure. The intensity of the peak, normalized to arbitrary units (a.u.), suggests a high degree of crystallinity or periodicity in the pore arrangement within the silica matrix. The absence of additional peaks beyond the primary one, particularly in the 2θ range of 2° to 5°, signifies the uniformity and homogeneity of the pore structure. The narrow width of the peak reflects a consistent pore size distribution, which is critical for applications requiring precise molecular sieving or controlled release, such as in drug delivery systems. The clear peak at this low-angle region typically corresponds to the long-range ordering of the mesopores, a characteristic feature of well-synthesized mesoporous materials. This XRD pattern confirms the successful synthesis of mesoporous silica nanoparticles with a uniform internal structure.

![XRD Pattern of Mesoporous Silica Nanoparticles](image)

Figure 2: Powder XRD patterns for mesoporous silica nanoparticles

3.3 Zeta Potential Measurements
Figure 3 illustrates the zeta potential measurements for three types of mesoporous silica nanoparticles: MSN (unmodified), MSN-NH₂ (amino-functionalized), and MSN-COOH (carboxyl-functionalized). The zeta potential, measured in millivolts (mV), indicates the surface charge of the nanoparticles, which is critical for their stability and interaction with biological systems. The unmodified MSN exhibits a zeta potential of approximately -25 mV, indicative of a negatively charged surface due to the presence of silanol groups. The MSN-NH₂ shows a shift to a positive zeta potential of around +10 mV, resulting from the introduction of amino groups that can protonate under physiological conditions, enhancing interaction with negatively charged cell membranes and biomolecules. In contrast, MSN-COOH displays a significantly more negative zeta potential, around -45 mV, due to the introduction of carboxyl groups, which deprotonate in neutral to basic pH conditions. These variations in zeta potential demonstrate the impact of surface functionalization on the electrostatic properties of the nanoparticles, influencing their stability in suspension and potential biological interactions. The negative charge on MSN-COOH could enhance colloidal stability for better uptake of drug molecule by the colorectal cancer cells.
3.4 Drug Release Profile

The pH-responsive cumulative in vitro drug release profile of Doxorubicin hydrochloride from a nanoparticle-based delivery system over a 48 h time period is shown in figure 4. The graph illustrates two distinct release profiles at pH 7.4 and pH 5.5, corresponding to physiological and mildly acidic conditions, respectively. At pH 7.4, which simulates the normal physiological environment, the drug release reaches approximately 32% of the total drug load after 48 h. This relatively lower release rate suggests a controlled release mechanism, minimizing premature drug release in normal tissues, which is advantageous for reducing systemic toxicity.

In contrast, at pH 5.5, representative of the acidic microenvironment often found in tumor tissues, the release of Doxorubicin hydrochloride is significantly higher, reaching about 67% within the same time frame. This enhanced release under acidic conditions indicates a responsive drug delivery system that preferentially releases the drug in the acidic tumor microenvironment, thereby increasing the local concentration of the drug at the target site.

The differential release profiles emphasize the potential effectiveness of this pH-sensitive delivery system in cancer treatment. By maximizing drug release in the tumor environment while limiting release in normal tissues, this system aims to enhance the therapeutic efficacy of Doxorubicin as an anticancer agent. The controlled release at neutral pH helps to mitigate adverse side effects and improve patient tolerance, while the increased release at acidic pH enhances the drug’s cytotoxic effect on cancer cells. This pH-responsive behavior underscores the system’s potential for targeted cancer therapy, offering a promising approach to go for animal studies in future.
4. Conclusion
This study demonstrated the successful synthesis, functionalization, and evaluation of mesoporous silica nanoparticles as a platform for targeted delivery of doxorubicin hydrochloride. The MSNs were synthesized using a modified sol-gel process and functionalized with amino and carboxyl groups, significantly influencing their physicochemical properties, including surface charge, as confirmed by zeta potential measurements. Characterization techniques such as SEM and XRD verified the production of well-defined spherical MSNs with uniform internal structure. The SEM analysis indicated a smooth surface with minimal aggregation, while XRD patterns revealed a distinct peak at around 3° (2θ), indicative of an ordered mesoporous structure critical for controlled drug release. Functionalization resulted in distinct surface charge while MSN-COOH showed a significantly negative zeta potential, enhancing colloidal stability. Drug loading and release studies underscored the efficacy of the pH-responsive delivery system, with DOX-loaded MSNs showing higher release at pH 5.5 compared to pH 7.4. This behavior is advantageous for cancer therapy as it promotes drug release in the acidic tumor microenvironment, enhancing therapeutic efficacy while reducing systemic toxicity. These findings highlight the potential of MSNs as a nanocarrier system for targeted cancer therapy, with surface functionalization allowing for tailored interactions with cellular targets and optimized drug release profiles. The pH-responsive release mechanism provides a viable strategy to enhance the therapeutic index of anticancer drugs like DOX, suggesting promising future cell studies as well as pre-clinical applications. This research contributes to the expanding field of nanoparticle-based drug delivery systems, underscoring their potential to revolutionize cancer treatment through more precise, efficient, and safer therapeutic approaches. Further studies, including in vivo evaluations and clinical trials, are necessary to fully realize clinical potential of the nanocarrier in cancer therapeutics.

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