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Targeted Drug Delivery Using 5-Fluorouracil Loaded Porous Silica Nanoparticles for Enhanced Osteosarcoma Treatment: An In Vitro Study on the MG-63 Cell Line

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Background: Osteosarcoma is a highly aggressive bone cancer, and effective drug delivery systems are crucial for enhancing the therapeutic efficacy of anticancer agents like 5-Fluorouracil (5-FU). Porous silica nanoparticles (PSNs) offer a promising solution for targeted drug delivery due to their high surface area, larger pore sizes, and high biocompatibility.this aids in sustained drug release. Objective: This study aimed to synthesize 5-FU-loaded PSNs and evaluate their potential as an effective drug delivery system for the treatment of osteosarcoma, focusing on their cytotoxic effects on MG-63 osteosarcoma cells. Materials and Methods: 5-FU was incorporated into PSNs using the sol-gel method. Characterization of the synthesized nanoparticles was performed through Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDX), and UV-Visible spectroscopy. The cytotoxic effects of the 5-FU-loaded PSNs were assessed using MTT assays at 24, 48, and 72 hours post-treatment. Results: SEM analysis revealed irregularly shaped agglomerates of PSNs with sizes ranging from 500 nm to 2 µm, confirming successful drug loading. EDX analysis demonstrated the presence of key elements, including fluorine, indicating the incorporation of 5-FU. MTT assay results indicated a time-dependent decrease in cell viability, with the PSN 5-FU group showing significant cytotoxicity (79% viability at 72 hours) compared to the control group (100% viability). Conclusion: The study demonstrates that 5-FU-loaded

PSNs effectively inhibit the proliferation of MG-63 osteosarcoma cells, supporting their potential as a novel drug delivery system for enhancing the therapeutic efficacy of 5-FU in osteosarcoma treatment. Future research should focus on optimizing the formulation and evaluating in vivo efficacy.
Keywords: 5-fluorouracil, chemotherapy, cancer therapy, osteosarcoma, porous silica nanoparticles, targeted drug delivery.

1. Introduction

Osteosarcoma is a type of primary osseous neoplasm that predominantly affects the metaphyseal region of long bones. It accounts for 40-60% of primary tumors of bone (1). Osteosarcoma has a predominance to affect the metaphysis of long bones with increased prevalence in children and adolescents due to skeletal growth (2). Despite advances in treatment, including chemotherapy and surgery, patient outcomes remain suboptimal due to drug resistance, systemic toxicity, and poor drug bioavailability. Standard chemotherapy regimens for osteosarcoma involve high doses of drugs such as methotrexate, doxorubicin, and 5-Fluorouracil (5-FU) (3). However, the therapeutic efficacy of these drugs is limited by their rapid clearance and lack of specificity, leading to harmful side effects (4).

5-FU is a pyrimidine analog, it inhibits thymidylate synthase and ultimately disrupts DNA synthesis causing apoptosis in rapidly proliferating cancer cells (5). As an effective agent in the treatment of colorectal, breast, and head and neck cancers, 5-FU was first synthesized more than a half-century ago; however, its clinical utility has been hindered by poor bioavailability and dose-dependent systemic toxicity when used against osteosarcoma (6). The development of multidrug resistance (MDR) remains a main obstacle in the treatment of cancer. MDR of tumor cells during chemotherapy can be attributed to various factors such as genetic factors, increased reparative capacity of DNA, enhanced effluent of drugs and elevated metabolism of xenobiotics (7).

Previous studies suggest that the nano carriers such as lipid based nanocarriers, mesoporous silica nanoparticles (MSN), silver nanoparticles can be a durable and stable carrier for chemotherapeutic drugs like 5-FU delivery to improve selectively drug-related cytotoxicity for use against various cancers, although those data remain in vitro; therefore further investigation is needed (8). Among these, porous silica nanoparticles (PSNs) stand out due to their large surface area, tunable pore size, and biocompatibility. PSNs can encapsulate a wide range of drugs, including hydrophobic and hydrophilic agents, and offer controlled and sustained release, which could potentially overcome the limitations of traditional chemotherapy (9).

The aim of this study was to investigate the efficacy of 5-FU-loaded PSNs in an in vitro setting using the MG-63 cell line for the treatment of osteosarcoma.

2. Materials and Methods

Synthesis of 5-Fluorouracil-Loaded Silica Nanoparticles

Silica nanoparticles were synthesized using the sol-gel method. A solution of 2 mL of

tetraethoxysilane (TEOS) in 5 mL of ethanol was prepared under continuous stirring for 30 minutes. Hydrolysis was initiated by adding 0.5 mL of HCl (0.1 M) and 2 mL of deionized water dropwise to the TEOS solution, and the mixture was stirred for 2 hours. The reaction mixture was allowed to age at room temperature for 12 hours to promote particle formation. Subsequently, 5-fluorouracil (5-FU) was incorporated by adding it to the aged solution. The solvent was evaporated at room temperature by air drying for 24 hours, yielding 5-FU-loaded silica nanoparticles in powder form, which were collected for further analyses

Characterization of Nanoparticles

1. Scanning Electron Microscopy (SEM):

The morphology and size of the synthesized nanoparticles were characterized using SEM (insert model and manufacturer if known). Images were obtained to assess the size distribution and surface characteristics of the particles.

2. Energy Dispersive X-ray (EDX) Analysis:

EDX analysis was performed to determine the elemental composition and confirm the successful incorporation of 5-FU into the silica nanoparticles.

3. UV-Visible Spectroscopy:

UV-visible spectral analysis was conducted to verify the presence of 5-fluorouracil within the nanoparticles. The absorption spectrum of the 5-FU-loaded nanoparticles was measured using a UV-visible spectrophotometer in the wavelength range of 200–800 nm.

MTT Assay for Cytotoxicity

The cytotoxicity of the 5-FU-loaded silica nanoparticles was assessed using the MTT assay on a human osteosarcoma cell line MG-63. The cells were seeded in 96-well plates and incubated with different concentrations of the nanoparticles for 24 hours. After incubation, MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added, and the formation of formazan crystals was measured by dissolving them in DMSO and reading the absorbance at 570 nm using a microplate reader. The cell viability was calculated as a percentage compared to untreated control cells.

3. Results

Scanning Electron Microscopy (SEM) Image of Silica Nanoparticles Loaded with 5-Fluorouracil (5-FU)

The surface morphology of the synthesized silica nanoparticles loaded with 5-fluorouracil (5-FU) was characterized using scanning electron microscopy (SEM) (Fig. 1). The SEM image reveals irregularly shaped agglomerates of nanoparticles with sizes ranging from 500 nm to 2 µm. The rough, spiky texture of the particles suggests successful drug loading onto the surface or within the pores of the silica matrix. The clustering of particles may be attributed to the interaction between 5-FU and the silica, which can induce aggregation during drying. Such morphology is typical of silica nanoparticles due to their high surface area and porous structure, which are ideal for drug adsorption and delivery.

The observed morphology confirms the successful formation of silica nanoparticles and suggests they are suitable for drug delivery applications, providing adequate surface area for effective drug loading.

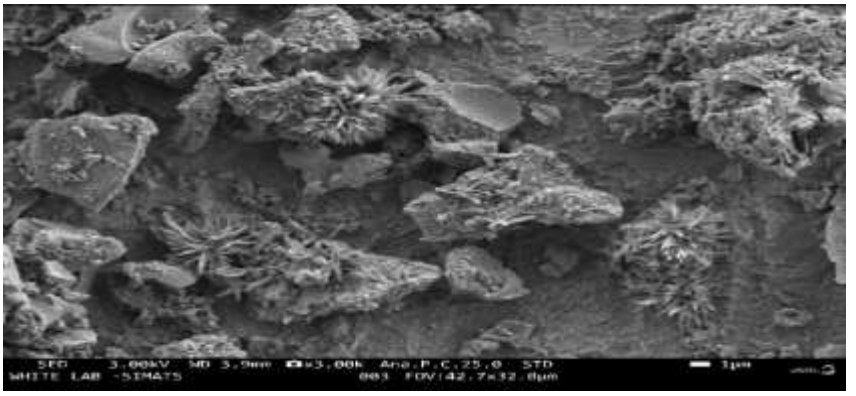


Figure 1: Scanning Electron Microscopy (SEM) Image of Silica Nanoparticles Loaded with 5-Fluorouracil (5-FU)

Energy Dispersive X-ray Spectroscopy (EDX) for Elemental Composition Analysis

To confirm the presence of 5-FU and other components, energy dispersive X-ray spectroscopy (EDX) analysis was performed on the nanoparticles (Fig. 2). The spectrum shows distinct peaks for oxygen (O), silicon (Si), and carbon (C), corresponding to the silica matrix and the organic drug (5-FU). Notably, the presence of fluorine (F), a key component of 5-FU, confirms the successful loading of the drug onto the silica particles. Additional peaks for sodium (Na) and titanium (Ti) were detected, which could indicate impurities or trace elements introduced during the synthesis process.

The quantitative analysis of elemental composition reveals that the sample contains:

- Oxygen (O): 48.2%
- Carbon (C): 19.5%
- Silicon (Si): 14.5%
- Sodium (Na): 7.6%
- Titanium (Ti): 5.8%
- Fluorine (F): 3.9%
- Aluminum (Al): 0.5%

48.2 wt% oxygen and 14.5 wt% silicon, consistent with silica (SiO_2). The carbon (19.5 wt%) and fluorine (3.9 wt%) are attributed to 5-FU. Trace amounts of sodium (7.6 wt%) and titanium (5.8 wt%) were also present. The EDX analysis confirms the elemental composition of the silica nanoparticles and verifies the presence of 5-FU, providing evidence of successful drug incorporation.

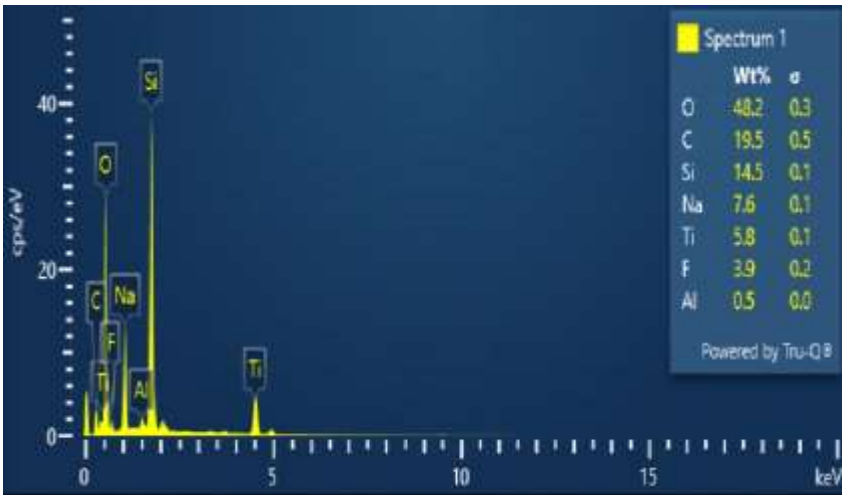


Figure 2: Energy Dispersive X-ray Spectroscopy (EDX) for Elemental Composition Analysis

UV-Visible Absorption Spectra of Silica Nanoparticles with 5-FU

UV-visible spectroscopy was employed to evaluate the optical properties of the synthesized nanoparticles and assess the interaction between 5-FU and the silica matrix (Fig. 3). The black curve represents the absorbance spectrum of pure 5-FU (5F), showing characteristic peaks around 265 nm. The blue curve represents the 5-FU-loaded silica nanoparticles (5F+PS). A slight shift in the absorbance peak and reduction in intensity were observed in the 5F+PS spectrum, suggesting an interaction between 5-FU and the silica matrix, possibly through hydrogen bonding or electrostatic interactions.

The slight blue shift in the absorbance peak of 5-FU after loading onto the silica indicates that the electronic environment of 5-FU has been altered due to its interaction with the nanoparticle matrix. This interaction may affect the release kinetics of the drug, enhancing its controlled release potential.

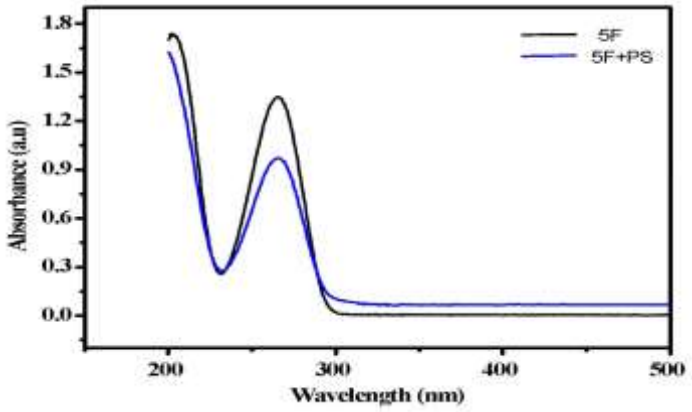


Figure 3: UV-Visible Absorption Spectra of Silica Nanoparticles with 5-FU

Microscopic Image of Cells Before and After Treatment with 5-FU-Loaded Silica Nanoparticles

A phase-contrast microscopic image of the untreated control cells (MG-63 cell line) is shown in Figure 4A. The cells exhibit typical adherent morphology, with elongated spindle-shaped structures and a high degree of confluency. The cells appear to be healthy, with well-defined cell boundaries and a spread-out arrangement on the culture surface. This morphology is characteristic of actively proliferating cells, indicating that the culture conditions were optimal for cell growth before drug treatment. The normal appearance of cells in this image serves as a baseline for comparison with treated cells, demonstrating that the cells were in a healthy and proliferative state prior to the addition of the drug.

Following treatment with 5-FU-loaded silica nanoparticles, significant changes in cellular morphology were observed (Figure 4B) in the cell line. The cells exhibit rounding, shrinkage, and detachment from the substrate, all of which are hallmarks of apoptosis or drug-induced cytotoxicity. Fewer cells are visible, and many appear to be clumped or undergoing cell death, likely due to the cytotoxic effects of 5-FU. This is consistent with the known mechanism of 5-FU, which induces cell cycle arrest and apoptosis in cancer cells.

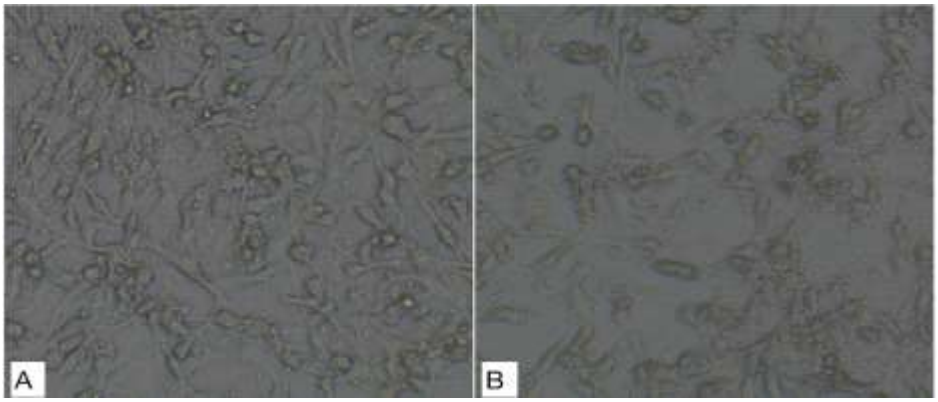
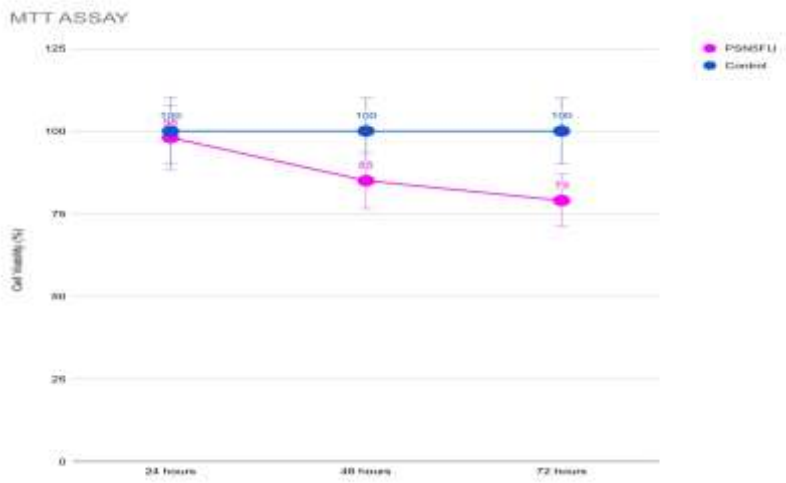


Figure 4A&4B: Microscopic Image of Cells Before and After Treatment with 5-FU-Loaded Silica Nanoparticles

The observed changes in cell morphology after treatment indicate that the 5-FU-loaded silica nanoparticles are effective in inhibiting cell proliferation and inducing cell death, supporting their potential use in cancer therapy. The comparison with the untreated control confirms the specificity and effectiveness of the drug delivery system.

MTT Assay for Cell Viability Analysis

The cytotoxic effect of 5-FU loaded porous silica nanoparticles on the MG63 cell line was assessed using the MTT assay at different time points: 24, 48, and 72 hours. The cell viability percentages of both the control and the PSN5FU-treated groups are presented in Graph 1.



Graph 1: MTT Assay of 5-FU loaded PSN

At 24 hours, the cell viability for the control group was 100%, while the PSN5FU-treated group showed a slight reduction to 98%. By 48 hours, the cell viability for the PSN5FU group decreased further to 85%, compared to the control group, which maintained 100% viability. After 72 hours, the PSN5FU group exhibited a significant decline in cell viability to 79%, while the control group continued to maintain 100% viability.

The results indicate a time-dependent decrease in cell viability in the PSN5FU-treated group, suggesting that the 5-FU-loaded PSNs have a sustained cytotoxic effect on the cancer cells over time. In contrast, the control group remained unaffected across all time points, confirming that the observed cytotoxicity is specifically due to the PSN5FU treatment. The statistically significant reduction in cell viability at 48 and 72 hours ($p < 0.05$) implies the potential of PSN5FU as an effective drug delivery system for prolonged anticancer activity.

4. Discussion:

The study aimed to investigate the efficacy of 5-FU loaded porous silica nanoparticles for treating osteosarcoma, utilizing the MG-63 osteosarcoma cell line. Techniques including SEM, UV-spectroscopy, and MTT assay were employed to analyze the physicochemical properties, drug loading efficiency, and cytotoxicity of the 5-FU-loaded PSNs. The results showed significant improvements in cytotoxic effects compared to the blank control group.

SEM analysis of the PSNs confirmed their porous architecture, which is a crucial factor for drug loading and controlled release. The nanoscale morphology, along with a large surface area, ensured high drug-loading efficiency. In comparison to the study by Tavkoli et al, where MSN were smaller than the particles in our study and measured 196.7 nm. This morphological characteristic was conducive to drug delivery, our results align well with existing literature that highlights the importance of surface area in improving drug-loading capacities. Edalatian et al, developed a nanocarrier system in which the nanoparticles were loaded with doxorubicin (DOX). Our study used PSN as they have a flexible and adaptable

pore size, since 5-FU is a small molecule drug, the larger and more diverse pore size distribution of PSNs can still provide efficient drug loading and release without requiring the uniform pore structure characteristic of MSNs, additionally PSN are more readily available than MSN. (10).

The surface morphology of the synthesized 5-FU-loaded PSNs was characterized using SEM, revealing nanoparticle agglomerates with rough and spiky textures. Kim et al, synthesized silica nanoparticles for doxorubicin delivery and observed rough surface textures attributed to drug adsorption on the nanoparticle surface. The size range observed ranged from 95 nm to 450nm (11). However, variations in particle size are expected due to differences in synthesis parameters and drug incorporation methods. The clustering and aggregation observed in our study might be attributed to electrostatic interactions between 5-FU and the silica matrix.

The UV-visible absorption spectra of the 5-FU-loaded PSNs showed a slight shift in the absorbance peak of 5-FU compared to its free form. This shift suggests an interaction between 5-FU and the silica matrix, potentially through hydrogen bonding or electrostatic forces. A similar shift in absorbance peaks was reported by Q. Fu et al, who studied the interaction of paclitaxel with MSNs (12). The substantial pore volume of silica nanoparticles allows for the encapsulation of a significant quantity of anticancer drugs, while the supramolecular structures function as a cap, facilitating both drug capture and release (13). With enhanced pharmacokinetics, improved therapeutic efficacy, and excellent stability, SNPs are regarded as one of the most effective carriers for drug delivery (14).

The observed changes in cell morphology after treatment with 5-FU-loaded PSNs, including rounding, shrinkage, and detachment, are indicative of apoptosis. This is supported by existing literature, where similar apoptotic features were observed in breast cancer cells treated with silica nanoparticles (15). The induction of apoptosis is a key mechanism of 5-FU, as it disrupts DNA synthesis and causes cell cycle arrest. In our present study, continued cytotoxic effects of 5-FU were noted at different time periods, hence it can be concluded that 5-FU maintains its mechanism of action even when loaded onto the PSNs, providing evidence that the drug retains its activity and therapeutic potential within the nanoparticle matrix. The enhanced cytotoxicity can be attributed to the controlled release of 5-FU from the PSNs, which resulted in prolonged drug exposure to cancer cells. This finding is in line with O. Udofot et al, where in 5-FU loaded with liposomal nanoparticles demonstrated improved cytotoxicity by ensuring sustained drug release and enhanced cellular uptake (16). Cheng et al, developed a nanocarrier system in which MSNs were loaded with DOX. In vivo studies analysis that the MSNs-DOX-PDA-TPGS formulation effectively overcomes multidrug resistance, outperforming both free DOX and DOX-loaded nanoparticles that lacked TPGS ligand modifications and can be used to deliver both hydrophilic and hydrophobic drugs (17).

Free 5-FU, although widely used in cancer treatment, has several limitations, including rapid systemic clearance, off-target toxicity, and poor bioavailability. The encapsulation of 5-FU in PSNs addresses many of these issues by enhancing the drug's pharmacokinetic profile, reducing toxicity, and providing targeted delivery to cancer cells (18). Previously numerous studies have used nanoparticles for oral cancer (19,20), as well as various diseases and

conditions (21-24).

The results of this study suggest that 5-FU-loaded PSNs have the potential to improve the treatment of osteosarcoma by overcoming the limitations of conventional chemotherapy.

The high surface area and porous structure of the nanoparticles enable high drug loading and controlled release, which can enhance the therapeutic index while minimizing systemic toxicity. However, challenges such as large-scale production, long-term stability, and the potential for immune responses must be addressed before clinical translation. Preclinical studies in animal models are needed to further investigate the biodistribution, metabolism, and excretion of PSNs, as well as their long-term safety. Future studies should focus on optimizing the surface functionalization of PSNs to achieve targeted delivery to osteosarcoma cells, potentially through the use of specific ligands or antibodies.

5. Conclusion

The results of this study demonstrate that 5-FU-loaded porous silica nanoparticles effectively inhibit the proliferation of MG-63 osteosarcoma cells in vitro. The successful incorporation of 5-FU was confirmed through SEM, EDX, and UV-visible spectroscopy analyses, while the MTT assay revealed a time-dependent cytotoxic effect. The findings align with existing literature on nanoparticle-based drug delivery systems, further supporting the potential of PSNs as promising carriers for 5-FU in osteosarcoma and other cancer therapies. Future research should aim to optimize the formulation for targeted delivery and evaluate its in vivo efficacy to establish its therapeutic potential in clinical settings.

STATEMENTS:

CONFLICT OF INTEREST: None declared

FUNDING STATEMENT: None to declare

DATA AVAILABILITY STATEMENT: The raw data can be obtained from the first author upon request.

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