Enzyme-Catalyzed Synthesis of Selenium-Doped Manganese Phosphate for Synergistic Therapy of Drug-Resisted Colorectal Tumor

Manman pei
Anhui University of Science and Technology

Kaiyuan Liu
Shanghai Tenth People's Hospital

Xiao Qu
Shanghai Tenth People's Hospital

Kairuo Wang
Shanghai Tenth People's Hospital

Qian Chen
Shanghai Tenth People's Hospital

Yuanyuan Zhang
Shanghai Tenth People's Hospital

Xinyue Wang
Shanghai Tenth People's Hospital

Zheng Wang
Anhui University of Science and Technology

Xinyao Li
Shanghai Tenth People's Hospital

Feng Chen
Shanghai Tenth People's Hospital

Huanlong Qin
Shanghai Tenth People's Hospital

Yang Zhang (✉ zhangyang0202@tongji.edu.cn)
Taizhou Central Hospital

Research Article

Keywords: Colorectal tumor, Multidrug resistance, Chemodynamic therapy, Enzyme-Catalyzed, Synergistic Therapy

Posted Date: August 18th, 2022
Abstract

Postoperative chemotherapy for colorectal cancer often causes multidrug resistance (MDR), which seriously affects the therapeutic effect and has been an urgent problem to be solved. Herein, selenium-doped manganese phosphate (Se-MnP) nanoparticles with amorphous structure have been prepared by a bioinspired enzyme-catalyzed strategy, using alkaline phosphatase, fructose disodium diphosphate. Se-MnP have an organic-inorganic hybrid composition, which is assembled from smaller-scale nanoclusters. Se-MnP has showed good Fenton reaction activity in chemodynamic therapy (CDT) due to the presence of manganese ions. Moreover, results from in vitro and in vivo studies demonstrated that Se-MnP as an effective drug carrier of oxaliplatin (OX) can reverse multidrug resistance of colorectal cancer cells and simultaneously induce casparase-mediated apoptosis of colorectal cancer cells. The Se-MnP reverse the MDR of colorectal cancer by down-regulating the expression of MDR-related ABC (ATP binding cassette) transporters proteins (ABCB1 and ABCC1). Finally, the in vivo studies demonstrated that OX-loaded Se-MnP can significantly inhibit OX-resistant HCT116 (HCT116/DR) tumor growth in nude mice. Considering the facile method of preparation and biomimetic chemical properties, the Se-MnP with the multiple functions will be a promising candidate for treating colorectal tumors with MDR characteristics.

1. Introduction

Colorectal cancer (CRC) is one of the most common and lethal malignancies of the digestive system [1]. According to data released by the World Health Organization's International Agency for Research on Cancer (WHO IARC) in 2020, the number of new colorectal cancer cases ranked third in the world, while the death rate ranked second in the world [2, 3]. The changed diet, reduced activity, heavy alcohol consumption, and smoking are thought to be the main reasons for the raised colorectal cancer rates [2]. Currently, adjuvant chemotherapy is one of the most commonly used treatments for non-metastatic colorectal cancer, and a combination of 5-fluorouracil, oxaliplatin, and leucovorin has been used as the standard chemotherapy strategy [4–6]. Among them, oxaliplatin-based chemotherapy plays an important role in the treatment of colorectal cancer [7–9]. However, the generation of drug resistance has become one of the principal reasons for the failure of chemotherapy [10, 11]. Various drug resistance problems in colorectal cancer seriously impeded the clinical chemotherapy effect and reduced the life expectancy of patients [10, 12].

Multi-drug resistance (MDR), which is the phenomenon of showing resistance to various anticancer drugs, is one of the major barriers in chemotherapy [13]. The research on biological mechanisms and treatments of tumor's MDR is a hot topic and frontier of tumor pharmacology, which is a major problem that restricts the radical cure of tumors and leads to the death of patients. Many therapeutic strategies have been explored to address the critical issues of MDR [14–17]. Unsurprisingly, the sequential therapy and combination therapy of several chemotherapeutic agents with non-overlapping mechanisms of action are considered effective solutions for MDR[10, 18–21]. But the two strategies have largely plateaued within the last decades, and cannot solve the problem completely [22, 23]. Indeed, some new therapeutic strategies of immunological methods developed by targeting key tumor features (e.g., anti-
CTLA4 and anti-PD-1/ PD-L1) also ultimately induce tumor MDR, comparing with conventional chemotherapy [24, 25].

Up to now, a variety of mechanisms have been reported to attribute to multidrug resistance, such as enhanced drug efflux, increased DNA damage repair, reduced apoptosis, elevated autophagy, and/or altered drug metabolism [26–28]. Among them, the overexpression of ATP-binding cassette (ABC) transporter [29, 30], which function as active drug efflux pumps, is the primary mechanism of drug resistance, leading to decreased intracellular drug accumulation [31, 32]. Although several inhibitors of ABC transporter had been investigated, their clinical usage was still limited, due to the uncertain active mechanism and the potential interactions with other chemo-drugs [33–35]. Therefore, it is urgent to develop an effective drug delivery system to increase the therapeutic uptake of drugs at the tumor site and potentially reverse the tumor MDR [36].

Nanostructured drug delivery systems (NDDSs) have controllable chemical properties, size, rapid clearance, and the potential in sustained or controlled drug release, which hold a promising way to reverse the MDR of colorectal cancer cells [37]. Comparing with traditional drug delivery system, NDDSs have many advantages, including EPR effect, biodegradable, biocompatibility, PH sensitivity, and so on. Many NDDSs manufactured using the components similar with the target tissue of body is ideal for solving the problem of potential hazards [38]. The payload and effective endocytosis of the NDDSs could be controlled by adjusting the constituent and structure of NDDSs, which may be favorable to avoid premature leakage of drug and to target drug delivery.

In this study, we synthesized selenium-doped manganese phosphate (Se-MnP) nanoparticles with an amorphous structure by an alkaline phosphatase-catalyzed biomimetic strategy, which were further used as nanocarriers for OX to treat drug-resistant colorectal cancer. The oxaliplatin-resistant colorectal cancer cell line HCT116/DR and its parent strain HCT116 have been used to investigate the reversal effect of the OX@Se-MnP. The Se released from OX@Se-MnP after being endocytosed by tumor cells was proved to down-regulate ABC transporter which cam pump the oxaliplatin molecules from intracellular to extracellular spaces. Simultaneously, Se and Mn$^{2+}$ could produce excessive ROS through both STAT3/JNK pathway and Fenton-like reaction, inducing mitochondrial damage. Both the downregulation of ABC protein and mitochondrial damage lead to effectively reversing drug resistance, providing a synergistic therapy of drug-resisted colorectal tumor (Scheme 1).

2. Materials And Methods

2.1 Preparation of Se-MnP and MnP

The nanoparticles of Se-MnP were synthesized by an enzymatic reaction, using an organic phosphorylated molecules of Fructose disodium diphosphate ($C_{6}H_{12}Na_{2}O_{12}P_{2}$, Na$_2$FDP) (Sigma-Aldrich Co., US) to instead traditional phosphate salts. In a typical process in synthesis of Se-MnP, 0.66 g of Na$_2$FDP was dissolved in 40 mL of deionized water, 0.02 g of Na$_2$SeO$_3$ (Sigma-Aldrich Co., US) was
dissolved in 40 mL of deionized water, separately. Then, the above solutions were mixed and stirred for 5 minutes at 37°C within a water bath. Thereafter, 1 mL of ALP aqueous solution (20 U/mL) was added to the mixed solution and magnetically stirred. Then, 0.19 g of MnCl$_2$·4H$_2$O (Aladdin Biochemical Technology Co., China) dissolved in 20 mL of water was dropwise added into the above solution. During the whole reaction process, the pH value of the reaction solution was kept between 8.0 and 8.5 by continually adding a dilute NaOH aqueous solution (0.5 M). Finally, the as-prepared samples were collected by centrifugation and washed with ethanol twice, deionized water twice, and freeze-dried into powders for further use. The MnP without Se element was used as a control sample. The synthesis procedure of the MnP sample is as follows: 0.66 g of Na$_2$FDP was dissolved in 80 mL of deionized water and then mixed with 1 mL of ALP (20 U/mL) aqueous solution. The rest of process is same to the preparation of Se-MnP sample.

2.2 Drug loading

The typical drug loading experiment was performed as follows: the dried powder (10 mg) of the as-prepared Se-MnP was dispersed in 5 mL solution with 10 mg oxaliplatin (OX). The suspension was treated in an ultrasonic bath for 1 h, and then shaken in a sealed vessel at 37 oC for 24 h, followed by centrifugation and drying to obtain the Se-MnP drug delivery system.

2.3 Characterization

X-ray powder diffraction (XRD) patterns were recorded using an X-ray diffractometer (Rigaku D/max 2550V, Cu Kα radiation, λ = 1.54178 Å). Fourier transforms infrared (FTIR) spectra were obtained with an FTIR spectrometer (FTIR-7600, Lambda Scientific, Australia). Scanning electron microscopy (SEM) was performed with a scanning electron microscope (Hitachi S4800, Japan), while Transmission electron microscopy (TEM) was carried out with a field-emission transmission electron microscope (FEI Talos F200X G2, USA). Thermogravimetric (TG) analysis was carried out with a simultaneous thermal analyzer (STA-409/PC, Netzsch, Germany) at a heating rate of 10 °C min$^{-1}$ under flowing air.

2.4 ·OH generation by Mn$^{2+}$mediated Fentonlike reaction

Methylene blue (MB, 10 µg mL$^{-1}$), H$_2$O$_2$ (10 mM), and Se-MnP (400 µg/mL) was reacted at 37°C for 30 min. The UV absorption spectrum of the sample was measured with a UV spectrophotometer (UV-2600, SHIMADZU, Japan) to verify that the ·OH generated by the Fenton-like reaction induced MB degradation.

2.5 Statistical Analysis

One-way analysis of variance was carried out using SPSS 21.0 with Tukey’s multiple comparison tests to determine the significant difference among three or more groups. The level of significance was set as p < 0.05, p < 0.01, and p < 0.001.

3. Results And Discussion
3.1 Synthesis and characterization

Scheme 1 (A) illustrates the preparation process and formation mechanism of Se-MnP. Se-MnP were synthesized through an enzymatic reaction-inspired strategy, using a phosphorus source of Fructose disodium diphosphate (C$_6$H$_{12}$Na$_2$O$_{12}$P$_2$, Na$_2$FDP) to instead the traditional phosphate salts. The biomolecule of FDP can be gradually hydrolyzed into F6P, Fructose, and dissociative PO$_{4}^{3-}$ ions in an aqueous solution under an enzymatic reaction generated by ALP enzyme. Meanwhile, the free Mn$^{2+}$ ions can react with the hydrolyzed PO$_{4}^{3-}$ and SeO$_3^{2-}$ ions to form a precipitate of Se-MnP clusters. In this process, the FDP and its dephosphorylated products integrate with the primary Se-MnP clusters to prevent the crystallization of clusters, and Se-MnP nanoparticles are obtained by an assembly or aggregate effect of these clusters.

In the current study, a Se-MnP NDDS were synthesized with a green, simple, and efficient enzyme-based catalytic method, using Na$_2$FDP and Na$_2$SeO$_3$ as anion sources. It should be mentioned that FDP biomolecules and their dephosphorylated products are native matters that exist in the human body, which means they have excellent biocompatibility. After reaction, FDP biomolecules are integrated into the MnP and Se-MnP to control their structure and crystal phase. The micrographs of Se-MnP from scanning electron microscope (SEM) and transmission electron microscopy (TEM) are displayed in Fig. 1A, which demonstrate a nanoparticle structure with diameters of approximately 50 ~ 100 nm. The MnP without Se element was used as a control sample (Fig. S1). Then, we studied the chemical and crystal properties of Se-MnP by Fourier transform infrared (FTIR) spectra and X-ray diffraction (XRD). The existence of FDP biomolecules and their derivatives is revealed by the FTIR spectra. The absorption peaks at 1078 and 549 cm$^{-1}$ can be attributed to the asymmetric stretching vibration of the PO$_{4}^{3-}$ groups.[39] The peaks at 1209, 1579 and 3336 cm$^{-1}$ of OX also are appearing in the spectra of OX@Se-MnP, which indicate successful OX loading; the results give clear evidence that the asprepared Se-MnP are inorganic-organic complex. (Fig. 1B). The elemental mapping of Mn, P, O, Se and C elements confirms the successful synthesis of Se-MnP with good dispersity of Se element (Fig. 1C).[40] In XRD patterns (Fig. 1D) of Se-MnP and OX@Se-MnP samples, there was a broad hump at the 2$\theta$ value of approximately 30°, while no more diffraction peak appeared, indicating an amorphous crystal structure of samples. The valence state of OX@Se-MnP was analyzed by X-ray photoelectron spectroscopy (XPS), which clearly shows the signals of manganese (Mn), selenium (Se), and platinum (Pt) (Fig. 1E). The binding energy of Se 3d at 51.2 eV is corresponding to metallic selenium (Se$^0$) (Fig. 1F) [40], and the Pt 4f peak at 72.6 eV is attributed to PtO (Fig. 1G), further confirming the existence of OX molecules in OX@Se-MnP. As shown in Fig. 1H, the zeta potential changes from −11.3 mV to −11.8 mV after loading with OX, owing to its negative potential (−7.48 mV). The dynamic light scattering (DLS) measurement indicates the obtained Se-MnP of around 92 nm (Fig. 1I) in consistent with the observation of TEM images.

Moreover, the result of thermogravimetric (TG) analysis of Se-MnP and OX@Se-MnP are given in Fig. S2. After loading OX molecules, the weight loss of OX@Se-MnP is obviously higher than Se-MnP. The 2.9% of weight loss in the TG curve of OX@Se-MnP is mainly resulted from the oxygenolysis of OX molecules.
The stability of Se-MnP in aqueous solution with different pH values of 7.4, 6.5 and 5.0 for 24 h was studied (Fig. S3), which clearly indicate the good stability of samples in solution with high pH value and obvious degradability in acid solution with low pH values. In addition, the UV absorption spectrum of the sample was measured with a UV spectrophotometer to verify that the Fenton-like reaction of Se-MnP sample (Fig. 1J). The decreased absorption peaks at wavelength of 650–700 nm of Se-MnP group with H$_2$O$_2$ clearly indicate the occurrence of Fenton-like reaction which induces the formation of ·OH and degradation of MB molecules.

### 3.2 Cytotoxicity and cytophagocytosis experiments of OX@Se-MnP

The cytotoxicity of OX and Se-MnP with respect to human colorectal cancer HCT116 cells and Oxaliplatin-drug-resistant HCT116 (HCT116/DR) were evaluated using a CCK-8 assay (Fig.S4, S5). In the 48-hour toxicity test, almost 90% of the HCT116 cells could be killed when the OX concentration was greater than 15 µg/mL, while more than half of the drug-resistant HCT116/DR cells were still alive. The IC$_{50}$ of HCT116/DR is 4.9 times larger than that of HCT116 (Fig.S5, Table S1). At the same concentration, Se-MnP is more toxic to drug-resistant strains; the IC$_{50}$ (HCT116/DR) is 0.64 of IC$_{50}$ (HCT116) (Fig.S3, Table S1). The IC$_{50}$ (half maximal inhibitory concentration) values were calculated and are summarized in Table S1.

As a bifunctional alkylating agent, OX could interfere with DNA replication and ultimately induce apoptosis of tumor cells [41–43]. Despite oxaliplatin-based chemotherapy being successfully applied for colorectal cancer therapy, OX could lead to serious side effects, such as neurotoxicity due to non-specific uptake [44–46]. Moreover, it is less effective for tumor cells with MDR. The as-prepared Se-MnP is suitable for drug-loading due to the small size and stable amorphous phase. Therefore, we further investigated the properties of Se-MnP as a nanocarrier for OX to compensate for these deficiencies. The drug loading (DL%) calculated according to the formula is 12%, and the encapsulation efficiency (EE%) is 22%.

\[
DL\% = \frac{\text{Weight of loaded drug}}{\text{Weight of nanoparticle and drug}} \times 100 \\
EE\% = \frac{\text{Weight of loaded drug}}{\text{Weight of feeding drug}} \times 100
\]

Throughout the separate co-culture of colorectal cancer cells with free OX, free Se-MnP, and OX@Se-MnP for 48 h, it was clear that OX@Se-MnP showed superior cytotoxicity to HCT116/DR compared to free OX or Se-MnP (Fig. 2A). Free OX (20 µg/mL) or Se-MnP (100 µg/mL) alone could reduce the cell viability of HCT116/DR cells to approximately 63% and 42%, respectively. In contrast, tumor cells were eliminated by nearly 90% in the OX@Se-MnP group, indicating a synergistic anti-tumor effect of OX and Se-MnP.

As shown in Fig. 2B, the cytotoxicity of OX@Se-MnP was in proportion to its concentration. After 24 h and 48 h co-culture with HCT116/DR cells, OX@Se-MnP showed a significant cell killing effect when concentration was over 50 µg/mL. As shown in Fig. 2C, OX@Se-MnP led to more significant cell damage than free OX alone under equivalent concentrations, indicating its potential for reversing drug resistance. Figure 2D illustrates that PBS (Control) and OX showed little cytotoxicity to drug-resistant cells, Se-MnP and OX@Se-MnP promoted more apoptosis of colorectal cancer resistant HCT116/DR cells, consistent
with CCK-8 assay results. The results of the confocal laser scanning microscope (CLSM) show the intracellular uptake of the FITC-OX@Se-MnP since the green fluorescence of FITC-labeled NPs could be allowed for the tracking of the NPs inside the cells (Fig. 2E), confirming that OX@Se-MnP can be uptaken by HCT116/DR cells at an early stage (2 hours) which is beneficial for accumulating in the tumor tissue.

### 3.3 Mechanism of reversing MDR of HCT116/DR cells by Se-MnP

The results of the CCK-8 assay were also examined to ensure the drug resistance reversal effect of Se-MnP by using HCT116 and HCT116/DR cells. Firstly, the IC50 of OX for HCT116/DR cells was calculated to be 42.34 µg/mL, while that for HCT116/DR cells was only 8.63 µg/mL (Fig. S4 and Table S1). Furthermore, the cell apoptosis was validated by the flow cytometry using Annexin-V-FITC and a PI containing assay (Fig. 3B,C). It was apparent that Se-MnP significantly enhanced the cytotoxicity of OX for CRC.

It is known that reactive oxygen species could mediate damages to mitochondria, thereby inducing apoptosis [47–49]. Therefore, intracellular ROS overproduction-induced apoptosis was further explored by the ROS fluorescence probe 2',7'-dichlorofluorescein diacetate (DCFH-A) since it could be effortlessly oxidized by ROS to emit green fluorescence. According to the ROS Assay Kit, the results showed that OX exhibited little or no obvious detectable DCFH. However, the fluorescence intensity of DCFH was significantly enhanced in the HCT116/DR cells after being cultured with Se-MnP and OX@Se-MnP (Fig. 3D). In agreement with the CCK8 assay and the flow cytometry, these results indicated that the ROS overproduction induced by OX@Se-MnP has a significantly suppressive effect on cancer cells. Se and its compounds have been clinically applied for cancer prevention and treatment due to the trigger of a lethal generation of ROS in many kinds of cancers, such as malignant mesothelioma [50], prostate cancer [51], and liver cancer [52]. Se was reported to induce oxidative stress in tumor tissues and exert antitumor activity, including the inhibition of tumor growth, VEGF expression, and metastasis; increases the immune response; and decrease antitumor drug resistance [53]. In addition, Se was proved to activated STAT3/JNK pathway, which finally increased the ROS level [54]. The ROS assay in Fig. 3D suggests that ROS generation plays a vital role in the process of selenium-induced cell apoptosis. A possible molecular mechanism involved is Caspase-8/Caspase-3-dependent apoptosis via ROS-triggered way [55].

Mitochondrial damage may cause the depolarization of the mitochondria which led to the decrease of the membrane potential. The mitochondrial membrane potential (ΔΨm) was further investigated by JC-1 fluorescence probes, which could shift from red to green fluorescence during the loss of ΔΨm. As shown in Fig. 3F, OX@Se-MnP dramatically induces the loss of ΔΨm, indicating that OX@Se-MnP could probably induce the mitochondrial dysfunction, which activates ROS overproduction for cell apoptosis [40, 56].

We further evaluated the effect of Se-MnP on inhibiting ABC transporters, which play an important role in the MDR of cancer cells. According to studies, ABC transporters (ABCB1, ABCC1) are highly expressed in drug-resistant strains compared to parental strains [26, 30, 57, 58]. Represented by the ABCB1 protein and
ABCC1 protein in the ABC family of proteins, the expression level of ABCB1 and ABC1 in HCT116/DR cells was significantly inhibited when Se-MnP and OX@Se-MnP were involved. (Fig. 3E).

### 3.4 In vivo antitumor therapy

Finally, we investigated the in vivo antitumor efficacy and systemic toxicity of OX@Se-MnP using BALB/c nude mice with an MDR-colorectal tumor model subcutaneously (Fig. 4A). PBS and OX (20 µg/mL) were injected intratumorally as controls, while the treatment group was injected with Se-MnP (100 µg/mL) and OX@Se-MnP (120 µg/mL) every three days for the in vivo evaluation of antitumor therapy. At the end of treatment (Day 14), OX@Se-MnP tremendously reduced the tumor volume from 660 ± 140 to 120 ± 22 mm³ and tumor weight from 0.77 ± 0.27 to 0.15 ± 0.08 g, compared to the control group (Fig. 4B-D). On the sixth day after treatment, OX@Se-MnP significantly improved inhibition of tumor growth over the other groups, and the inhibition effect gradually became more and more remarkable with increasing treatment time (Fig. 4D). Furthermore, OX@Se-MnP showed a significant effect in inhibiting tumor growth compared to OX or Se-MnP. All mice survived at the end of experiment, and mice treated with Se-MnP or OX@Se-MnP showed no differences compared to the control group regarding to the body weight, indicating good biocompatibility of these samples (Fig. 4E).

Further, the heart, liver, spleen, lung, and kidneys were sectioned for Hematoxylin and eosin (H&E) staining to evaluate the toxicity of the nanoparticles. The results from the H&E staining assay revealed normal tissue morphology in treated groups indicating the absence of observable organ injuries (Fig. 4F). Throughout the separate coculturing of colorectal cancer drug-resistant cells with free OX, Se-MnP, and OX@Se-MnP, it was clear to see that OX@Se-MnP showed superior cytotoxicity compared to free OX or Se-MnP. The anticancer capacity of Se-MnP and the synergistic effect of Se and OX in OX@Se-MnP were further confirmed in the in-vivo experiment, where the tumor volume of the group treated by OX@Se-MnP was significantly smaller than that of the group treated with Se-MnP or OX alone.

Hematoxylin–eosin (HE) staining and immunohistochemical staining of the Ki67 assay, caspase-3, ABCB1, ABCC1, and ABCG2 were performed for the pathological analysis of tumor samples (Fig. 5). As shown in Fig. 5A, HCT116/DR tumor cells grew well in the control group with large and oval-shaped nuclei, and OX intratumor injection showed little effect on tumor growth. In contrast, tumors treated with Se-MnP and OX@Se-MnP exhibited the typical appearance of apoptotic tumor tissue with condensed nucleus chromatin. The expression level of Ki67 and caspase-3 were further analyzed in tumor samples. Ki67 is a well-known cellular marker for proliferation, whereas caspase-3 is commonly regarded as the central regulator of apoptosis [59, 60]. In the group of Se-MnP and OX@Se-MnP, the expression of Ki67 remarkably decreased while the expression of caspase-3 significantly increased compared to the control group. These results demonstrated that Se-MnP and OX@Se-MnP inhibited the proliferation of tumor cells via reducing the expression of Ki67, as well as promoted the apoptosis of tumor cells via activating the caspase-3 signaling pathway.

The expression of ABCB1, ABCC1, and ABCG2 in HCT116/DR tissues of mice treated with free OX, Se-MnP, and OX@Se-MnP was also studied. As shown in Fig. 5B, the expression levels of ABCB1, ABCC1,
and ABCG2 were high in the control groups and the groups treated with free OX via local injection. However, in the groups treated with Se-MnP and OX@Se-MnP, the expression of ABCB1, ABCC1 and ABCG2 were obviously reduced, showing prominent potential of Se-MnP to reverse the drug-resistance of tumors by downregulating the expression of ABC transporter.

ROS triggered the caspases-mediated apoptosis pathway, which can be verified in Fig. 3 and Fig. 5. Furthermore, systematic in vitro and in vivo results have demonstrated that Se-MnP could be selectively uptaken by colorectal cancer cells and continue to generate ·OH by the Fenton-like catalytic reaction, which subsequently triggers the caspases-mediated tumor apoptosis. Thus, Se-MnP induced cell apoptosis synchronously collaborated with oxaliplatin to cause cell death in the CCK8 assay, induce morphological changes, and restrain in-vivo tumor growth. These results indicate that OX@Se-MnP holds great potential as an advanced NDDS system for achieving efficient cancer treatment.

4. Conclusions

In summary, a series of Se-MnP based drug-carriers with an organic-inorganic hybrid constituent and amorphous structure were successfully produced, which was further used to overcome the MDR of tumor cells and enhance colorectal cancer therapy. In the in vitro experiments, Se-MnP showed a remarkable antitumor capacity, which could significantly generate ROS and synchronously induce the apoptosis of colorectal cancer cells. More importantly, Se-MnP could downregulate the expression of MDR-related ABC family proteins (ABCB1 and ABCC1) to reverse the MDR of HCT116/ DR tumor cells. Then, Se-MnP was used as an effective drug carrier for OX, enhancing the uptake of OX and synergistically inducing DNA damage and apoptosis in HCT116/ DR cells. Finally, the in vivo studies confirmed that Se-MnP and OX@Se-MnP could tremendously reverse MDR of tumor cells and induced tumor apoptosis to suppress tumor growth. The Se-MnP based drug carrier could be a promising drug carrier for colorectal tumor therapy by synchronously inducing apoptosis and reversing the MDR of tumor cells.

Declarations

Funding

This work was supported by the National Natural Science Foundation of China (82072051), Science and Technology Commission of Shanghai (20JC1411702 and 20ZR1456100), and Basic Study on Public Projects of Zhejiang Province (LGF20H060017).

Competing interests:

The authors declare no competing interests.

Author Contributions Statement

Yang Zhang, Huanlong Qin and Feng Chen: Conceptualization and Methodology. Manman Pei, Kaiyuan Liu and Xiao Qu: Data Collection and Analysis, Writing-Original draft preparation.
References


Scheme

Scheme 1 is available in the Supplementary Files section.

Figures
Synthesis and characterization of Se-MnP. (A) Scanning electron microscope (SEM, a) and Transmission electron microscopy (TEM, b) of Se-MnP. (B) FTIR spectra of Na$_2$SeO$_3$, MnP control sample, and Se-MnP microspheres before and after drug loading. (C) Elemental mapping and corresponding merged images of Se-MnP revealing the uniform distribution of Mn, P, O, Se, and C elements in the matrix. (D) XRD patterns of Na$_2$SeO$_3$, MnP, Se-MnP, OX@Se-MnP. (E) The survey XPS spectrum of OX@Se-MnP. (F, G) XPS
spectrum of Se 3d (F) and Pt 4f (G) in the OX@Se-MnP. (H) Zeta potentials of MnP, Se-MnP, OX, OX, OX@Se-MnP. (I) Hydrodynamic diameter distribution of MnP, Se-MnP, OX@Se-MnP. (J) UV-visible absorption spectra and photo (inset) of MB degradation. Scale bar: (C)10 μm

Figure 2

Cytotoxicity assessments of the OX@Se-MnP and uptake of FITC-TPOM by HCT116/DR cells. (A) Relative cell viabilities of HCT116/DR cells co-incubation with OX (20 μg/mL), Se-MnP (100 μg/mL), OX@Se-MnP (120 μg/mL) for 48 h. (B) Relative cell viabilities of HCT116/DR cells after 48 h co-incubation with OX@Se-MnP at varied concentrations. (C) Relative cell viabilities of HCT116/DR cells co-incubation with OX and OX@Se-MnP at varied concentrations after 24 h. (D) The images of CAM and PI stained HCT116/DR cells treated with PBS, OX (20 μg/mL), Se-MnP (100 μg/mL), OX@Se-MnP (120 μg/mL) for 48 h. (E) OX@Se-MnP were incubated with HCT116/DR cells. After incubating for 2 h, 4 h, 8 h, and 10 h, the cells were stained with DAPI and were observed by fluorescence microscope. Scale bar: (D) 100 μm (F) 10 μm. **p < 0.01, ***p < 0.001.
Figure 3

(A) A schematic representation of oxaliplatin acts on drug-resistant and non-resistant cells, and OX@Se-MnP acts on drug-resistant cells. (B) Flow cytometric analysis of HCT116/DR cells at 24 h after various treatments including PBS, OX, Se-MnP, OX@Se-MnP. (C) The percentage of apoptotic cells was determined by flow cytometry. (D) Fluorescence images of HCT116/DR cells stained with DCFH-DA. (E) The
expression levels of ABCC1 protein. (F) Fluorescence images of HCT116/DR cells stained with JC-1. Scale bars: (D) 10 μm, (F) 50 μm.

Figure 4

(A) Scheme illustration for the *in vivo* evaluation of antitumor therapy. (B) Photographic images of the tumor at the end of these treatments. (C) Average weights of tumors harvested at the end of these
treatments (n=5). (D) Relative tumor volume of mice after various treatments. (E) Average weights of mice. (F) Histological analysis of the main organs of untreated mice (PBS) and mice treated with OX:Se-MnP:OX@Se-MnP. *p < 0.05, ***p < 0.001.

Figure 5
(A) Histological analysis of tumor tissues stained with hematoxylin and eosin, Ki-67, and caspase-3 from these groups. (B) Histological analysis of tumor tissues stained with ABCB1, ABCC1, and ABCG2 from these groups.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SupportingInformation0808.docx
- Scheme1.tif