

Synthesis, Characterization, and In Vitro Radiosensitizing Evaluation of Au@Pt-Functionalized Single-Walled Carbon Nanotubes for Breast Cancer Radiotherapy

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Abstract

Radiation therapy, one of the most widely used cancer treatment modalities, is often limited by uncontrolled drug release and insufficient tumor-targeting efficiency. Herein, a novel nanocarrier based on oxygen-functionalized single-walled carbon nanotubes decorated with gold and platinum nanoparticles (O-SWCNTs-Au@Pt) was developed as an efficient X-ray radiosensitizer. The surface of the O-SWCNTs-Au@Pt hybrid was further functionalized with bovine serum albumin (BSA) and folic acid (FA) to enhance surface biocompatibility, prolong blood circulation, and enable active tumor targeting. The integration of these components resulted in a multifunctional nanoplatform, O-SWCNTs-Au@Pt-BSA-FA, which was synthesized for the first time using a simple, reproducible, and cost-effective approach and comprehensively characterized by various analytical techniques. The in vitro therapeutic efficacy of O-SWCNTs-Au@Pt-BSA-FA was evaluated in the 4T1 breast cancer cell line using the MTT assay in the presence and absence of X-ray irradiation. The developed nanoformulation exhibited high radiosensitivity, favorable biocompatibility, and rapid therapeutic activity with minimal cytotoxicity toward non-irradiated cells. Notably, treatment with O-SWCNTs-Au@Pt-BSA-FA combined with X-ray irradiation reduced 4T1 cell viability by up to 42%, demonstrating significantly greater cytotoxicity than O-SWCNTs-BSA and O-SWCNTs-Au@Pt-BSA. Owing to their unique physicochemical properties and excellent ability to penetrate biological membranes, SWCNTs represent an effective nanocarrier for the delivery of Au@Pt hybrids, thereby enhancing the therapeutic efficacy of X-ray-induced cancer treatment.

Introduction

Cancer remains one of the leading causes of mortality worldwide, with its incidence and complexity continuing to increase each year. Among the available therapeutic strategies, radiation therapy (RT) is one of the most effective and widely used treatment modalities, playing a role in the management of more than 50% of cancer patients during the course of their disease [1, 2]. RT employs high-energy X-rays or γ -rays to induce irreparable DNA damage either directly or indirectly through the generation of reactive oxygen species (ROS) and other free radicals, ultimately triggering cancer cell death. However, the therapeutic efficacy of RT is constrained by the narrow therapeutic window between tumor eradication and normal tissue toxicity. Delivering sufficiently high radiation doses to eliminate malignant cells often exceeds the tolerance of surrounding healthy tissues, resulting in severe adverse effects. Conversely, reducing the radiation dose to minimize normal tissue damage may compromise tumor control, allowing residual cancer cells to survive and increasing the risk of tumor recurrence [3, 4]. To overcome these limitations, considerable efforts have been devoted to improving the precision and selectivity of RT through the development of advanced treatment strategies that maximize radiation delivery to tumors while minimizing exposure to adjacent healthy tissues [5].

Radiosensitizers have emerged as an effective strategy to overcome the inherent limitations of conventional radiation therapy by amplifying radiation-induced damage in tumor cells, thereby enhancing therapeutic efficacy [6]. Based on their mechanisms of action, radiosensitizers can generally be classified into two categories. The first includes chemotherapeutic agents that increase cellular

sensitivity to ionizing radiation by modulating biological pathways involved in DNA repair, apoptosis, and oxidative stress. The second comprises materials that interact directly with incident radiation, thereby enhancing local energy deposition within tumors [7, 8]. Metal-based nanomaterials represent the most extensively investigated class of the second category because of their high atomic numbers (high-Z), which enable efficient absorption of ionizing radiation and promote the generation of secondary electrons and reactive oxygen species, thereby increasing localized radiation dose and tumor cell destruction [9, 10]. During the past two decades, metallic nanomaterials have attracted considerable attention owing to their unique physicochemical characteristics and versatile biomedical applications, including molecular imaging, disease diagnosis, targeted drug delivery, and cancer therapy [11]. Among the available high-Z metals, gold (Au) and platinum (Pt) have been widely explored as radiosensitizers because of their excellent radiation absorption capability, outstanding chemical stability, high biocompatibility, and relatively low toxicity [12–14]. Their ability to selectively enhance radiation-induced damage within tumors while reducing irradiation of surrounding healthy tissues makes them particularly attractive for improving the therapeutic index of RT [14].

To maximize the therapeutic potential of metallic radiosensitizers, a variety of nanocarrier systems—including mesoporous silica nanoparticles, graphene derivatives, polymeric nanoparticles, and carbon nanotubes (CNTs)—have been developed to improve their delivery efficiency and tumor accumulation. Among these platforms, CNTs have emerged as highly promising nanocarriers owing to their exceptionally high surface area, large drug-loading capacity, efficient cellular uptake, and remarkable ability to transport therapeutic agents across biological membranes [15, 16]. Furthermore, both covalent and non-covalent functionalization strategies significantly improve the aqueous dispersibility and biocompatibility of CNTs while reducing aggregation and minimizing their long-term accumulation in biological systems, thereby enhancing their suitability for biomedical applications [17, 18].

Recent advances in nanotechnology have demonstrated the remarkable potential of CNT-based nanoplateforms for improving the efficacy of cancer radiotherapy. Owing to their unique physicochemical characteristics, CNTs have been extensively engineered as multifunctional carriers for therapeutic radionuclides, metallic nanoparticles, and tumor-targeting ligands, resulting in enhanced tumor accumulation and improved therapeutic outcomes [19, 20]. Surface functionalization further improves their aqueous dispersibility, colloidal stability, biocompatibility, and selective internalization by cancer cells while minimizing nonspecific interactions with healthy tissues. In addition, the exceptionally high specific surface area of CNTs, together with their ability to interact with aromatic molecules through π - π stacking, enables efficient immobilization of metal-based radiosensitizers [16, 19]. In particular, gold- and platinum-based nanoparticles can be stably anchored onto functionalized CNTs, providing efficient high-Z-mediated radiation dose enhancement through increased secondary electron generation under X-ray irradiation. Collectively, these advances establish CNT-based nanoplateforms as highly promising candidates for the development of next-generation targeted radiosensitizers.

Motivated by these advances, we report for the first time the fabrication of a multifunctional metal-metal radiosensitizing nanoplateform based on oxygen-functionalized single-walled carbon nanotubes (O-

SWCNTs) decorated with Au@Pt hybrid nanoparticles. In this design, O-SWCNTs serve as an efficient nanocarrier, whereas the incorporated high-Z Au and Pt nanoparticles are intended to amplify localized energy deposition by generating secondary electrons upon X-ray irradiation, thereby improving the therapeutic performance of radiotherapy. To further optimize the biological properties of the nanosystem, the hybrid nanostructures were coated with bovine serum albumin (BSA), which enhances biocompatibility, colloidal stability, prolonged blood circulation, and low immunogenicity. In addition, folic acid (FA) was conjugated to the nanoparticle surface as an active targeting ligand to promote preferential uptake by folate receptor-overexpressing breast cancer cells. The cytotoxicity and radiosensitizing performance of the resulting O-SWCNTs–Au@Pt–BSA–FA nanoplatfrom were investigated in murine 4T1 breast cancer cells using the MTT assay in the presence and absence of X-ray irradiation (4 and 8 Gy). Compared with the corresponding control formulations, the combined treatment with O-SWCNTs–Au@Pt–BSA–FA and X-ray irradiation markedly decreased cancer cell viability, demonstrating a substantially enhanced radiosensitizing effect. These findings indicate that the proposed multifunctional CNT-based nanosystem constitutes a promising strategy for targeted radiosensitization and may provide an effective platform for improving the therapeutic outcomes of cancer radiotherapy.

Materials and methods

Materials

Hydrogen peroxide (H_2O_2), trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), and sodium borohydride (NaBH_4) were purchased from Merck (Darmstadt, Germany). Hydrogen tetrachloroaurate(III) hydrate (HAuCl_4), hexachloroplatinic(IV) acid hexahydrate ($\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$, ~ 0.40% Pt), bovine serum albumin (BSA), folic acid (FA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade and were used as received without further purification. Deionized water was used throughout all experiments.

Preparation and purification of SWCNTs

SWCNTs were synthesized by the chemical vapor deposition (CVD) method according to a previously reported procedure [21]. Briefly, 80 mg of Fe–Mo/ Al_2O_3 catalyst was accurately weighed, placed in a quartz boat, and positioned at the center of a horizontal quartz-tube reactor. Prior to the growth process, the reactor was purged with argon gas at a flow rate of 350 sccm while being heated to 680°C. Once the reaction temperature was reached, methane was introduced at a flow rate of 40 sccm for 30 min as the carbon source for SWCNT growth. After completion of the deposition process, the methane flow was discontinued, and the reactor was allowed to cool naturally to room temperature under a continuous argon atmosphere. The as-grown carbon product was then collected for further purification. The

synthesized SWCNTs were purified following a previously reported method [22]. Briefly, the as-prepared product was refluxed in 3 M NaOH solution for 6 h to remove the Al₂O₃ catalyst support, followed by refluxing in HNO₃ for 1 h to dissolve the residual metal catalyst particles. The purified SWCNTs were subsequently washed repeatedly with deionized water until a neutral pH was achieved and then dried in an oven at 110°C for 24 h.

Preparation of O-SWCNTs

O-SWCNTs were prepared by a hydrothermal oxidation method according to a previously reported procedure with slight modification [23]. Briefly, 0.2 g of purified SWCNTs was dispersed in 12 mL of hydrogen peroxide and transferred into a 25 mL Teflon-lined stainless-steel autoclave. The autoclave was sealed and heated at 180°C for 12 h to introduce oxygen-containing functional groups onto the surface of the SWCNTs. After naturally cooling to room temperature, the resulting O-SWCNTs were collected, thoroughly washed with deionized water to remove residual oxidizing agents and reaction by-products, and then dried in an oven at 80°C for 12 h before further use.

Preparation of Au@Pt functionalized O-SWCNTs (O-SWCNTs-Au@Pt)

O-SWCNTs decorated with Au@Pt hybrid nanoparticles (O-SWCNTs-Au@Pt) were synthesized through the in-situ reduction of Au and Pt precursors. Briefly, 0.9 mL of sodium citrate solution (1% w/v) was added dropwise to an aqueous solution containing 1036.36 µL of HAuCl₄ (0.45% w/v) and 11.75 µL of H₂PtCl₆·6H₂O (approximately 0.40% Pt) under continuous stirring. Subsequently, 4.7 mg of oxygen-functionalized SWCNTs (O-SWCNTs) was introduced into the reaction mixture and dispersed thoroughly. Freshly prepared NaBH₄ solution (0.21 mg mL⁻¹) was then added dropwise as the reducing agent, and the reaction was stirred for an additional 3 h at room temperature. The formation of Au@Pt hybrid nanoparticles was accompanied by a distinct color change of the reaction mixture, indicating the successful reduction of the metal precursors. Finally, the resulting O-SWCNTs-Au@Pt nanostructures were purified by dialysis against deionized water for 48 h to remove unreacted metal ions and residual reagents.

Preparation of FA Conjugated BSA (BSA-FA)

To activate the carboxyl groups of folic acid (FA), 47.5 mg of *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC), 30 mg of *N*-hydroxysuccinimide (NHS), and 25 mg of FA were dissolved in 2 mL of DMSO and allowed to react for 20 min at room temperature. The activated FA solution was then added dropwise to 5 mL of BSA solution (40 mg mL⁻¹) under continuous stirring. The pH of the reaction mixture was adjusted to 8.0 using an aqueous NaOH solution, and the reaction was allowed to proceed overnight at room temperature. The resulting BSA-FA conjugate was subsequently

purified by dialysis to remove unreacted reagents and low-molecular-weight by-products. Throughout the conjugation procedure, the reaction vessel was wrapped with aluminum foil to protect the light-sensitive FA from photodegradation.

Determination of BSA-FA and O-SWCNT-Au@Pt concentrations

To determine the concentration of the solutions, 2 mL of BSA-FA and O-SWCNT-Au@Pt were removed and poured into a watch glass to dry and then weighed to obtain a concentration.

Preparation of O-SWCNT-Au@Pt-BSA-FA NPs

The O-SWCNTs–Au@Pt–BSA–FA hybrid nanosystem was prepared through a simple and straightforward mixing process. Briefly, O-SWCNTs–Au@Pt and BSA–FA solutions were mixed at a mass ratio of 1:5 (O-SWCNTs–Au@Pt:BSA–FA) and stirred continuously at room temperature for 24 h to allow efficient surface functionalization. The resulting suspension was subsequently purified by five successive centrifugation cycles to remove unbound BSA–FA molecules and other residual impurities. The purified nanostructures were finally collected for further characterization and biological evaluation.

In vitro treatment efficacy

The cytotoxicity and radiosensitizing effects of the synthesized nanostructures were evaluated in the 4T1 murine breast cancer cell line using the MTT assay in the presence and absence of X-ray irradiation. Briefly, 4T1 cells were seeded into 96-well plates at a density of 1×10^5 cells per well in RPMI-1640 culture medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin, followed by incubation at 37°C in a humidified atmosphere containing 5% CO₂ for 24 h. After cell attachment, the culture medium was replaced with fresh medium containing different concentrations of O-SWCNTs–BSA, O-SWCNTs–Au@Pt–BSA, or O-SWCNTs–Au@Pt–BSA–FA nanostructures. Following 5 h of incubation, the nanoparticle-containing medium was removed, the cells were gently washed with phosphate-buffered saline (PBS), and fresh culture medium was added. The cells were subsequently exposed to X-ray irradiation at doses of 4 or 8 Gy and then incubated for an additional 24 h. Cell viability was determined using the MTT assay. Briefly, 20 µL of MTT solution was added to each well and the plates were incubated for 4 h to allow the formation of formazan crystals. The medium was then carefully removed, and 150 µL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate (ELISA) reader. Cell viability was calculated relative to the untreated control group, and all experiments were performed in five independent replicates.

Characterization of SWCNT, O-SWCNT-Au@Pt and O-SWCNT-Au@Pt-BSA-FA

The optical properties and qualitative characterization of the synthesized nanostructures were investigated using a UV–Visible spectrophotometer (Lambda 850, PerkinElmer, USA) over the wavelength range of 200–800 nm. The crystalline structure and phase composition were analyzed by X-ray diffraction (XRD) using an X'Pert PRO diffractometer (PANalytical, Netherlands) equipped with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$), operating at 40 kV and 30 mA over the 2θ range of $10\text{--}80^\circ$. Fourier transform infrared (FT-IR) spectra were recorded using a Mattson 1000 spectrophotometer in the spectral range of $400\text{--}4000 \text{ cm}^{-1}$ to identify the functional groups and confirm surface functionalization. The hydrodynamic size distribution of the nanostructures was determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Worcestershire, UK). The pH of the solutions was monitored using an 827 pH Lab meter (Metrohm, Switzerland). Zeta potential measurements were performed using a Zetasizer Nano ZS (Malvern Instruments Ltd., UK) based on electrophoretic light scattering and calculated according to the Helmholtz–Smoluchowski equation. The morphology and microstructure of the synthesized nanostructures were examined by transmission electron microscopy (TEM, JEM-2010F, JEOL, Japan), while their elemental composition and elemental distribution were analyzed by energy-dispersive X-ray spectroscopy (EDS, JED-300, JEOL, Japan).

Statistical analysis

The mean $\pm$ standard deviation was exploited to represent the measurement data and GraphPad Prism software (GraphPad Prism 8) was exerted for statistical analysis of the data.

Results and Discussion

To verify the successful fabrication of the O-SWCNTs–Au@Pt–BSA–FA nanosystem and monitor each functionalization step, UV–Vis absorption spectroscopy was employed. As shown in Fig. 1a, the characteristic absorption bands of Pt and Au nanoparticles were observed at 277 and 525 nm, respectively, corresponding to the intrinsic absorption of Pt nanoparticles and the surface plasmon resonance (SPR) of Au nanoparticles [24, 25]. Following the decoration of O-SWCNTs with Au@Pt hybrid nanoparticles, the absorption bands shifted to 285 and 530 nm, confirming the successful formation of the O-SWCNTs–Au@Pt nanostructure [26]. Bovine serum albumin (BSA) exhibited its characteristic absorption peak at approximately 275 nm, whereas folic acid (FA) displayed two distinct absorption bands centered at 285 and 358 nm [27]. After surface functionalization with BSA and FA, the UV–Vis spectrum of O-SWCNTs–Au@Pt–BSA–FA exhibited the characteristic absorption features of all constituent components. Specifically, the Au@Pt-related absorption bands appeared at 284 and 533 nm with slight red shifts, while the characteristic FA absorption bands were observed at 282 and 356 nm, showing a slight blue shift. These spectral changes provide strong evidence for the successful conjugation of BSA and FA onto the surface of the O-SWCNTs–Au@Pt nanostructure and confirm the successful fabrication of the final multifunctional nanosystem.

X-ray diffraction (XRD) analysis was performed to investigate the crystalline structure, phase composition, and average crystallite size of SWCNTs, O-SWCNTs–Au@Pt, and O-SWCNTs–Au@Pt–BSA–FA nanostructures. As shown in Fig. 1b, pristine SWCNTs exhibited the characteristic diffraction peaks indexed to the (002), (100), (101), and (103) crystal planes. Following the incorporation of Au@Pt hybrid nanoparticles, additional diffraction peaks corresponding to the (111), (200), (220), and (311) planes were observed, confirming the presence of the metallic phase. The observed diffraction peaks are in good agreement with the corresponding standard JCPDS reference cards (JCPDS No. 75-1621, JCPDS No. 65-2870, and JCPDS No. 04-0802), demonstrating the successful formation of the constituent nanomaterials.

Compared with O-SWCNTs–Au@Pt, the diffraction peaks of O-SWCNTs–Au@Pt–BSA–FA exhibited a noticeable decrease in intensity after surface modification with BSA and FA. In addition, the XRD pattern of the final nanoplatform displayed a more pronounced amorphous background than those of pristine SWCNTs and O-SWCNTs–Au@Pt. These changes are attributed to the amorphous nature of the protein and organic coating and provide strong evidence for the successful surface functionalization of the nanostructures.

The average crystallite size (D) was estimated using the Scherrer equation (Eq. 1):

$$D = K\lambda / \beta \cos\theta \quad \text{Eq. 1}$$

where K is the shape factor, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) of the diffraction peak expressed in radians, and θ is the Bragg diffraction angle.

The calculated crystallite sizes indicated a gradual increase following successive surface modification with BSA and FA. This increase may be attributed to the presence of the macromolecular coating and the resulting changes in crystallite growth and structural organization of the nanostructures. Together with the observed changes in diffraction intensity and amorphous background, these findings further support the successful functionalization of O-SWCNTs–Au@Pt with BSA and FA.

The FT-IR spectroscopy was employed to verify the successive functionalization steps and to confirm the chemical composition of the synthesized nanostructures. The FT-IR spectra of the intermediate products and the final O-SWCNTs–Au@Pt–BSA–FA nanosystem are presented in Fig. 2. The FT-IR spectrum of bovine serum albumin (BSA) exhibited two characteristic absorption bands at 1661.9 and 1537.5 cm^{-1} , corresponding to the amide I (C = O stretching) and amide II (N–H bending and C–N stretching) vibrations, respectively, which are characteristic of the protein structure. The spectra of AuNPs and PtNPs displayed characteristic absorption bands at 1663.9/1643.9 cm^{-1} and approximately 1150/1130 cm^{-1} , consistent with previous reports [28]. After conjugation of folic acid (FA) with BSA, the resulting BSA–FA spectrum retained the characteristic absorption bands of both components with slight shifts in peak positions. In particular, the amide I band shifted from 1661.9 cm^{-1} in pristine BSA to 1639.2 cm^{-1} in the BSA–FA conjugate, indicating interactions between BSA and FA molecules [28]. Moreover, a new absorption band appeared at 1395.3 cm^{-1} in the BSA–FA spectrum, corresponding to

the characteristic vibration of FA, further confirming successful conjugation. The FT-IR spectrum of the final O-SWCNTs–Au@Pt–BSA–FA nanoplatform exhibited the characteristic absorption bands associated with the O-SWCNTs–Au@Pt framework together with the distinctive bands of BSA and FA. The simultaneous presence of these characteristic vibrations, along with the observed peak shifts, provides strong evidence for the successful immobilization of the BSA–FA conjugate onto the surface of the O-SWCNTs–Au@Pt nanostructure [29, 30].

Dynamic light scattering (DLS) and zeta potential analyses were performed to determine the hydrodynamic size distribution and surface charge of SWCNTs, O-SWCNTs–Au@Pt, and O-SWCNTs–Au@Pt–BSA–FA nanostructures in aqueous suspension. In addition, the pH-dependent surface charge of the nanostructures was evaluated over the pH range of 2–10 by adjusting the medium with 100 mM HCl or NaOH. The average hydrodynamic diameters of the synthesized nanostructures are presented in Fig. 3a. Zeta potential is an important indicator of colloidal stability, as it reflects the electrostatic interactions between dispersed nanoparticles. Nanoparticles with a sufficiently high positive or negative zeta potential generally exhibit enhanced colloidal stability because the electrostatic repulsive forces between particles suppress aggregation. Consequently, changes in zeta potential during each functionalization step provide valuable evidence for successful surface modification of the nanostructures. Surface charge also plays an important role in the biological behavior of nanoparticles. Because the plasma membrane and blood components generally possess a net negative surface charge, negatively charged nanoparticles tend to exhibit reduced nonspecific electrostatic interactions with blood cells, which may contribute to improved colloidal stability in physiological media and prolonged circulation time. The zeta potential values of the synthesized nanostructures are shown in Fig. 3b. At the highest investigated pH, the zeta potentials of SWCNTs, O-SWCNTs–Au@Pt, and O-SWCNTs–Au@Pt–BSA–FA were – 26.00, – 38.36, and – 27.07 mV, respectively. The observed changes in surface charge after successive functionalization steps further support the successful fabrication of the final multifunctional nanoplatform.

The morphology and microstructure of SWCNTs, purified SWCNTs, O-SWCNTs–Au@Pt, and O-SWCNTs–Au@Pt–BSA–FA were investigated by transmission electron microscopy (TEM), as shown in Fig. 4. The purified SWCNTs retained their characteristic tubular morphology, while the O-SWCNTs–Au@Pt nanostructures exhibited a highly entangled CNT network with numerous Au@Pt nanoparticles uniformly distributed on the outer surfaces of the nanotubes (Fig. 4c,d). This homogeneous decoration demonstrates the successful immobilization of the metallic nanoparticles onto the oxygen-functionalized SWCNTs. The average particle sizes of the metallic nanoparticles were determined by measuring approximately 150 particles from the TEM images. The mean particle sizes of Au@Pt and O-SWCNTs–Au@Pt–BSA–FA were approximately 6 and 8 nm, respectively. The observed increase in particle size after BSA–FA functionalization can be attributed to the formation of an organic coating surrounding the nanoparticles and a slight tendency toward nanoparticle clustering following surface modification. High-resolution TEM (HR-TEM) images revealed well-resolved lattice fringes, indicating the high crystallinity of the metallic nanoparticles [31]. The measured interplanar spacings were 0.226 and 0.236 nm (Fig. 4e), corresponding to the (111) crystallographic planes of Pt (JCPDS No. 04-0802) and Au

(JCPDS No. 04-0754), respectively [32]. Furthermore, the selected-area electron diffraction (SAED) pattern (Fig. 4f) displayed a series of bright concentric diffraction rings, confirming the polycrystalline nature of the Au@Pt hybrid nanoparticles and further verifying their successful formation [33, 34].

The elemental composition and spatial distribution of the synthesized O-SWCNTs–Au@Pt and O-SWCNTs–Au@Pt–BSA–FA nanostructures were investigated by energy-dispersive EDS coupled with elemental mapping. As shown in Fig. 5c,d, the elemental maps reveal a homogeneous distribution of carbon (C), oxygen (O), gold (Au), and platinum (Pt) throughout both nanostructures, confirming the successful incorporation of the Au@Pt hybrid nanoparticles onto the oxygen-functionalized SWCNTs.

Notably, the EDS mapping of O-SWCNTs–Au@Pt–BSA–FA additionally revealed the presence of nitrogen (N), which is attributed to the BSA–FA coating. The appearance of this element, together with the uniform elemental distribution, provides strong evidence for the successful surface functionalization of the O-SWCNTs–Au@Pt nanostructure with the BSA–FA conjugate. Furthermore, the absence of detectable impurity elements in the EDS spectra indicates the high chemical purity of the synthesized multifunctional nanosystem.

The cytotoxicity and radiosensitizing performance of the synthesized nanostructures were evaluated using the MTT assay, a well-established quantitative method for assessing cell viability. Murine 4T1 breast cancer cells were selected as the *in vitro* model. To investigate the concentration-dependent therapeutic effects, cells were treated with O-SWCNTs–BSA, O-SWCNTs–Au@Pt–BSA, and O-SWCNTs–Au@Pt–BSA–FA at concentrations of 10, 30, and 90 $\mu\text{g mL}^{-1}$ in the presence or absence of X-ray irradiation (4 and 8 Gy). Cell viability was subsequently determined to evaluate the combined effects of the nanostructures and radiation treatment. As shown in Fig. 6, treatment with either X-ray irradiation alone or the nanostructures alone produced only a limited reduction in cell viability. In contrast, the combined treatment of the nanostructures with X-ray irradiation resulted in a substantially greater cytotoxic effect, demonstrating a clear radiosensitizing response. Furthermore, cell viability decreased progressively with increasing nanoparticle concentration and radiation dose, indicating a concentration- and dose-dependent therapeutic effect.

The cytotoxicity of O-SWCNTs–BSA was first examined. In the absence of irradiation, O-SWCNTs–BSA exhibited minimal intrinsic cytotoxicity toward 4T1 cells. However, when combined with X-ray irradiation (4 or 8 Gy), a significantly greater reduction in cell viability was observed. For example, treatment with O-SWCNTs–BSA at concentrations of 10, 30, and 90 $\mu\text{g mL}^{-1}$ resulted in cell viabilities of 80.03%, 76.20%, and 75.30%, respectively, demonstrating that the simultaneous application of radiation and the nanosystem enhanced the therapeutic response compared with either treatment alone. The incorporation of Au@Pt hybrid nanoparticles further improved the radiosensitizing performance of the nanopatform. Owing to the high atomic numbers of Au and Pt, the nanostructures can enhance local energy deposition under X-ray irradiation, thereby increasing radiation-induced cellular damage. Following further surface modification with folic acid, the O-SWCNTs–Au@Pt–BSA–FA nanosystem exhibited the strongest therapeutic effect among all investigated formulations. The enhanced efficacy is

attributed to the combined contribution of active folate receptor-mediated cellular uptake and the radiation dose enhancement provided by the Au@Pt hybrid nanoparticles.

Among all treatment groups, O-SWCNTs–Au@Pt–BSA–FA combined with X-ray irradiation produced the greatest reduction in cell viability, reaching approximately 42% at the highest nanoparticle concentration and radiation dose. These results demonstrate that the multifunctional nanoplatform possesses superior radiosensitizing activity compared with O-SWCNTs–BSA and O-SWCNTs–Au@Pt–BSA. Although the underlying mechanism was not directly investigated in the present study, the enhanced therapeutic effect is likely associated with increased local energy deposition and the generation of secondary electrons by the high-Z Au@Pt nanoparticles under X-ray irradiation, leading to amplified radiation-induced cellular damage.

Statistical analysis further confirmed the enhanced therapeutic efficacy of the O-SWCNTs–Au@Pt–BSA–FA nanoplatform. Compared with the X-ray (8 Gy) group, treatment with O-SWCNTs–Au@Pt–BSA–FA plus X-ray (8 Gy) at nanoparticle concentrations of 10, 30, and 90 $\mu\text{g mL}^{-1}$ showed statistically significant differences, with *P* values of 0.0915 (ns), 0.05 (*), < 0.001 (***) and < 0.0001 (****), respectively. Overall, increasing both the nanoparticle concentration and radiation dose resulted in a progressive decrease in cell viability, confirming the concentration-dependent radiosensitizing capability of the synthesized multifunctional nanoplatform.

Conclusion

In this study, a simple, reproducible, and cost-effective strategy was developed for the fabrication of highly stable O-SWCNTs, O-SWCNTs–Au@Pt, and O-SWCNTs–Au@Pt–BSA–FA nanoplatforms. The synthesized nanostructures were evaluated as multifunctional radiosensitizers using the 4T1 murine breast cancer cell line, and their therapeutic performance was assessed by the MTT assay under X-ray irradiation. Among the investigated formulations, O-SWCNTs–Au@Pt–BSA–FA exhibited the highest radiosensitizing efficiency, producing a significantly greater reduction in cell viability than O-SWCNTs–BSA and O-SWCNTs–Au@Pt–BSA. The enhanced therapeutic efficacy is attributed to the synergistic effects of the high-Z Au@Pt hybrid nanoparticles, which amplify radiation-induced energy deposition, together with folic acid-mediated active targeting that promotes cellular uptake. The radiosensitizing performance of the nanoplatform was found to be dependent on both nanoparticle concentration and radiation dose. Under the highest treatment conditions, the combined administration of O-SWCNTs–Au@Pt–BSA–FA and X-ray irradiation reduced 4T1 cell viability to approximately 42%. Overall, these findings demonstrate that the proposed multifunctional O-SWCNTs–Au@Pt–BSA–FA nanosystem is a promising candidate for targeted cancer radiotherapy. The combination of efficient X-ray dose enhancement, favorable biocompatibility, and active tumor-targeting capability provides a versatile platform for the development of next-generation nanoradiosensitizers with improved therapeutic efficacy.

Declarations

Author contribution

Study conception: Afsoon Aghaei and Babak Pashaei; material preparation: Maryam Shaterian and Hossein Danafar; methodology: Afsoon Aghaei, Maryam Shaterian, and Hossein Danafar; data collection: Blaž Likozar, Andraž Šuligoj, and Saso Gyergyek; data analysis: Afsoon Aghaei, Hossein Danafar, and Babak Pashaei; visualization: Maryam Shaterian and Hossein Danafar; writing-original draft: Afsoon Aghaei and Babak Pashaei; supervision: Afsoon Aghaei and Babak Pashaei; writing-review and editing: Babak Pashaei. All authors read and approved the final manuscript.

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Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate Not applicable.

Consent for publication All authors consent for the publication.

Conflict of interest The authors declare that they have no conflict of interest.

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Figures

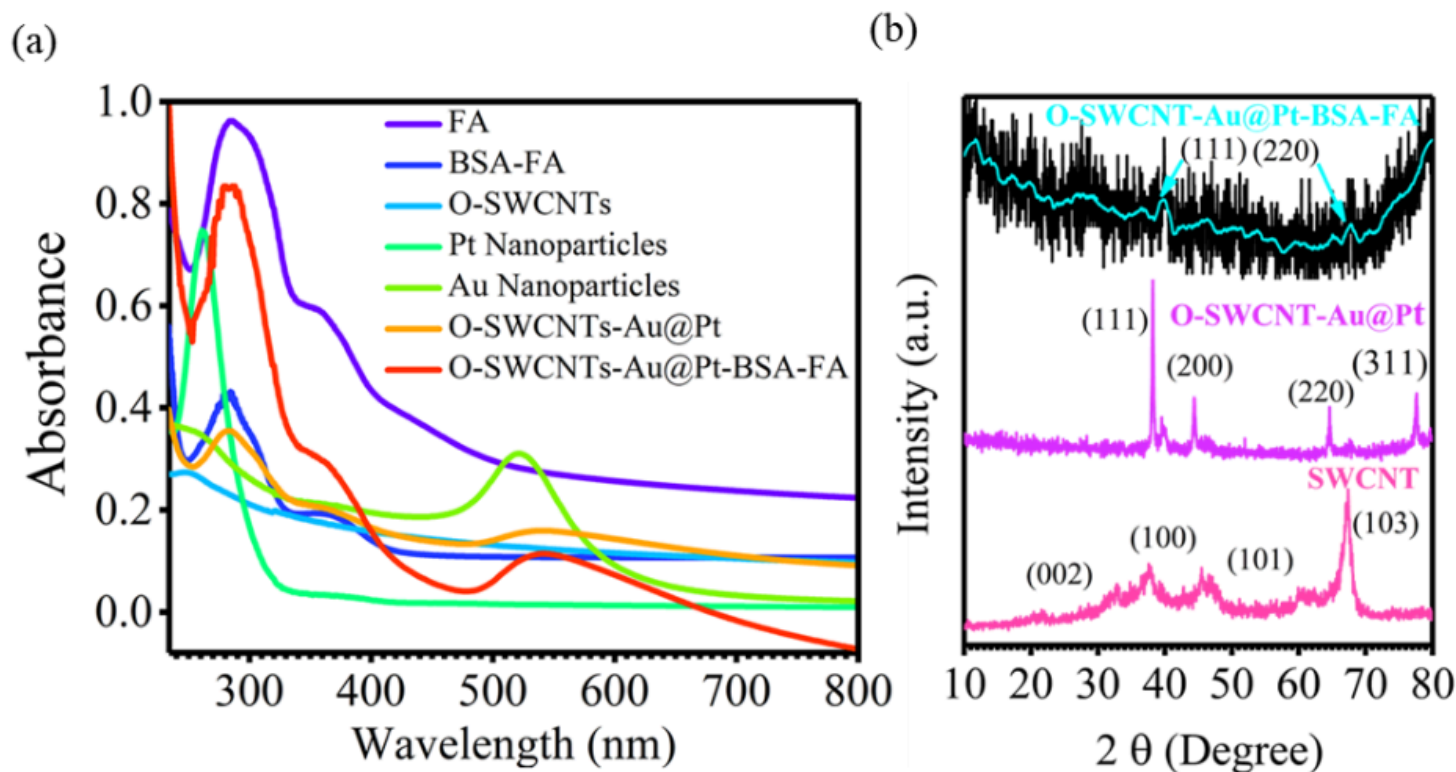


Figure 1

(a) UV-vis spectra of FA, BSA-FA, O-SWCNT, PtNPs, AuNPs, O-SWCNTs-Au@Pt, and O-SWCNTs-Au@Pt-BSA-FA (b) XRD patterns of SWCNT, O-SWCNT-Au@Pt, and O-SWCNT-Au@Pt-BSA-FA.

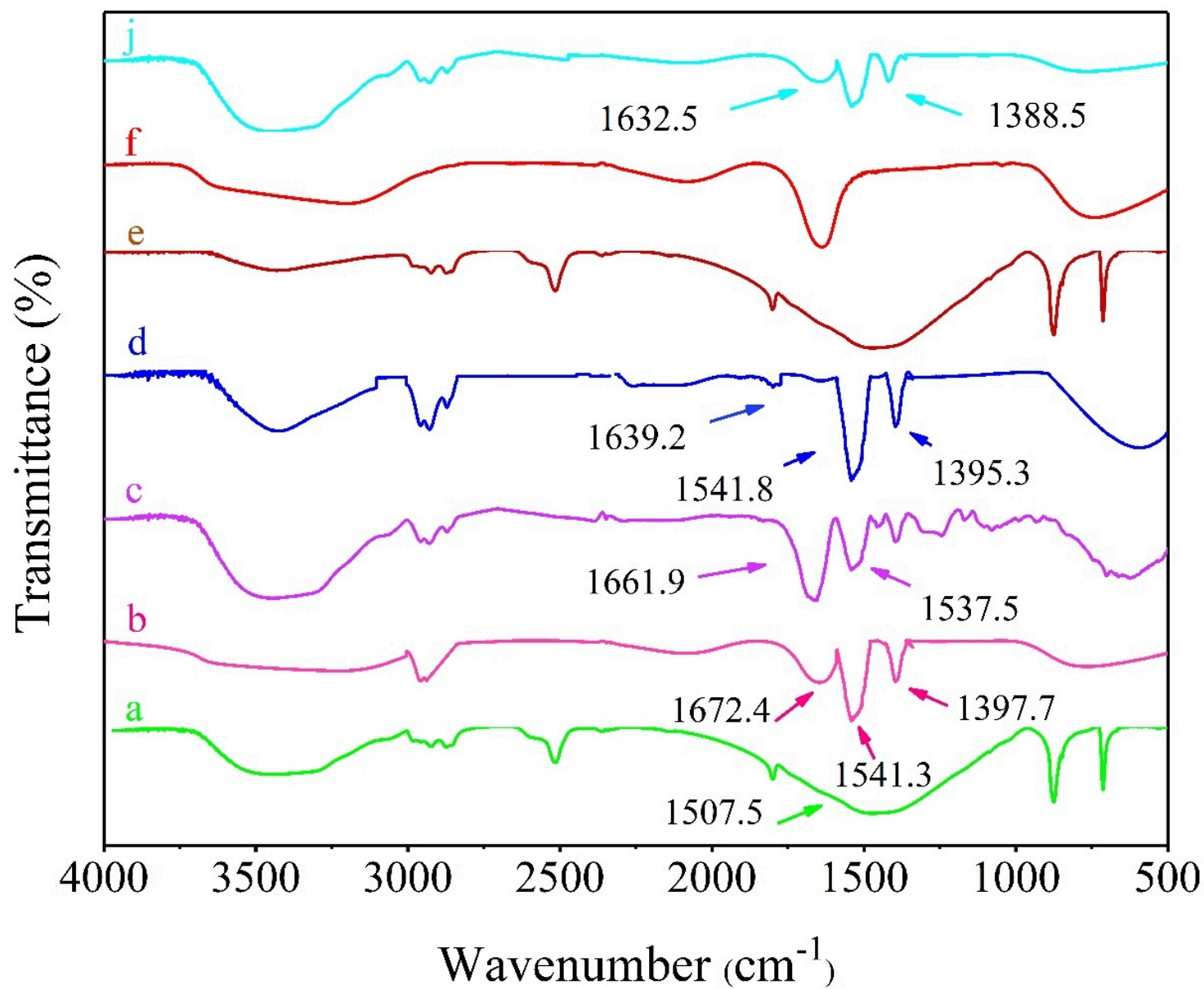


Figure 2

FT-IR spectra of a) SWCNT b) FA c) BSA d) BSA-FA e) O-SWCNT-Pt f) O-SWCNT-Au j) O-SWCNT-Au@Pt-BSA-FA.

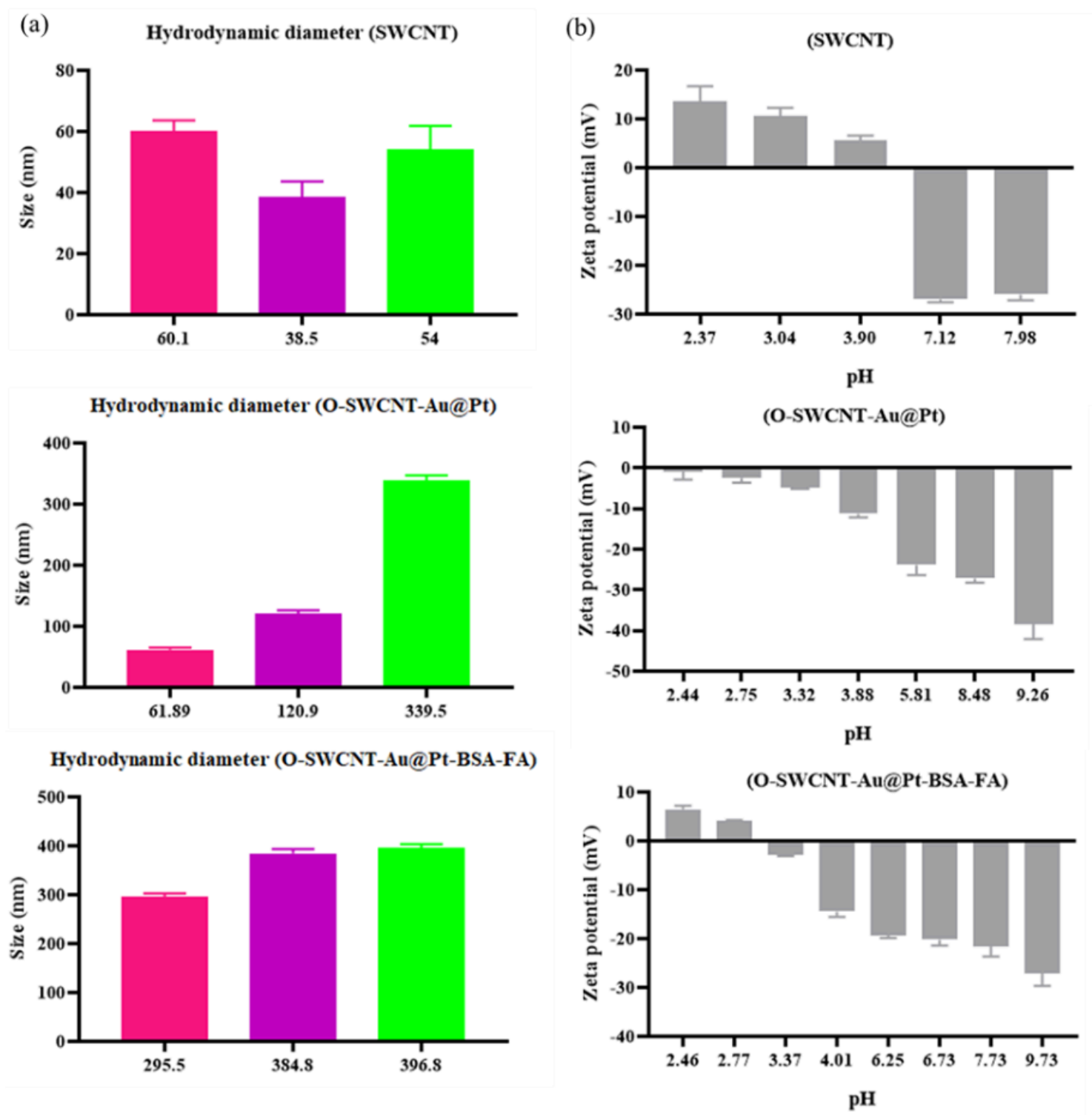


Figure 3

(a) Hydrodynamic size and (b) Surface charge of the SWCNTs, O-SWCNTs-Au@Pt and O-SWCNTs-Au@Pt-BSA-FA.

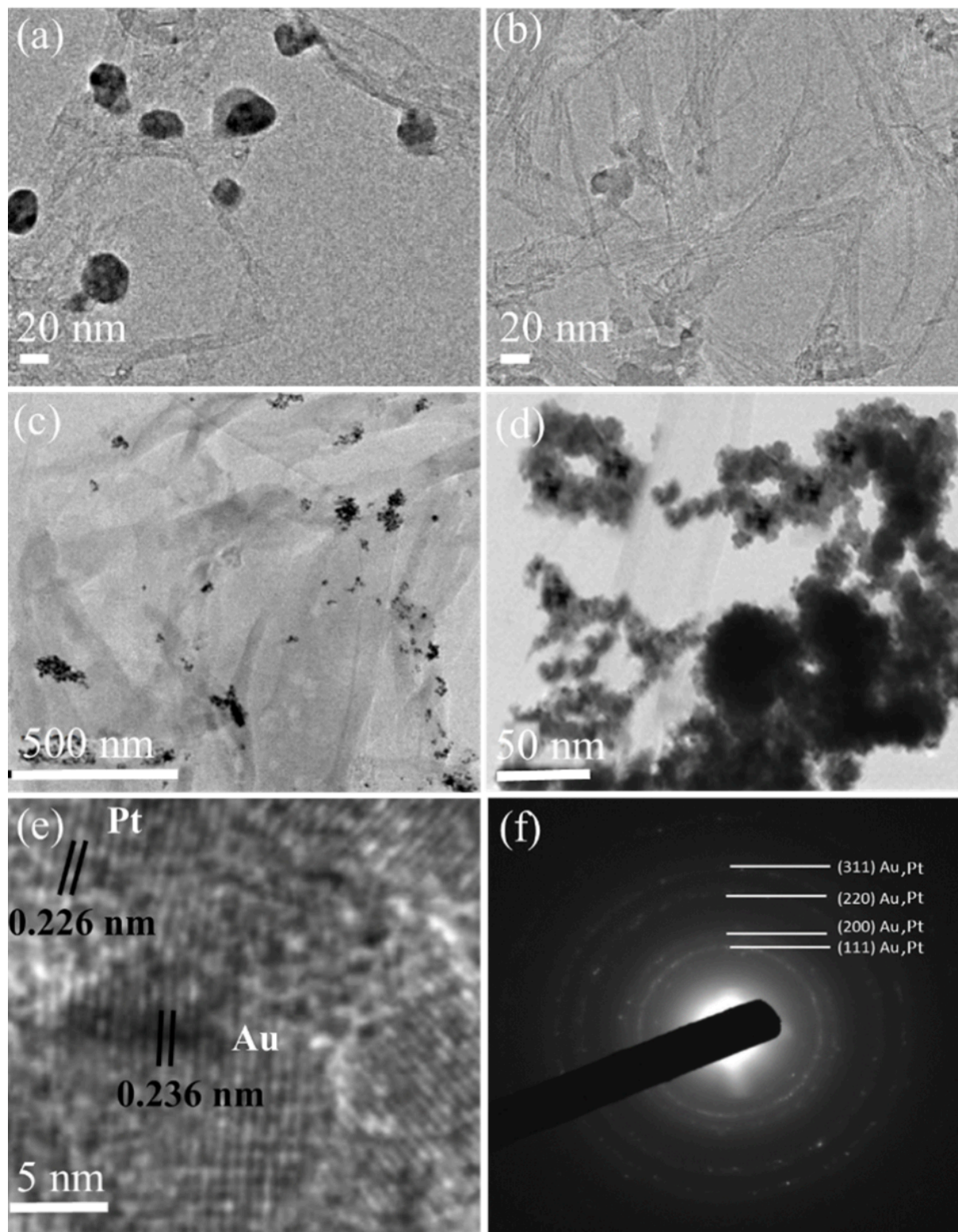


Figure 4

HR-TEM images of (a) SWCNTs (b) pure SWCNTs (c) O-SWCNTs-Au@Pt (d) O-SWCNTs-Au@Pt-BSA-FA (e) HR-TEM of SWCNTs-Au@Pt-BSA-FA (f) The SAED pattern SWCNTs-Au@Pt-BSA-FA.

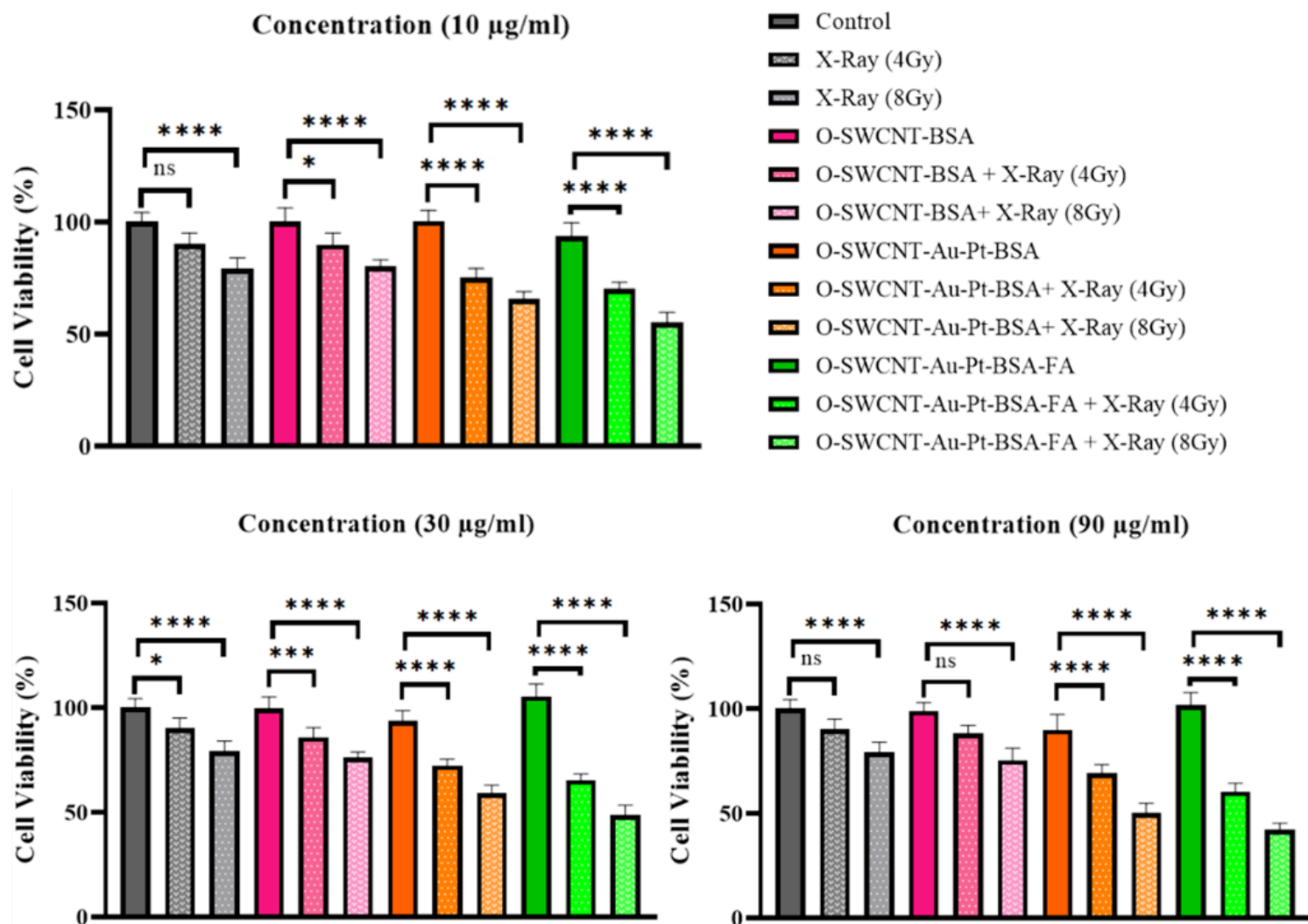


Figure 5

EDS of (a) O-SWCNTs-Au@Pt (b) O-SWCNTs-Au@Pt-BSA-FA, and elemental maps of (c) O-SWCNTs-Au@Pt (d) O-SWCNTs-Au@Pt-BSA-FA.

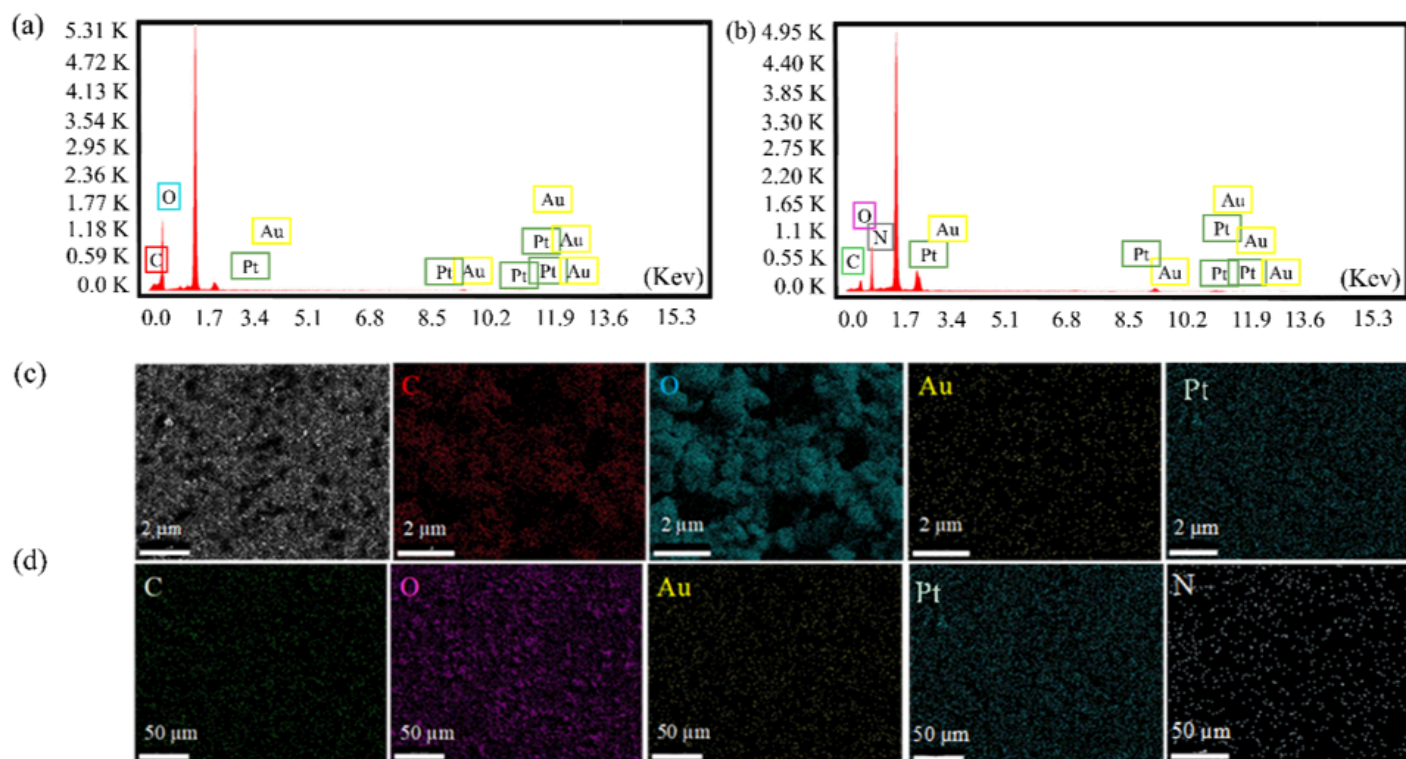


Figure 6

Cell viability of 4T1 breast cancer cells treated with different concentrations (10, 30, and 90 $\mu\text{g mL}^{-1}$) of the synthesized nanostructures in the absence and presence of X-ray irradiation (4 and 8 Gy), as determined by the MTT assay. Data are presented as mean $\pm$ SD ($n = 5$). Statistical significance is indicated as ns (not significant), $P < 0.05$ (*), $P < 0.001$ (***), and $P < 0.0001$ (****).