Zinc Sulfide Nanoparticle-decorated Fibre Mesh to Enable Localized H₂S-amplified Chemotherapy

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Zinc Sulfide Nanoparticle-decorated Fibre Mesh to Enable Localized H₂S-amplified Chemotherapy

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For the first time, we report the design and fabrication of a ZnS nanoparticle-decorated silica fibre mesh (ZnS@SiO₂) for localized H₂S-amplified chemotherapy. With incorporation of DOX, implanted ZnS@SiO₂ fibres enable sufficient on-site drug dosage and intracellular H₂S content, inducing significant *in vitro* and *in vivo* tumour inhibition.

While current systemic drug delivery systems (SDDSs) for chemotherapy still face challenges such as burst drug release, low targeting efficiency and inevitable clearance during circulation ^{1,2}. Localized drug delivery systems (LDDSs), in forms of films, hydrogels, microarrays, rods or drug-eluting wafers, have been developed for targeted chemotherapy ^{3, 4}. Electrospun fibres offer the potential for superior performance in localized chemotherapy due to their unique properties such as high surface area, flexibility and so on ⁵⁻⁷. Additional benefits include sustained and continuous release of chemotherapeutic drugs, providing a high local drug concentration at the tumour site while maintaining a low drug level in the biological system. For this reason, intensive research has been carried out on fibre-based LDDSs for chemotherapy ¹, and recently there was a move towards drug-loaded nanoparticle-fibre assemblies, in which nanoparticles can be taken-up by cancer cells, underwent intracellular drug release. Meanwhile, there has been rapid

advances in nanomedicine and nanotechnology for more efficient anticancer purposes. Among these emerging modalities, gas therapy has been explored utilizing the generation of therapeutic gases ⁸ such as nitric oxide (NO) ⁹, carbon monoxide (CO) ¹⁰, hydrogen (H₂) ¹¹ and hydrogen sulfide (H₂S) ^{12,13} to induce cell death. As the third gas transmitter after NO and CO ¹⁴. H₂S can be generated endogenously by the catalysis of H₂S-producing enzymes ¹⁵. Endogenous or low levels of H₂S may lead to pro-cancer effects while the presence of high H₂S levels may induce cancer inhibition ¹⁶. Interestingly, studies also suggested that excessive H₂S can increase the oxidative stress build-up in cancer cells by suppression of the enzyme catalase (CAT) ¹⁷. In addition, ZnS can actively react with H⁺ in the acid environment, and thus offers the potential for effective H₂S generation ^{18, 19}. Meanwhile, doxorubicin (DOX), a broad-spectrum drug used in chemotherapy, could also induce the generation of reactive oxygen species (ROS) besides its capability in causing cancer cell DNA dysfunction ²⁰. The suppression of CAT leads to over-production of H₂O₂, increasing the ROS level and promoting DNA damage and cell necrosis ²¹. Therefore, it would be logical to combine DOX and H₂S gas in one therapeutic platform to achieve H₂S-enhanced chemotherapy. However, to the best of our knowledge, no such investigation has yet been reported.

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Electronic Supplementary Information (ESI) available: N₂ absorption and desorption isotherm curves of SiO₂ fibres and ZnS@SiO₂ fibres. TEM images of ZnS nanoparticles released from ZnS@SiO₂ fibres. DOX loading efficiency and capacity of ZnS@SiO₂ fibres. SEM images of ZnS@SiO₂ fibres after incubation with PBS solution at pH=5.4 for different time. Bright field images of Huh7 cells after incubation with various samples at pH = 6.0 for 12 h. Photographs of representative mice after treatment. See DOI: 10.1039/x0xx00000x

Fig. 1 Schematic illustration of DOX-ZnS@SiO₂ fibrous mesh for localized H₂S-amplified chemotherapy.

Fig. 2 (a) Schematic illustration of the formation mechanism of ZnS@SiO₂ fibres; SEM images of (b) as-spun SiO₂ and (c) ZnS@SiO₂ fibres; (d) TEM image, (e) EDS elemental mapping and (f) XRD pattern of ZnS@SiO₂ fibres.

In the present study, we aimed to develop an enhanced chemotherapy platform combining the benefits of both gas therapy and localized drug release, comprising doxorubicin (DOX) loaded ZnS nanoparticles assembled silica fibres (DOX-ZnS@SiO₂) (as shown in Fig. 1). The *in vitro* and *in vivo* anticancer performance of the DOX-ZnS@SiO₂ fibrous mesh was also revealed.

ZnS@SiO₂ composite fibres were synthesized via hydrothermal growth of ZnS nanoparticles on the surface of silica nanofibres (procedure as demonstrated in Fig. 2a). A flexible silica fibrous membrane was prepared via electrospinning and subsequent heat treatment according to a modified sol-gel method reported previously²². After calcination at 800°C for 3 h, the as-prepared SiO₂ nanofibres presented a fibrous texture and smooth surface morphology (Fig. 2b). Flexible silica nanofibres with negative charged surface and inactivity during hydrothermal process enabled the assembly of ZnS nanoparticles²³.

Following the hydrothermal synthesis of ZnS nanoparticles reported previously²⁴, ZnS nuclei formed in the precursor solution, and were subsequently absorbed and grew on the surface of silica fibres. ZnS nanoparticles with an average diameter of ~110 nm were uniformly assembled on the surface of silica fibres (Fig. 2c, d). In consequence, the diameter of the composite fibres increased from 510 nm to 720 nm, and a slight decrease in surface area was observed (Fig. S1). ZnS nanoparticles liberated from ZnS@SiO₂ fibres were composed of nanocrystals, which increased the pore size of the composite fibres (Fig. S2). Meanwhile, element mapping images showed a homogeneous distribution of sulphur and zinc in ZnS@SiO₂ composite fibres (Fig. 2e), confirming the successful growth of ZnS nanoparticles on the surface of silica nanofibres. A representative XRD pattern of the as-prepared ZnS@SiO₂ composite fibres is shown in Fig. 2f. The broad peak appeared from 15° to 35° on the diffraction pattern was ascribed to SiO₂ due to its amorphous nature. Three main diffraction peaks are visible and these match well with the primary peaks of the

Fig. 3 (a) Cumulative H₂S gas release of ZnS@SiO₂ fibrous mesh in solutions different pH; (b) Zeta potentials of DOX, ZnS@SiO₂ fibres and DOX-ZnS@SiO₂ fibres; (c) UV-Vis spectra of DOX solution, ZnS@SiO₂ fibres and DOX-ZnS@SiO₂ fibres loaded in DOX solutions with different concentrations; (d) Cumulative DOX release profile of DOX-ZnS@SiO₂ fibres in PBS at different pH values.

characteristic diffraction patterns of wurtzite ZnS (PDF# 12-0688), indicating the assembly of ZnS on the surface of SiO₂ nanofibres. The ZnS@SiO₂ composite fibres synthesized demonstrated a high degree of ZnS nanoparticle loading, and this laid the foundation for the construction of a H₂S-enhanced chemotherapy platform.

The *in vitro* H₂S gas release profile of the ZnS@SiO₂ composite fibres was investigated at different pH values. The concentration of H₂S gas in aqueous solution was measured using a spectrophotometric procedure and quantified with a standard curve (Fig. S3). Under neutral conditions, the mean cumulative concentration of H₂S gas was 4.9 μM after the first 4 h and 5.5 μM after 16 h. In comparison, under acidic conditions (designed to simulate the mild acid tumour microenvironment) the mean cumulative concentration of H₂S after 16 h reached 43.3 μM and 80.6 μM at pH = 6.2 and pH = 5.4, respectively (Fig. 3a). These results demonstrate that, in the presence of hydrogen ions (H⁺), ZnS nanoparticles on the surface of ZnS@SiO₂ composite fibres could react with H⁺ to produce H₂S gas which is a prerequisite for H₂S-enhanced chemotherapy.

Encouraged by the favourable structure of ZnS@SiO₂ fibres, DOX was used as a model drug and loaded on the fibrous mesh for chemotherapy. As shown in Fig. 3b, the mean zeta potentials of DOX and ZnS@SiO₂ fibres were 6.2 mV and -27.1 mV, implying that it is feasible to load DOX by electrostatic absorption. After drug loading, the mean zeta potential of DOX-ZnS@SiO₂ fibres became positive (3.4 mV), indicating the successful loading of DOX. UV-Vis spectra of DOX loaded ZnS@SiO₂ composite fibres (Fig. 3c) revealed that the DOX loading capacity increased with the increasing DOX concentration, while the loading efficiency decreased sharply (Fig. S5). Considering the adverse effects and therapeutic effects²⁵, the loading concentration of DOX solution was set at 100 μg/ml for subsequent studies.

Fig. 4 Relative viability of (a) 7702 cells incubated with ZnS@SiO₂ fibres for 24 h at pH = 7.4 and (b) Huh7 cancer cells incubated with DOX-ZnS@SiO₂ fibres with different concentrations at pH = 6.0 for 24 h; (c) Relative cell viability, (d) DOX fluorescent images and (e) WSP-1 fluorescent images of Huh7 cancer cells incubated with (i) pure culture medium, (ii) DOX (4.5 µg/mL), (iii) ZnS@SiO₂ fibres (100 µg/mL) at pH = 7.4, (iv) DOX-ZnS@SiO₂ fibres (100 µg/mL) at pH = 7.4, (v) ZnS@SiO₂ fibres at pH = 6.0, (vi) DOX-ZnS@SiO₂ fibres at pH = 6.0. Scale Bar: 100 µm; (f) Mechanism illustration of the H₂S-enhanced anticancer effect achieved by DOX-ZnS@SiO₂ fibres.

The *in vitro* DOX release profile of DOX-ZnS@SiO₂ composite fibres was investigated (Fig. 3d). At pH = 7.4, only 15.9% of DOX was released in the initial 24 h, which could be attributed to free diffusion of DOX in the composite fibres. In contrast, 80.6% and 52.4% of DOX in the composite fibres were released in the same period at pH = 5.4 and pH = 6.2, respectively. There are two reasons for the pH-dependent release profile. As discussed previously, under acidic conditions, ZnS nanoparticles react with H⁺ ions to release H₂S gas. As the reaction proceeds, it is suggested that the ZnS nanoparticles are liberated from silica fibres (Fig. S6). The hydrodynamic diameter of the release ZnS nanoparticles was ~190 nm with polymer dispersion index of 0.289 (Fig. S7). Moreover, H⁺ ions are likely to destroy the electrostatic interaction between ZnS nanoparticles and DOX molecules, also accelerating the drug-release.

The *in vitro* performance of DOX-ZnS@SiO₂ composite fibres was investigated using Human HL-7702 liver cells and Human Huh-7 liver cancer cells. As shown in Fig. 4a, no clear toxicity was observed when the concentration of ZnS@SiO₂ fibre was increased up to 200 µg/mL, indicating its biocompatible. This observation was thought to be due to the fact that, under neutral conditions, the concentration of H₂S released from ZnS@SiO₂ fibres is not sufficiently high to induce normal cells death. To uncover the anticancer performance, Huh7 liver cancer cells were incubated with DOX-ZnS@SiO₂ fibres under different concentrations. The pH of the culture media was adjusted to 6.0 by adding hydrochloric acid²⁶. Incubation for 24 h at concentration of DOX-ZnS@SiO₂ fibres up to 200 µg/mL resulted in 68.2% Huh7 cell death and the IC₅₀ value was ~23.6 µg/mL (Fig. 4b). To further investigate the underlying

Fig. 5 (a) Schematic illustration of the implantation procedures of DOX-ZnS@SiO₂ fibres at the tumor site; (b) body weight and (c) tumor volume variations of mice after various treatments (n=5); Digital photographs of (d) representative tumors and (e) H&E-stained tumor slices collected from different groups of mice after 14-day treatment. Scale bar: 1000 µm

influence of H₂S gas induced, the following systems were used to treat Huh7 cells: (i) pure culture medium; (ii) culture medium containing DOX of 4.5 µg/mL; (iii) ZnS@SiO₂ fibres of 100 µg/mL at neutral pH; (iv) DOX-ZnS@SiO₂ fibres of 100 µg/mL at neutral pH; (v) ZnS@SiO₂ fibres of 100 µg/mL at pH = 6.0; (vi) DOX-ZnS@SiO₂ fibres of 100 µg/mL at pH = 6.0. The concentration of DOX in Group (ii) maintained at the same level as that for the DOX-ZnS@SiO₂ fibres in Group (iv) and Group (vi). However, it should be noted that this is relatively low in comparison with that reported in DOX chemotherapy²³. As shown in Fig. 4c, after 48 h incubation, for free DOX alone (ii) only 39.5% Huh7 cell death occurred, and ZnS@SiO₂ fibres induced virtually no cell necrosis under neutral pH condition. In comparison, although certain H₂S gas may potentially release extracellularly, the cell viability (Group v) decreased to ~77.1% after incubation with ZnS@SiO₂ fibres at pH = 6.0 for 48 h. This could be attributed to the release of high concentrations of on-site H₂S gas in the acidic environment, consequently inducing cell death. The cell viability of DOX-ZnS@SiO₂ fibres decreased from ~68.6% (Group iv) to ~24.8% (Group vi) after 48 h when the culture medium varied from the neutral to pH=6, implying its amplified *in vitro* anticancer performance by the H₂S induced in an acid condition.

The examination using flow cytometry indicates that the tumour cell can effectively uptake ZnS nanoparticles released from the composite fibres during the incubation for 24 h (Fig. S8). The synergistic anticancer performance of the DOX-ZnS@SiO₂ fibre mesh, was investigated by evaluating the intracellular presence of DOX and H₂S. Under excitation at 480 nm, DOX presents intense red fluorescence at 590 nm. After 12 h incubation, as expected, Huh7 cells treated with the DOX-ZnS@SiO₂ fibrous mesh at pH=6.0 showed higher intracellular level of DOX even than those treated with pure DOX (Fig. 4d, Fig. S9). This may be associated with the continuous release of DOX from the DOX loaded fibres under acidic conditions, while pure DOX was consumed rapidly during the incubation process. In addition, using WSP-1 as a probe, cells incubated with

ZnS@SiO₂ and DOX-ZnS@SiO₂ fibres encountered significant H₂S gas release under acidic conditions (Fig. 4e, Fig. S10). On the contrary, no H₂S gas was released under neutral pH conditions.

Based on the *in vitro* findings, it is proposed that, under an acidic condition, DOX-loaded ZnS nanoparticles on the surface of silica fibres are released and taken-up by Huh7 cells. Subsequently, both DOX and H₂S gas are released intracellularly. The H₂S at the certain concentration may amplify the anticancer efficacy of DOX (Fig. 4f). This is attributed to that H₂S gas can effectively suppress the activity of catalase which is the key enzyme for H₂O₂ decomposition²⁶. Thus, increased oxidative stress at the tumour site leads to cell apoptosis and improved efficacy of DOX.

The *in vivo* therapeutic performance of DOX-ZnS@SiO₂ fibre mesh was investigated using a Huh7 mouse tumour model. ZnS@SiO₂ fibres and DOX-ZnS@SiO₂ fibres were implanted in the tumour site via minimally invasive surgery, respectively (Fig. 5a). Compared with the control group (Group 1), no clear weight variation was observed in the mice treated with intratumorally injected DOX (Group 3), ZnS@SiO₂ fibres (Group 2) and DOX-ZnS@SiO₂ fibres (Group 4), indicating that there was no acute toxicity associated with the treatment systems (Fig. 5b). On day 14, the tumour progression of mice treated with low dosage of DOX was partially inhibited (Fig. 5c). Mice treated with ZnS@SiO₂ fibres showed considerable tumour suppression compared with the control group (Fig. S11 and Fig. 5d). However, for those treated with DOX-ZnS@SiO₂ fibrous mesh, the tumour sizes shrank after 14 days treatment. Furthermore, H&E-stained microscopy slices of tumors indicated that the tumour tissues treated with DOX-ZnS@SiO₂ fibres were more seriously damaged than those with DOX only (Fig. 5e). However, in the control groups with ZnS@SiO₂ fibres, the tumour tissues retained their normal pathology. Overall, the *in vivo* results suggest that the use of a DOX-ZnS@SiO₂ fibre mesh offers an effectively enhanced chemotherapeutic efficacy.

In this study, for the first time, a fibrous silica mesh with an active surface comprising ZnS nanoparticles is synthesized to enable H₂S gas combined chemotherapy in a localized manner. ZnS nanoparticles, used as a H₂S-generating donor, were assembled on the surface of electrospun SiO₂ nanofibres. Under acidic conditions, ZnS@SiO₂ nanofibres released H₂S gas due to the reaction of ZnS with H⁺ ions. The cumulative H₂S gas concentration was up to 80.63 μM at pH= 5.4, which was significantly higher than that in normal tissue. ZnS nanoparticles may liberate from ZnS@SiO₂ fibres in the presence of H⁺ ions and can be effectively taken-up by cancer cells. The DOX loaded ZnS@SiO₂ fibres exhibited a pH-dependent drug release profile. The *in vitro* study demonstrated that, due to the combined effects for intracellular DOX and H₂S induction, DOX-ZnS@SiO₂ fibres had exceptional *in vitro* and *in vivo* performance when compared with pure DOX or ZnS@SiO₂ fibres. This study offers a distinctive approach to materials design by combining fibres and particles for LDDSs and will pave the way not only for enhanced chemotherapy, but also other modalities of ROS-based cancer treatment.

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Conflicts of interest

There are no conflicts to declare.

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