Electronic Supplementary Material (ESI) for Biomaterials Science.

SUPPORTING INFORMATION FOR THE MANUSCRIPT

Amplification of Ferroptosis with Liposomal Nanoreactor Cooperates with Low-Toxic Doxorubicin Apoptosis for Enhanced Tumor Chemotherapy

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1. Quantitative determination of iron content

For quantitative determination of iron content, 20 mg LiMOFs was digested with 20 mL pH 2 HCl solution under vortex. Then, the solution was transferred into a 50 mL volumetric flask and the flask was replenished with pH 2 HCl to the scale mark. Samples (n=3) were withdrawn and filtered with 0.45 μm membranes after the mild shake. The o-phenanthroline method was employed to determine the assay of iron.

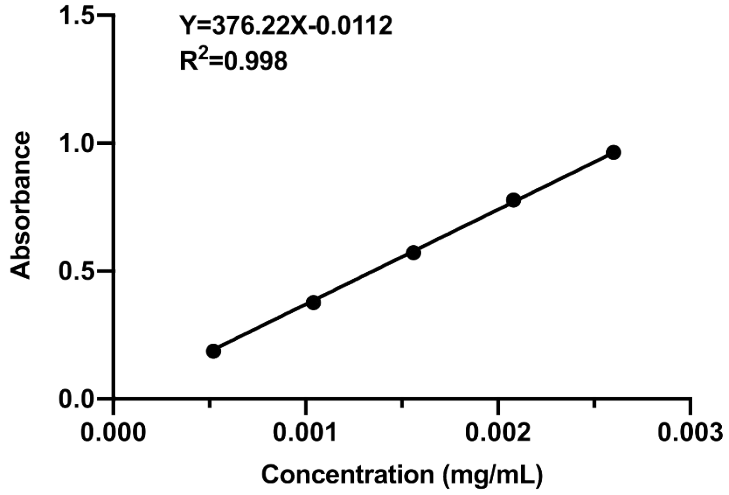

Fig. S1 UV-Vis Standard curve of Fe solution

2. Stability test of ART in LiMOFs

5 mg ART and 50 mg LiMOFs were accurately taken and placed into a 50 mL centrifuge tube. The centrifuge tube was then filled with 40 mL pH 7.4 PBS and sealed to be stored under room temperature. 1 mL sample was collected from the tube at day 1, 5, and 10 respectively, and then mixed with 4 mL 0.2% NaOH solution. The whole mixture was placed under 60 ℃ for 30 min. Vortex was employed on it every 10 min. After cooling down to room temperature, the solution absorbance was detected by the UV-Vis method. The same method was carried out to test the stability of free ART in pH 7.4 PBS.

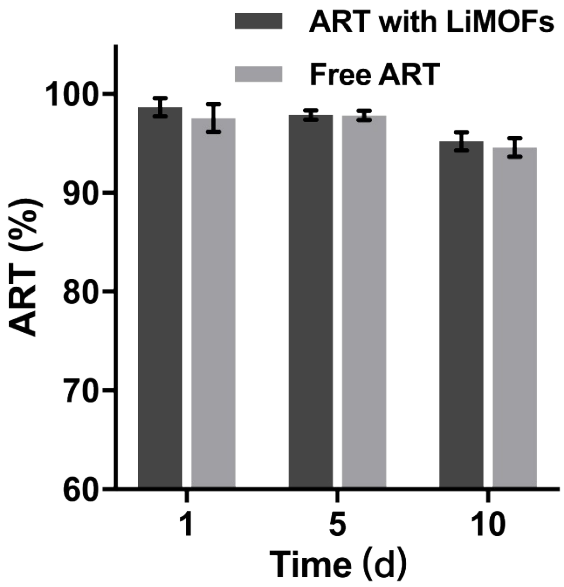

Fig. S2 ART percentage in LiMOFs suspension within 10 days. Data were presented as mean±SD (*n*=3).

3. Fluorescence intensity of LPO

C11-BODIPY$^{581/591}$ was used to measure LPO in cells. The fluorescence was monitored by CLSM and the fluorescence intensity was quantified using ImageJ software.

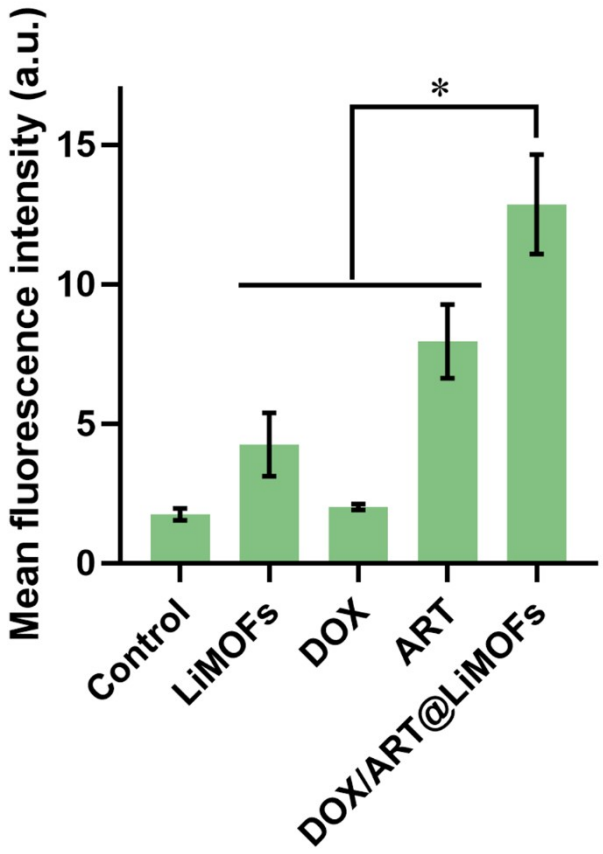

Fig. S3 Fluorescence intensity of LPO stained with fluorescent BODIPY-C11 (*$P < 0.05$ *vs.* DOX/ART@LiMOFs).

4. *In vitro* synergistic effect study of DOX and ART

The combined treatment effect of DOX and ART on the 4T1 cell line was also characterized by MTT assay. The quantitative analysis of the combined therapeutic effects of DOX and ART in the DOX/ART@LiMOFs NPs system was performed using the combination index (CI) theorem of Chou-Talalay (Chou et al., 1994), which offers quantitative definition of the antagonism (CI > 1), synergism (CI < 1), and additive effect (CI = 1), and has been widely used to analysis the synergism/antagonism effect in drug combination (Chung et al., 2014). The general equation for the combination index $^{n}(CI)x$ for n drugs at x% inhibition is described as follows:

$$n(CI)x = \sum_{j}^{n} = \frac{(D)i}{(Dx)j}$$

where $(D)_i$ is the concentrations of *n* components used in combination to achieve x% drug effect, and $(Dx)_j$ is the concentrations of each drug alone to achieve the same effect.

Table S1 The cooperativity index (CI) of DOX and ART in different cell lines

Cell	CI
4T1	0.197
A549	0.689
IMR-32	0.409

5. Tumor inhibition rate(%)

After the treatment cycle, the tumor mass was dissected and weighed, and the tumor mass inhibition rate was calculated. Tumor inhibition rate = (M control group - M experimental group) / M control group × 100%.

Table S2 Comparison of tumor inhibition rate of mice in each group.

Group	Tumor inhibition rate(%)
NS	--
ART	27.88 ± 10.71**
ART@LiMOFs	33.27 ± 4.06**
DOX+ART	43.32 ± 6.38*
DOX/ART@LiMOFs	58.31 ± 6.77

Data are shown as the means ± SD (n = 3) (*P < 0.05 and **P < 0.01 *vs.* DOX/ART@LiMOFs group).

6. In vivo synergistic effect study of DOX and ART

The combined therapeutic effect of DOX and ART on 4T1 tumor-bearing mice was also characterized by tumor inhibition experiments. Two-drug interaction index (CDI): CDI=AB/(A*B). AB is the ratio of the tumor weight between the two-drug combination group and the control group, and A or B is the ratio of the tumor weight of each drug alone group and the control group. When CDI<1, the two drugs are synergistic.

The combined treatment effect of DOX and ART in 4T1 tumor-bearing mice was also evaluated by the interaction index (CDI). The results are shown in Table S3, the CDI is less than 1, indicating that the preparation constructed by the combination of DOX and ART has a synergistic effect in vivo, which is consistent with the in vitro evaluation results.

Table S3 The cooperativity index (CDI) of DOX and ART in 4T1 tumor-bearing mice

Tumor-bearing model	CDI	p-value
4T1	0.726	$p<0.05$