Electronic Supplementary Material (ESI) for Biomaterials Science.

Electronic Supplementary Information for:

Supramolecular copolymer modified statins-loaded discoidal rHDLs for atherosclerotic anti-inflammatory therapy by cholesterol efflux and M2 macrophage polarization

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S1. Determination of drug content

The gained sample was centrifugated by 10000 rpm for 10 min, and the content of the drug was determined by HPLC (Shimadzu, Japan). The HPLC system included a Shim-pack V-ODS column (5 μm, 150 × 4.6 mm), a UV-DAD detector (Shimadzu, Japan) operated at 237 nm and a pump (LC-20AD). The mobile phase was acetonitrile /water (60/40, v/v). The flow rate was 1.0 mL/min and the column temperature was 30°C.

S2. Determination of attachment efficiency of PF/TC

PF/TC-AT-d-rHDL was subjected to ultrafiltration centrifugation[1] to gain the supernatant containing free PF/TC. The content of PF/TC was quantitatively measured by determining the content of iron element using ICP-MS (ICAP-Qc, Thermo, U.S.A.).[2] The attachment efficiency of PF/TC in PF/TC-AT-d-rHDL was calculated using following equation:

$$AE\ (\%) = \frac{M_0 - M_1}{M_0} \times 100\ \%$$

M_0 represented the content of PF/TC in PF/TC-AT-d-rHDL, and M1 represented the content of free PF/TC.

S3. Storage stability of PF/TC-AT-d-rHDL *in vitro*

The prepared PF/TC-AT-d-rHDL was stored at 4 ℃ for 7 days. The particle size and zeta potential of the gained sample collected at a predetermined time intervals were determined by ZetaPlus particle size and zeta potential analyzer (Brookhaven Instruments, U.S.A.).

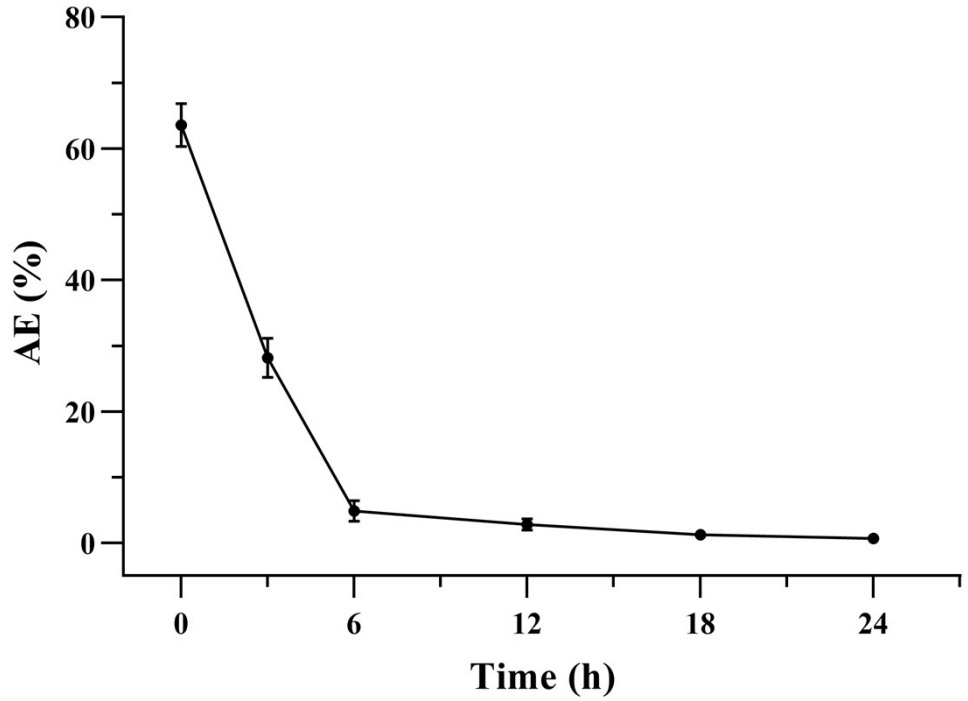

Fig. S1. AE of PF/TC in PF/TC-AT-d-rHDL after reacting with H_2O_2 for different times (Mean ± SD, n = 3).

The addition of H_2O_2 decreased the AE value, which could be explained by the reason that H_2O_2 oxidized the Fc into Fc^+ to dissociate the supramolecular copolymer modified in the carrier and further increased the content of the iron element in the supernatant. The significant reduction of AE within 6 h (from 63.58 ± 3.26% to 4.86 ± 1.56%) illustrated that PF/TC-AT-d-rHDL possessed high ROS-sensitivity and the modified PF/TC could rapidly dissociate in presence of H_2O_2.

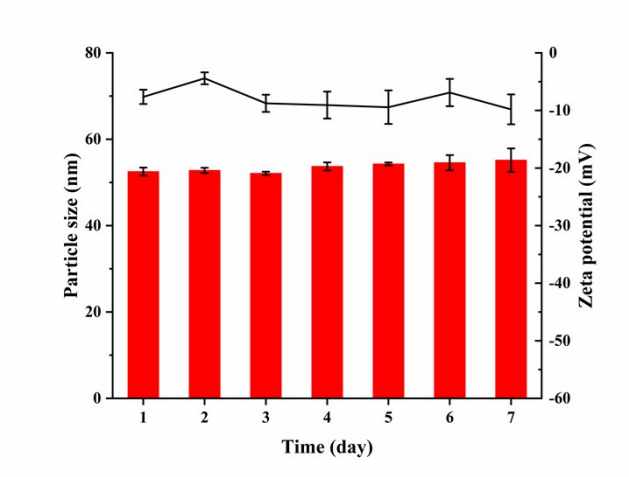

Fig. S2. The in vitro storage ability of PF/TC-AT-d-rHDL during 7-days' storage (Mean ± SD, n = 3).

There were no obvious changes in the particle size and zeta potential of PF/TC-AT-d-rHDL after storing the carrier at 4 ℃ for 7 days, which demonstrated that the PF/TC-AT-d-rHDL possessed excellent storage stability *in vitro*.

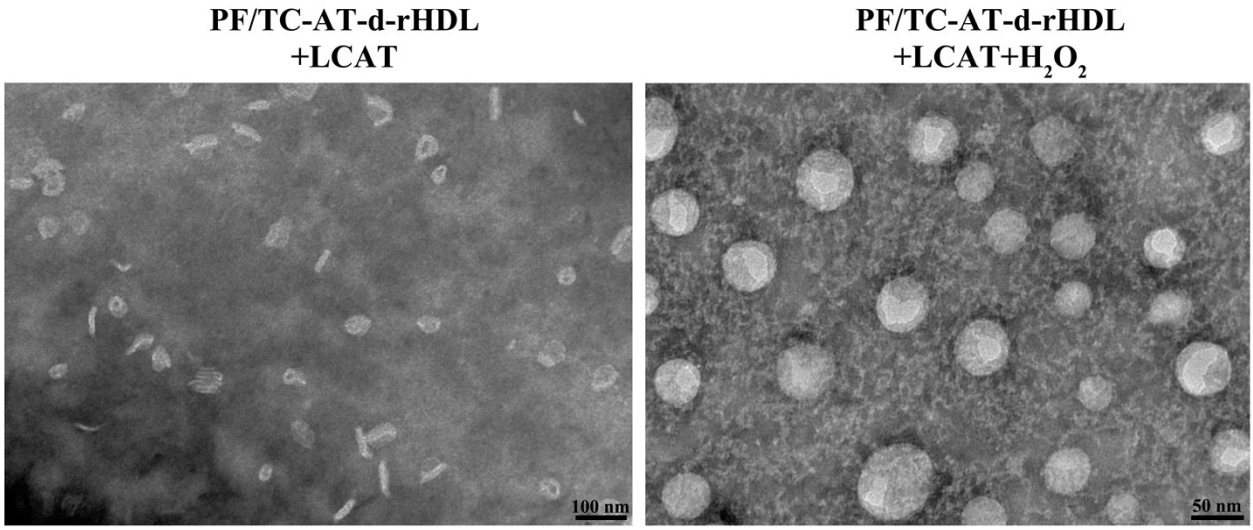

Fig. S3. The TEM images of PF/TC-AT-d-rHDL after incubating with LCAT (1 μg mL^{-1}) or LCAT (1 μg mL^{-1}) + H_2O_2 (1 mM).

In a larger view, the addition of H_2O_2 did trigger the morphological transformation of the PF/TC-AT-d-rHDL from discoidal into a spherical one in the presence of LCAT, which further revealed that H_2O_2 could restore the remodeling behavior of the carrier.

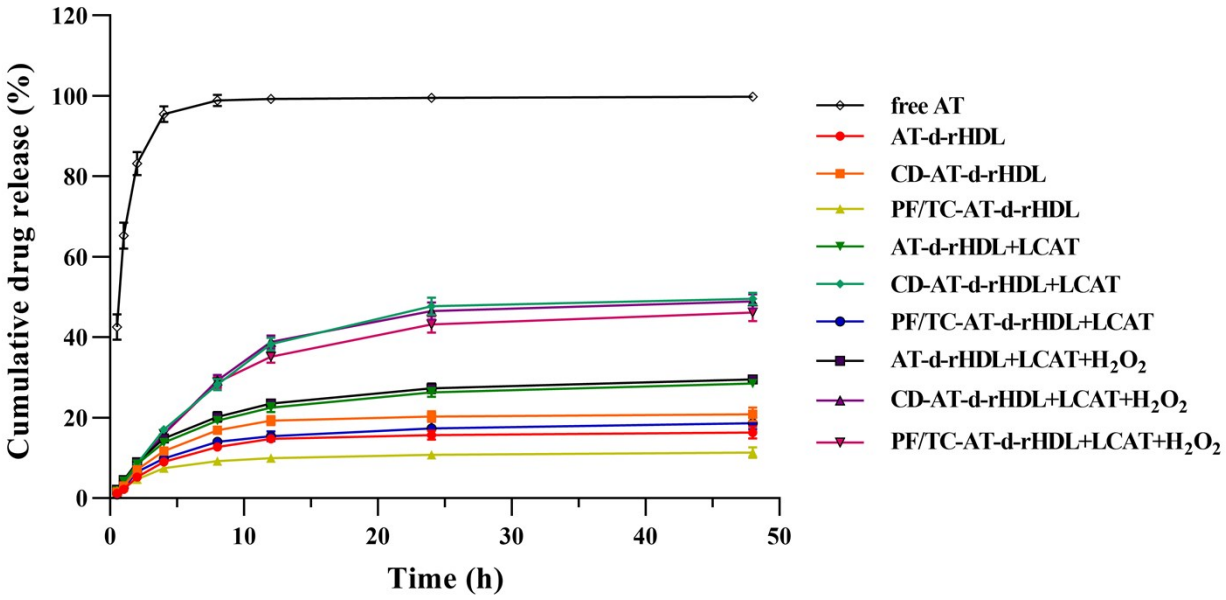

Figure S4. The cumulative drug release in the system of free drugs and different preparations (Mean ± SD, n = 3).

The cumulative release of free AT from dialysis bag was more than 90 % in 4 h, indicating that dialysis bags did not affect the release of free drugs and proved the feasibility of the dialysis bag method in investigating the responsive drug release behaviors. All preparations exhibited the sustained drug release behaviours. The difference of drug release among these preparations was highly consistent with their remodeling extent, which illustrated that cholesterol efflux from cholesterol donor mediated by the d-rHDL nanoparticle could trigger the remodelling behaviour of the carrier to release encapsulated drugs.

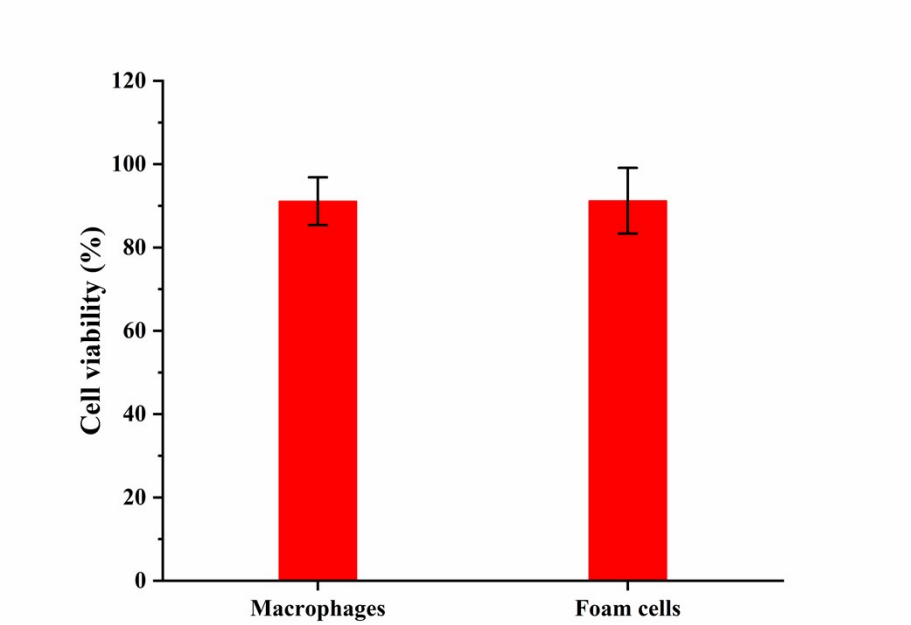

Fig. S5. Cell viability of H_2O_2 at the concentration of 1 mM on macrophages and foam cell (Mean ± SD, n = 5).

In view of the physiological concentration of H_2O_2 in pathological lesions,[3] the biocompatibility of 1 mM H_2O_2 was investigated. The high cell viability revealed that 1 mM H_2O_2 had no obvious cytotoxicity on macrophages and foam cells, providing that such concentration of H_2O_2 was safely used in cell-related experiments.

References

(1) X. Gu, S. Yu, Q. Peng, M. Ma, Y. Hu and B. Zhou, *Anal. Biochem.*, 2020, **588**, 113475.

(2) B. Jackson, S. Harper, L. Smith and J. Flinn, *Anal. Bioanal. Chem.*, 2006, **384**, 951–957.

(3) E. Schröder, and P. Eaton, *Curr. Opin. Pharmacol.*, 2008, **8**, 153–159.