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Supporting Information

Atorvastatin-based nanoparticles-enclosed glyceryl monostearate as a targeted drug carrier for inhibition of atherosclerosis lesions

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SUPPLEMENTARY RESULTS

Stability of ATR nanoparticles through the DLS analysis

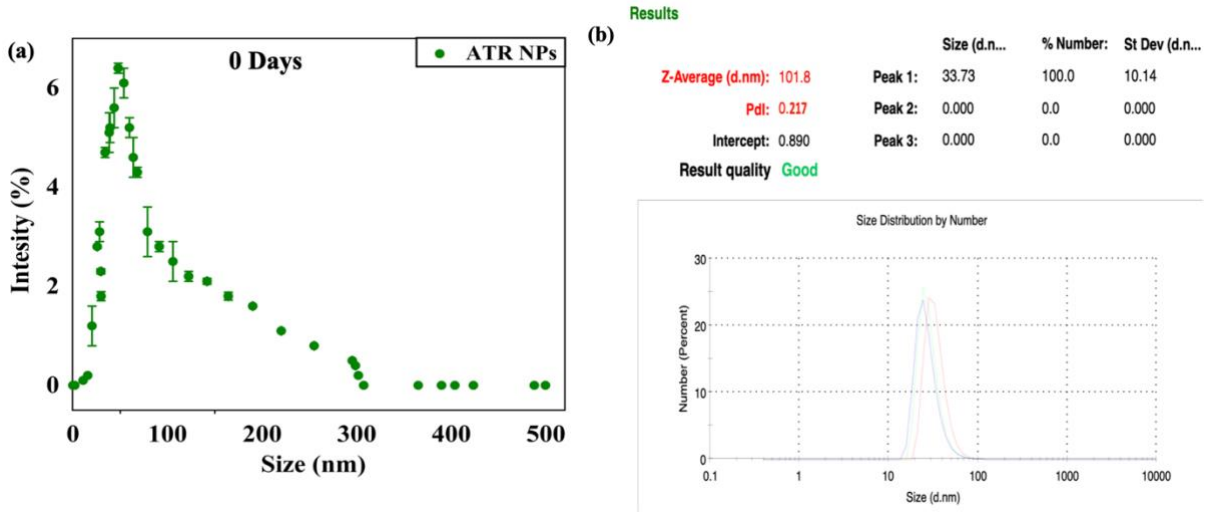


Fig. S-1: (a& b) Representative particle size intensity curve and distribution of ATR NPs at 0 days.

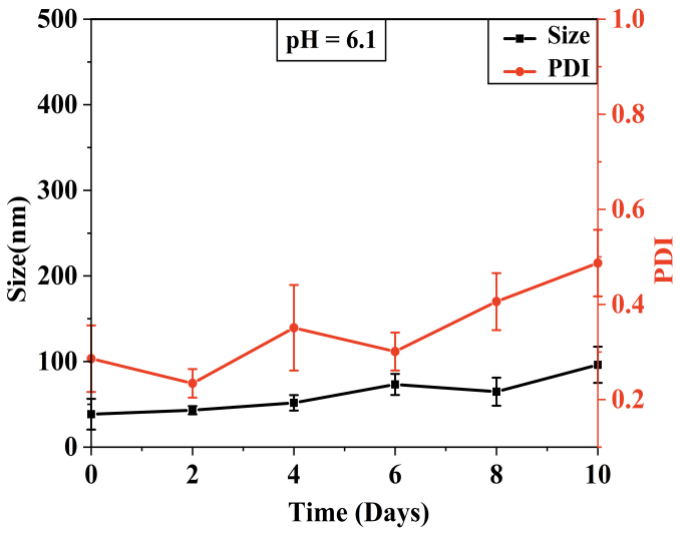
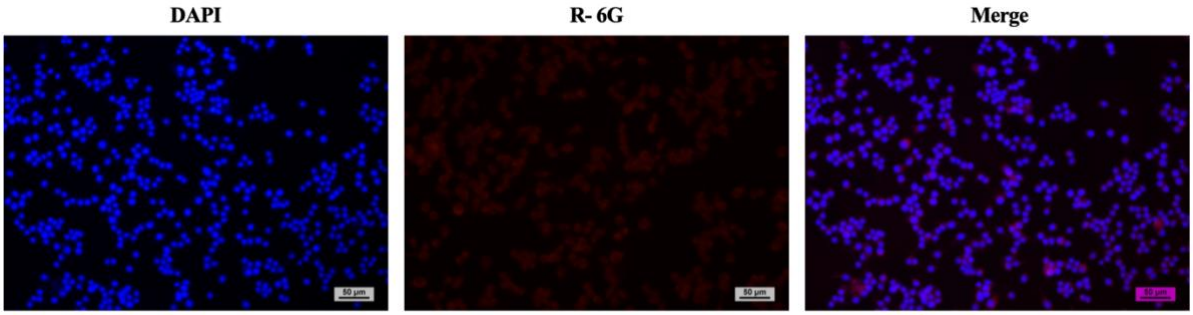


Fig. S-2: Particle stability of ATR NPs of slightly acidic early/maturing endosomes (pH=6.1) at 0 to 10 days via dynamic light-scattering analysis.

Cellular uptake of pure ATR drugs

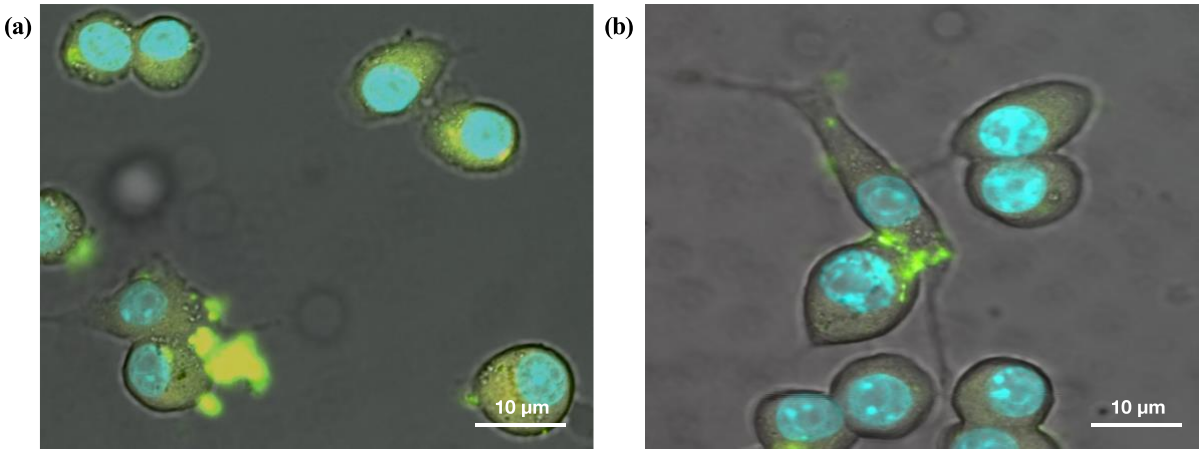
Therefore, we investigated the uptake of ATR nanoparticles (NPs) in foam cells and macrophages. To study hydrophobic interactions, we utilized the red fluorescent dye Rhodamine-6G (R-6G) to measure cellular uptake in ATR NPs and pure ATR, focusing particularly on the lipid core and R-GG. As depicted in red, fluorescence declined in foam cells treated with pure ATR tagged with R-6G for 4 hours in Fig. S-3.



Pure ATR tagged with R-6G in Foam cells

Fig. S-3: Fluorescent microscopy images displayed cellular internalization in foam cells treated with pure ATR at 4 h. (scale bar: 50 μm).

Cellular co-localization of ATR NPs drugs treated chloroquine



ATR NPs Chloroquine ATR NPs

Fig. S-4: Effect of chloroquine on ATR Nanoparticle in lysosome through the confocal microscope (a) without chloroquine and with chloroquine.

Effect of ATR NPs on Scavenger Receptor Surface

Method :

The RAW 264.7 macrophage cell line was cultured in 6-well plates at a density of 1×10^5 cells per well and incubated in 1 mL of cell culture medium for 12 hours. Subsequently, the cells were treated with ox-LDL to induce foam cell formation and then exposed to nano drugs (pure ATR and ATR NPs) or left untreated for 24 hours. This treatment aimed to evaluate the impact of these nano drugs on the expression of the foam cell surface scavenger receptor. The medium was discarded, and the macrophage cells were washed with PBS following a 30-minute fixation in 4% paraformaldehyde (PFA). The cells were then incubated for 10 minutes at 20°C in a solution containing 0.2% Triton X-100, followed by blocking with 2% bovine serum albumin (BSA) in PBS. At room temperature, the cells were incubated with polyclonal anti-rabbit CD36 antibody (Thermo-Fisher) for 4 hours. After washing, the cells were

incubated for 1 hour with the secondary antibody, goat anti-rabbit Alexa Fluor 488. The cell surfaces were then cleaned, involving two PBS washes, air drying, application of mounting media, and subsequent observation at 10× magnification using a confocal fluorescence microscope.

Results :

CD36 has been revealed to substantially impact atherosclerotic lesion progress and modified lipid deposits after the ATR-loaded nanoparticles in cardiovascular disease. To determine the efficiency of different ATR NPs in inducing scavenger receptor expression on macrophages in the presence of OX-LDL-induced foam cells, CD36 expression was measured following cotreatment with pure ATR NPs on foam cells. We quantitatively assessed the modulation of CD36 protein expression, a key determinant of lipid uptake, as depicted in Figure 5 (b). The upregulation of CD36 within foam cells was significantly elevated. After being exposed to ATR NPs and pure ATR for 24 hours, foam cells showed decreased expression levels of scavenger receptors and CD36, a lipid receptor protein in Fig. S-5 (c) and S-5 (d), and Quantification of the CD36 surface receptor in green accumulation in cells are expressed as average ± SD (n = 3); *** $p < 0.001$) at Fig. S-5. These findings show that ATR NPs and pure ATR inhibit foam cell growth via lowering lipid receptor expression.

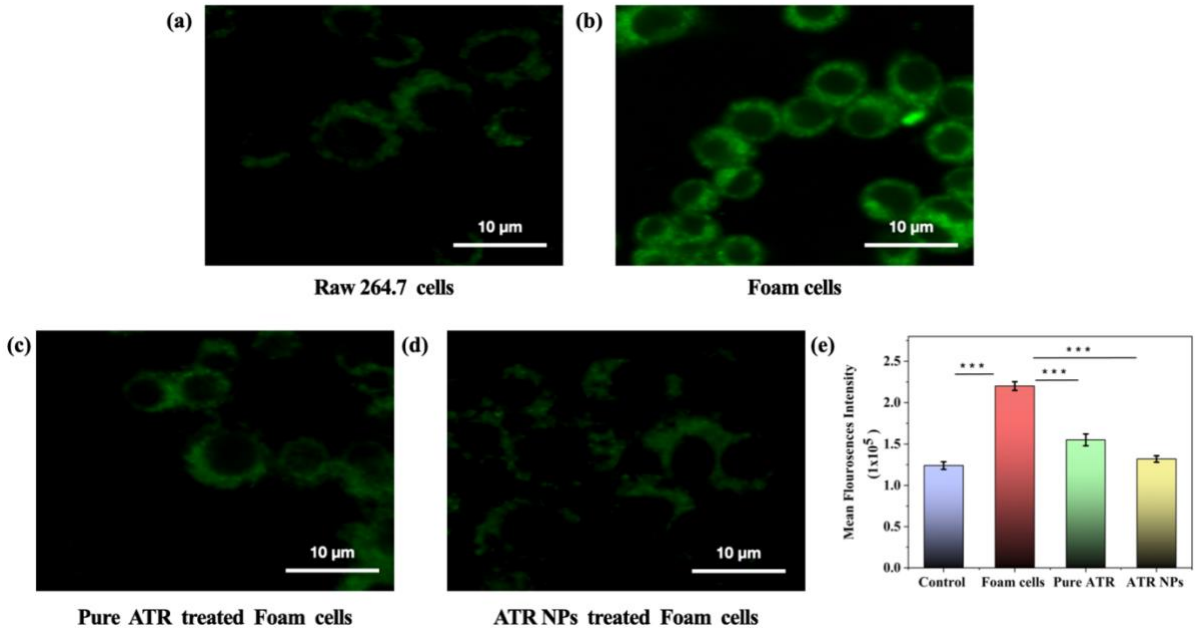


Fig. S-5: Illustrates immunofluorescence micrographs illustrating CD36 in green in (a) normal macrophages and (b) understanding the mechanisms for uncontrolled ox-LDL uptake in atherosclerosis foam cells. Furthermore, (c) ATR NPs and (d) pure ATR inhibit ox-LDL uptake in foam atherosclerosis, mostly for uncontrolled ox-LDL uptake, (e) Quantification of the CD36 surface receptor in green accumulation in cells are expressed as average ± SD (n = 3); *** $p < 0.001$).