Supplementary Information

Preparation and Study on Anti-tumor Effect of Chitosan-coated Oleanolic Acid Liposomes

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Part S1. Cytotoxicity studies

In *vitro* cytotoxicity testing was done using the direct contact method with the test samples based on ISO 10993-5 standards.[1, 2] The test samples (OA solution, blank liposomes, OA liposomes, chitosan-coated liposomes without OA and chitosan-coated OA liposomes) and the control sample (phosphate-buffered saline (pH 7.4)) were placed on appropriate well of h9c2 rat myocardial cells in triplicate. Among them, the concentrations of OA was 100 μg/mL.

Briefly, the h9c2 rat myocardial cells were seeded in 96-well flat-bottomed plates at a seeding density of 2.0×10^4 cells/well and grown for 24 hours at 37 °C in a 5% CO_2 atmosphere. At that time, the cells were attached and grew. The test samples and control sample were filtered through sterile 0.22 μm filter membrane. Each sample

was done in triplicate (100 µL / well) into the appropriate wells of culture dishes. The h9c2 rat myocardial cells were incubated for 24 hours under the conditions mentioned above with the drugs, and the cells' viability was analyzed using the MTT agent of all the formulations. After 24 hours, the medium was discarded and the cells were incubated with 200 µL fresh medium containing 0.5mg/mL MTT for 4 hours. After removed the unreduced MTT, 150 µL of DMSO was added to each well to dissolve the formazan crystals. The absorbances of the samples were measured at 490 nm using a microplate reader. The assays were performed for three times. The relative growth rates (RGR) of cells were calculated according to the following equation. The toxicity of the samples was evaluated.

$$\text{RGR\%} = \frac{\text{The absorbance value of test samples}}{\text{The absorbance value of control sample}} \times 100\%$$

Preliminary cytotoxicity evaluation was done to assess the potential of test samples for biomedical applications. In order to detect the cytotoxicity of the OA solution, blank liposomes, OA liposomes, chitosan-coated liposomes without OA and chitosan-coated OA liposomes, an MTT assay was performed. The results of cells' relative growth rates were shown in Fig. S1. The relative growth rates were 94.24% ± 1.37%, 103.45% ± 3.53%, 93.6% ± 1.07%, 100.83% ± 3.09%, 102.64% ± 3.23%, respectively. The samples were found to be non-cytotoxic to normal cells and had good biocompatibility.[1, 2]

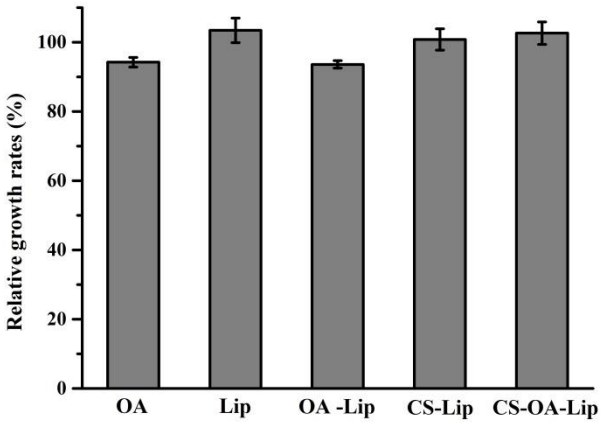

Fig. S1. Relative growth rates of the h9c2 rat myocardial cells. All values are expresses as mean ± SD (n=3).

Abbreviations: OA (OA solution); Lip (blank liposomes); OA-Lip (OA liposomes); CS-Lip (chitosan-coated liposomes without OA); CS-OA-Lip (chitosan-coated OA liposomes).

References

1. S. Dawlee, A. Jayakrishnan and M. Jayabalan, *Journal of Materials Science: Materials in Medicine*, 2009, **20**, 243-250.
2. Biological evaluation of medical devices. Part 5. Tests for cytotoxicity: In vitro methods. ISO-10993-5, 1992.