Supporting information

Enhanced anticancer effect of lysozyme-functionalized metformin-loaded shellac nanoparticles on a 3D cell model: Role of the nanoparticle and payload concentrations

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(*Biomaterials Science 2024*)

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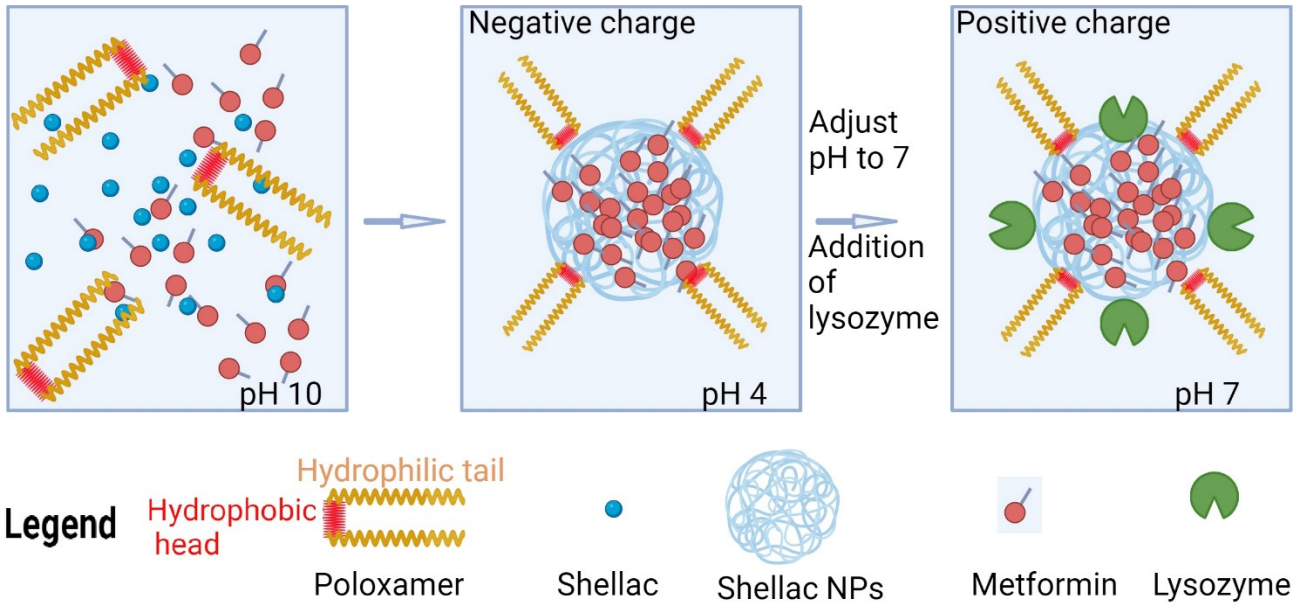

Figure S1. Schematic for the preparation of the lysozyme coated metformin-loaded shellac NPs. Stage 1 includes co-precipitation of an aqueous solution of metformin, Poloxamer 407 and shellac induced by a pH drop from 10 to 4. The metformin-loaded nanoparticles are sterically stabilized by the Poloxamer 407. Stage 2 includes adjusting the pH to 7 and coating with a cationic protease (lysozyme) achieved by electrostatically driven adsorption.

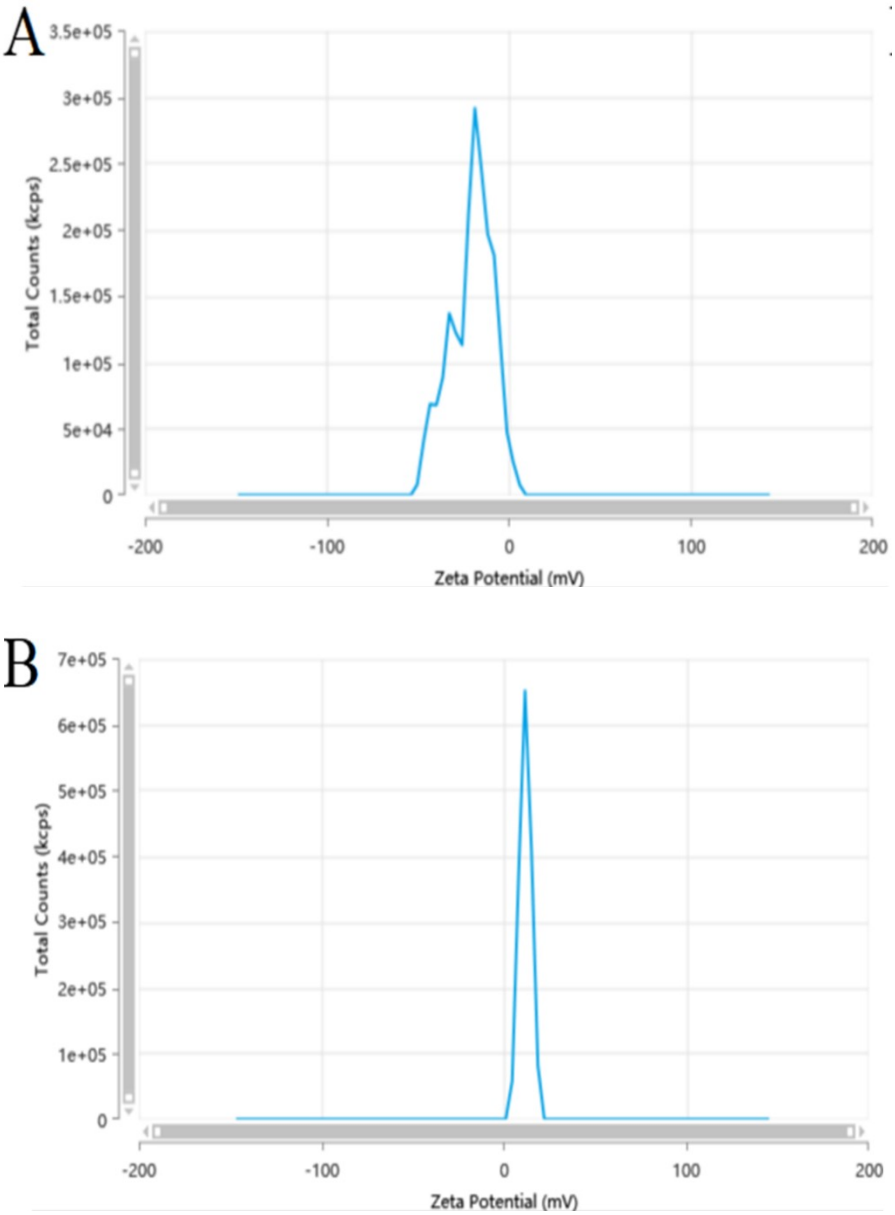

Figure S2: Zeta-potential distribution of 0.2 wt% Shellac-0.1 wt% Metformin-0.25wt% P407 coated at different concentration of lysozyme: (A) 0 wt% lysozyme; (B) 0.25 wt% lysozyme.

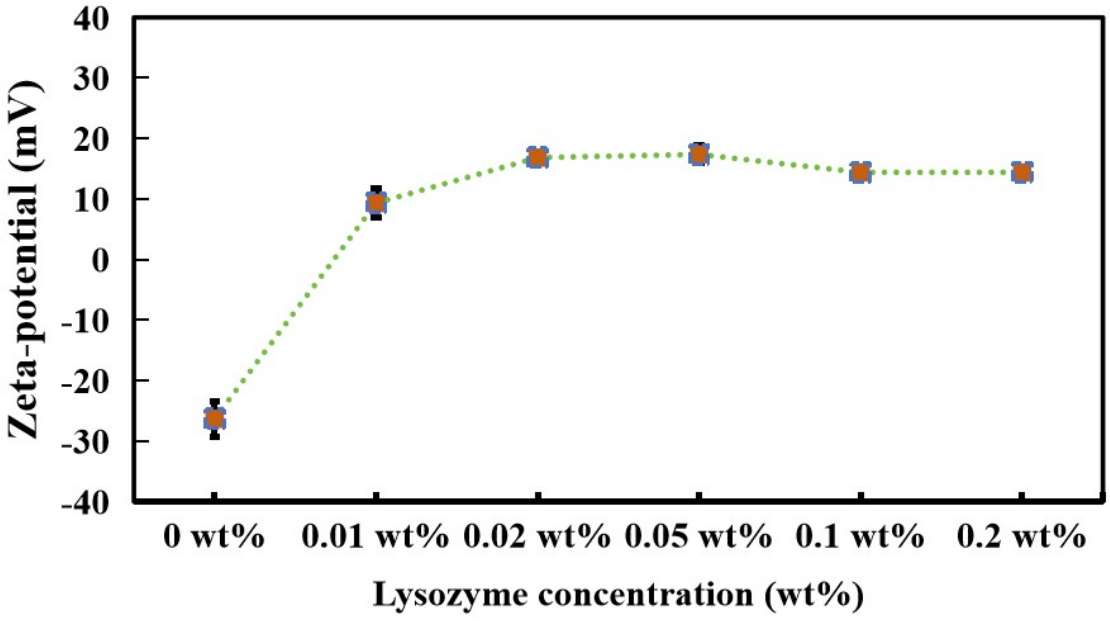

Figure S3: Zeta-potential of 0.2wt% Shellac-0.1 wt% Metformin-0.25wt% P407 coated at different concentration of lysozyme.

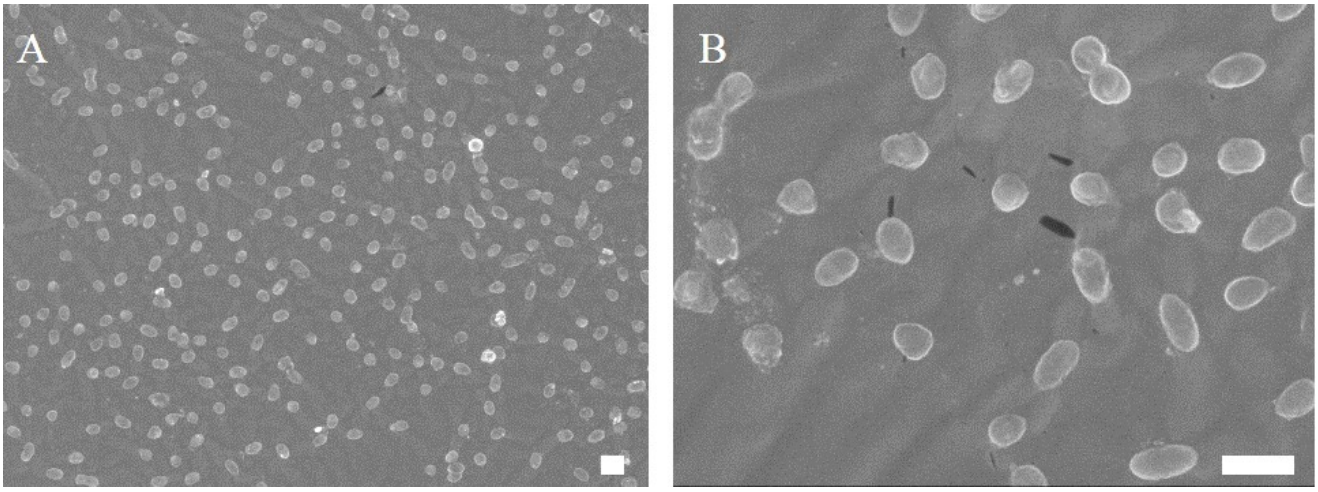

Figure S4: SEM observation of the 1000 times diluted 0.2wt% Shellac-0.25wt% Lysozyme- 0.2wt% Metformin- 0.25wt% P407 NPs with different magnification. The scale bar is 100 nm.

Estimate of the metformin loading per shellac nanoparticle

A rough estimate of the number of metformin molecules per nanoparticle can be obtained by estimation of the number of nanoparticles per 100 g of dispersion at fixed amount of. With shellac density being 1.035 g/cm^3 and average particle diameter 60 nm and shellac concentration 0.2 wt%, the number of particles is

$$N_p = 100 \times 0.2/100/1.035/(4\pi/3 \times (30 \times 10^{-7})^3) = 1.71 \times 10^{15}$$

For overall concentrations of metformin of 0.1 wt% and 90% encapsulation efficiency, one can estimate the total number of metformin molecules encapsulated in 100 g of dispersion:

$$N_{met} = 100 \times 0.1/100/129 \times 6.02 \times 10^{23} \times (90/100) = 4.65 \times 10^{20}$$

Thus, a typical number of metformin molecules per shellac nanoparticle is

$$N_{met\_per\_particle} = N_{met} / N_p = 1.71 \times 10^{20} / 2.96 \times 10^{15} = 2.45 \times 10^5$$

This estimate is done without taking into account the contribution of the encapsulated metformin to the size of the nanoparticle.