

Supplementary Materials:

1. Materials and Methods

1.1 Synthesis of PB analogue and PB@Lipo

For synthesis of PB analogue, PB analogue were prepared by hydrothermal method. Firstly, $K_3[Fe(CN)_6]$ (1.0 mM), $MnCl_2$ (0.75 mM) with PVP (3 g) were dissolved in 40 mL HCl (0.01 M) solution and stirred for 1 h at room temperature until it became yellow transparent solution. Then placed the solution in an electric oven at 80 °C for 24 h. After 24 h, the PB analogues were obtained by centrifugation frequently and washed with DI water and ethanol several times. The precipitation was dried under vacuum (Figure 1A). For synthesis of PB@Lipo, a reverse-phase mixture was prepared by mixing chloroform, isopropyl ether, and methanol (6:6:1). Then, a lipid mixture was obtained from the resulting mixture by simultaneously adding hydrogenated soybean phospholipids (15.9 μ M), cholesterol (6.4 μ M), and 1,2-distearoyl-sn-glycero-3-phosphor ethanolamine-N-biotinyl[poly(ethylene glycol)]-2000 (0.2 μ M). Meanwhile, 1.0 mL of PB analogues aqueous solution (5 mg/mL) was added to the lipid mixture (1 mL). After shaking for 5 min on a shaker, the solvent was evaporated at 45 °C under nitrogen to form a gel-like suspension. Finally, PB@Lipo were dispersed in PBS (1.0 mL, pH 7.4) and stored at 4 °C until use(Figure 1B)[1] .

1.2 Characterization of PB@Lipo

The microstructure and physical characteristics of PB@Lipo were observed using a JEM-2100F transmission electron microscope (TEM). The size and zetapotential of PB@Lipo was measured using dynamic light scattering (DLS, Nano ZS90 Zetasizer, Malvern). The absorption spectrum was recorded using a himadzu UV-3600 ultraviolet-visible spectrophotometer. Determination of catalase (CAT) enzyme activity in tissues using a specific spectrophotometric kit. Glutathione peroxidase activity was determined using the Glutathione Peroxidase (GPx) Kit.

1.3 Models and treatments for full-thickness skin defects

Prior to the experiment, mice were acclimatized in cages for 7 days. Twenty-seven 6-8 week old C57BL/6 mice were randomly divided into CON, PBS and PB@Lipo groups of 9 mice each. 12 h of light and 12 h of dark alternated daily. Additionally, food and drinking were available. After anesthetizing mice with 3% sodium pentobarbital, the back skin of mice was rectangular full-thickness excision with a size of 1.5cm*1.5cm. Mice in the PBS group were treated with PBS on days 0, 2, 4, 6, 8, 10 and 12, and PB@Lipo (0.1 mg/mL, 20uL) was applied to the wounds of mice in the PB@Lipo group. Meanwhile, the wounds were observed and photographed on days 0, 3, 7 and 14. After the mice were sacrificed with an excess of 3% pentobarbital sodium on the 14th day, the heart, liver, spleen, lung, kidney and wound skin were collected and fixed in 4% paraformaldehyde solution.

1.4 Hematoxylin-eosin (H&E) staining

Conventional sucrose dehydration, OCT embedding and frozen sectioning were performed on heart, liver, spleen, lung, kidney and wound tissue. According to the instructions of the hematoxylin-eosin staining kit (Solarbio), the sections were stained sequentially with hematoxylin, differentiation solution and eosin. After rapid dehydration, the sections were sealed with neutral gum for further observation by light microscopy.

1.5 Masson trichrome staining

Wound tissue was subjected to conventional sucrose dehydration, OCT embedding and frozen sectioning. The sections were stained with hematoxylin staining solution, acid ethanol differentiation solution, Masson blue solution, Lichun red magenta staining solution, weak acid solution, phosphomolybdenum acid solution, aniline blue staining solution according to the instructions of the Masson's Trichrome Stain Kit (Solarbio). After rapid dehydration, the sections were sealed with neutral gum for further observation by light microscopy.

1.6 Immunofluorescent staining

Wound tissue was subjected to conventional sucrose dehydration, OCT embedding followed by frozen sections. Afterwards, the obtained sections were perforated with 0.3% Triton X-100 for 15 minutes, blocked with goat serum (1:10) for 2 hours. Then, anti-CD86 (1:200, Affinity) antibody, anti-ARG-1 (1:200, Affinity) antibody, anti-COL-1 (1:200, Affinity) antibody, and anti-COL-3 (1:200, Affinity) antibody were added to incubate overnight at 4°. At final, secondary antibody (Goat Anti-Rabbit IgG (H+L) FITC-conjugated, 1:1000, Affinity) was added to incubate for 2 hours at room temperature. After sealing with anti-fluorescent quenching agent DAPI, fluorescence images were observed under a light microscope.

1.7 Statistical analysis

Results were expressed as mean \pm standard deviation (SD).

One-way analysis of variance (ANOVA) was used for statistical analysis. $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) were statistically significant.

References

- [1] L.-J. Zhi, A.-L. Sun, D. Tang, *In situ* amplified photothermal immunoassay for neuron-specific enolase with enhanced sensitivity using Prussian blue nanoparticle-loaded liposomes, *Analyst*. 145 (2020) 4164–4172. <https://doi.org/10.1039/D0AN00417K>.