

Ultrasound-triggered immunogenic nanotherapeutics for optimizing osteosarcoma therapy by enhancement of tumor microenvironment responsive delivery

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1. A macroscopic photograph of Ce6 NPs@Gel with different concentration and ratio of CMC and OD.

(a)



(b)

$$V_{\text{CMC}(3.33\%)} : V_{\text{Ce6 NPs}(300\mu\text{g/ml})} : V_{\text{OD}(5\%)} = 1:1:1$$

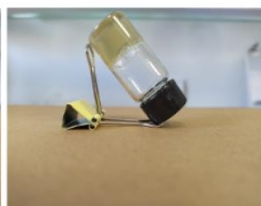


(c) $V_{\text{CMC}(1.67\%)} : V_{\text{OD}(5\%)} = 1:1$

$V_{\text{CMC}(1.67\%)} : V_{\text{OD}(5\%)} = 1:2$

$V_{\text{CMC}(1.67\%)} : V_{\text{OD}(5\%)} = 1:3$

$V_{\text{CMC}(1.67\%)} : V_{\text{OD}(2.5\%)} = 1:3$



$V_{\text{CMC}(1.67\%)} : V_{\text{OD}(2.5\%)} = 1:1$

$V_{\text{CMC}(1.67\%)} : V_{\text{OD}(2.5\%)} = 1:2$

$V_{\text{CMC}(1.11\%)} : V_{\text{OD}(2.5\%)} = 1:2$

$V_{\text{CMC}(1.67\%)} : V_{\text{OD}(5\%)} = 2:1$

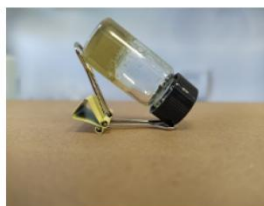


Fig. S1. A macroscopic photograph of Ce6 NPs@Gel with different concentration and ratio of CMC and OD.

2. The chemical composition and electronic structure of Ce6 NPs@Gel

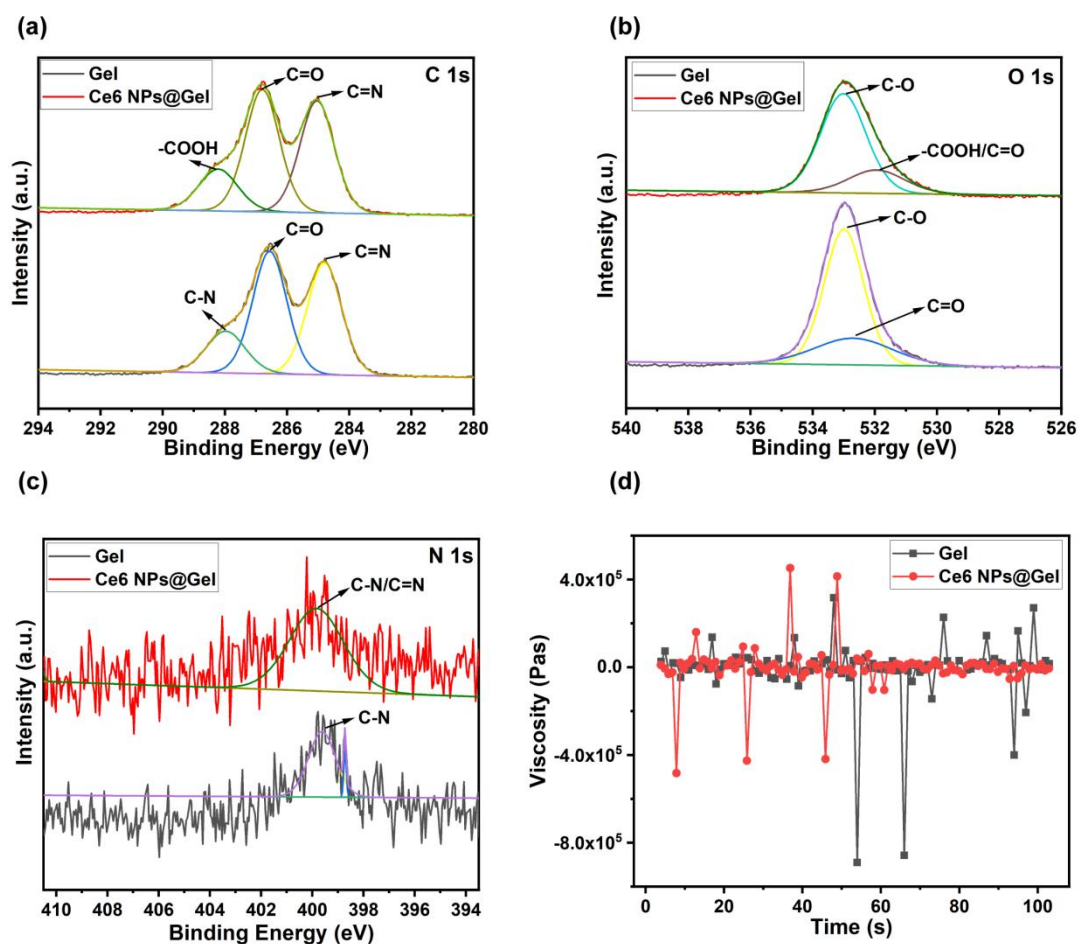


Fig. S2 The chemical composition and electronic structure of Ce6 NPs@Gel **a, b, c** C 1s, O 1s and N 1s XPS spectrum of Gel and Ce6 NPs@Gel. **d** Viscosity of Gel and Ce6 NPs@Gel at different time points.

3. Cellular uptake of the nanoparticles in LM8 cell lines

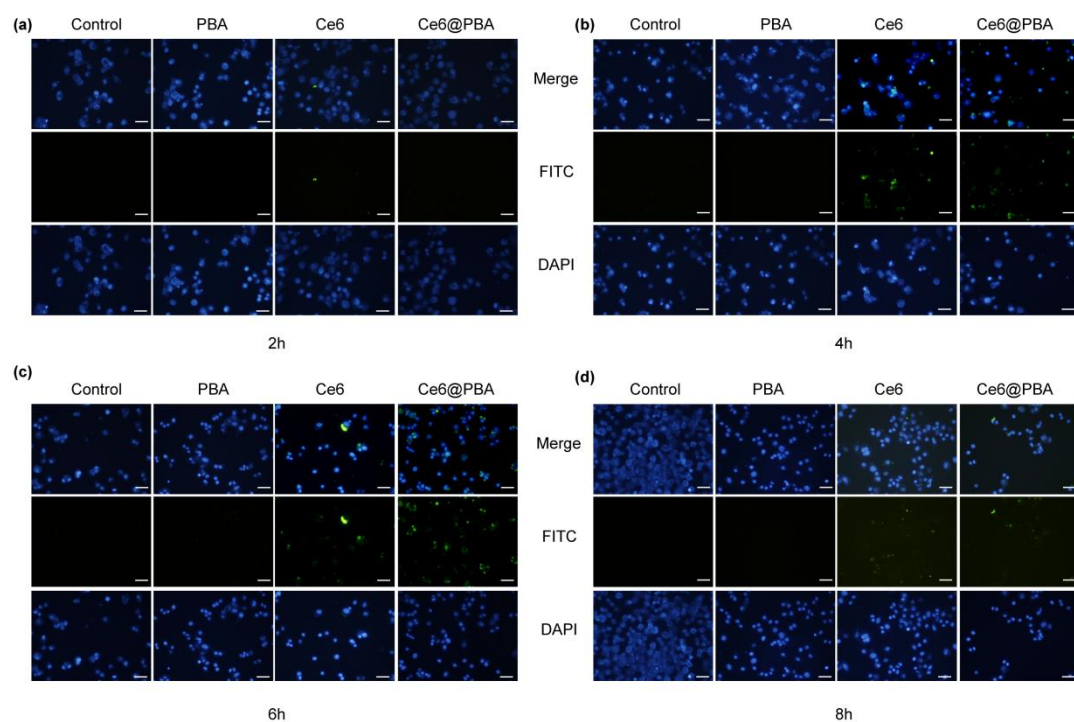


Fig. S3 Cellular uptake of the nanoparticles in LM8 cell lines **a, b, c, d** Cells were incubated with, and detected for Control, PBA, Ce6 and Ce6 NPs for the indicated time intervals (0h, 2h, 4h, 6h and 8h) by immunofluorescence staining. Scale bar, 10 mm.

4. In vivo biocompatibility of Ce6 NPs@Gel + Ultrasound in mice

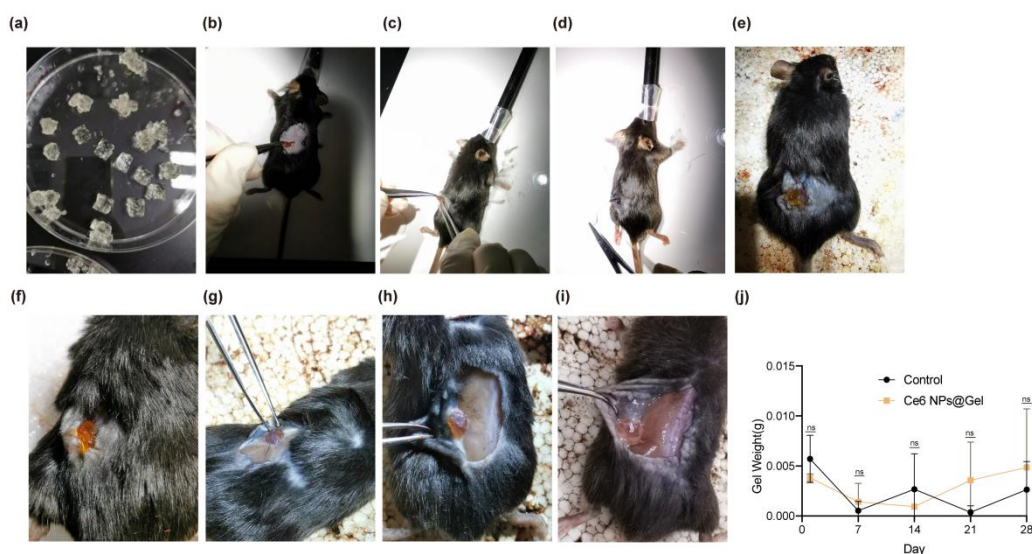


Fig. S4 In vivo biocompatibility of Ce6 NPs@Gel + Ultrasound in mice **a, b, c, d, e, f,**

g, h, i Establishing in vivo model of gel formation and retention after subcutaneous

injection of Ce6 NPs@gel solution in the back of C3H mice. Mice were anesthetized

and gel from its back were collected and weighed at day1, 7, 14, 21 and 28. **j**

Quantitative analysis of the gel weight and body weight was analyzed and presented

as mean $\pm$ SD (n=40) and ns represented no significance, *p < 0.05, **p < 0.01,

p < 0.001, *p < 0.0001.