Supporting Information

Biosilicified Oncolytic Adenovirus for Cancer Viral Gene Therapy

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OA-Trail Mineralization Induced by Different Concentrations of PEI

The different concentrations of PEI (0.05 mg/mL, 0.1 mg/mL or 0.2 mg/mL) were used to induce OA silicified encapsulation, and the zeta potential of PEI-coated OA were examined using a Zetasizer analyzer.

With increasing concentration of PEI in solution, the zeta potential value of PEI-coated OA increased first and then remained at 7.31 mV when the PEI concentration in solution was above 0.2 mg/mL **(Figure S3A)**, indicating a saturation encapsulation of PEI on OA under this surface coverage ratio. However, the aggregation of virus-silica mixture was observed **(Figure S3B; Figure S3C)** when the PEI concentration of 0.2 mg/mL was used to induce the OA mineralization in solution. The zeta potential of OA-PEI was low (-3.02 mV) when the PEI concentration of 0.05 mg/mL was used **(Figure S3D)**, and the OA mineralization solution was clear **(Figure S3E)**. Moreover, no mineralized virus particles were observed according to the TEM image **(Figure S3F)**, indicated that lower PEI concentration (0.05 mg/mL) cannot induce virus mineralization. Notably, the zeta potential value of PEI-coated OA is close to zero (-0.52 mV) after the PEI concentration of 0.1 mg/mL was used to coat

on OA **(Figure S3G)**. After the mineralization, the treated virus particles could be well dispersed in the solution and observed by TEM **(Figure S3H; Figure S3I)**.

Besides, under this condition (PEI, 0.1mg/mL), it could form an ideal mineralized virus with low cytotoxicity **(Figure S7)**. These results confirmed that the PEI concentration of 0.1 mg/mL in solution was suitable for the mineralization of OA.

Degradation of "silica coating"

To investigate the degradationo of "silica coating" of OA-Trail@SiO_2 in lysosome, the pH of the silicified virus solution was adjusted to 5.6 (pH value in the lysosome). As shown in **Figure S4A**, the centrifugation of the silicified virus solution in pH 7.4 showed obvious precipitation, but the precipitation almost completely disappeared under pH 5.6 condition. Then, silicified virus solution sample (pH 5.6) was observed under TEM. There are many small silica particles in the field of vision **(Figure S4B)**, and the typical virus morphology could be observed after negtive staining **(Figure S4C)**. These results indicated that the silica coating of OA@SiO_2 was degraded and OA was released under pH 5.6 conditions. Besides, there have many reports about the degradation of silica, and the degradation process was demonstrated *in vitro* and *in vivo*.[1-3]

OA@SiO_2 Reduced Hepatotoxicity *In Vivo*

To investigate the hepatotoxicity of OA-Trail@SiO_2, C57BL/6 mice were treated with OA-Trail or OA-Trail@SiO_2 by intravenous injection, and the serum and liver were harvested after 4 days. The results showed that the ALT, AST and ALP levels in OA-Trail@SiO_2-treated mice were significantly lower than those in native OA-Trail-treated mice. It should be noted that the ALT and AST levels of the OA-Trail@SiO_2 and PEI-SiO_2-treated mice were not higher than those of PBS-treated mice **(Figure S9A, B)**. Additionally, immunohistochemical results showed a lower virus concentration in the liver in OA-Trail@SiO_2-treated mice than in OA-Trail-treated mice **(Figure S9D)**, and H&E staining of liver sections from OA-Trail@SiO_2-treated mice showed no obvious damage **(Figure S10)**. These results indicated that OA-Trail@SiO_2 exhibited significantly decreased hepatotoxicity following intravenous injection.

Table S1. Zeta potential value of each step during OA-Trail biomineralization.

	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Mean Value	Standard Deviation
OA	*-10.9 mV*	*-10.6 mV*	*-11.5 mV*	*-9.94 mV*	*-9.51 mV*	*-10.3 mV*	*-10.46 mV*	*0.71*
OA@PEI	*0.74 mV*	*-1.22 mV*	*-2.73 mV*	*0.18 mV*	*0.11 mV*	*-0.17 mV*	*-0.52 mV*	*1.15*
OA@SiO_2	*-6.73 mV*	*-7.22 mV*	*-6.18 mV*	*-4.58 mV*	*-5.22 mV*	*-6.47 mV*	*-6.07 mV*	*0.9*

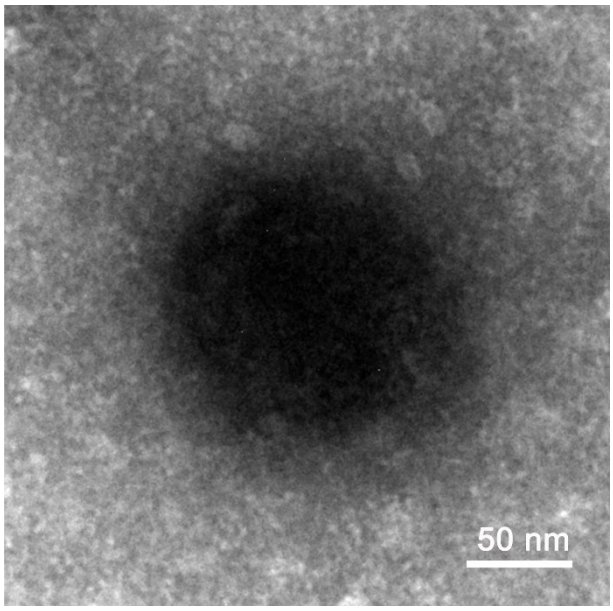

Figure S1. TEM images of negatively stained OA-Trail@SiO_2.

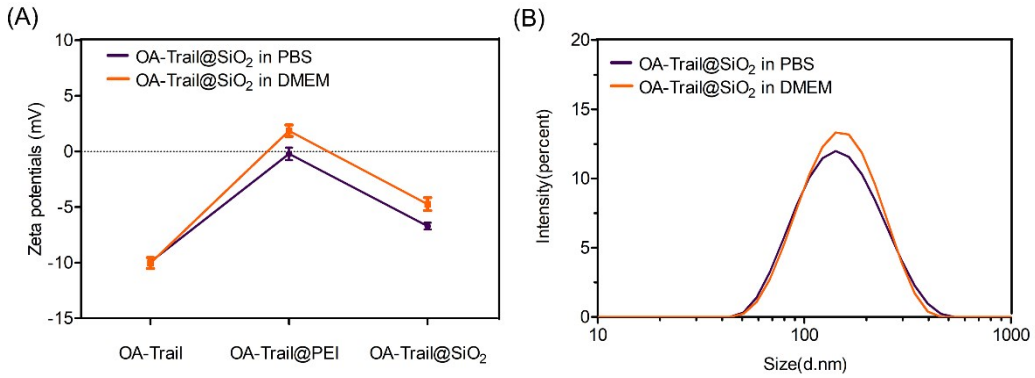

Figure S2. (A) Zeta potential changes during OA-Trail biomineralization in PBS and DMEM. (B) DLS size profiles of OA-Trail@SiO_2 in PBS and DMEM.

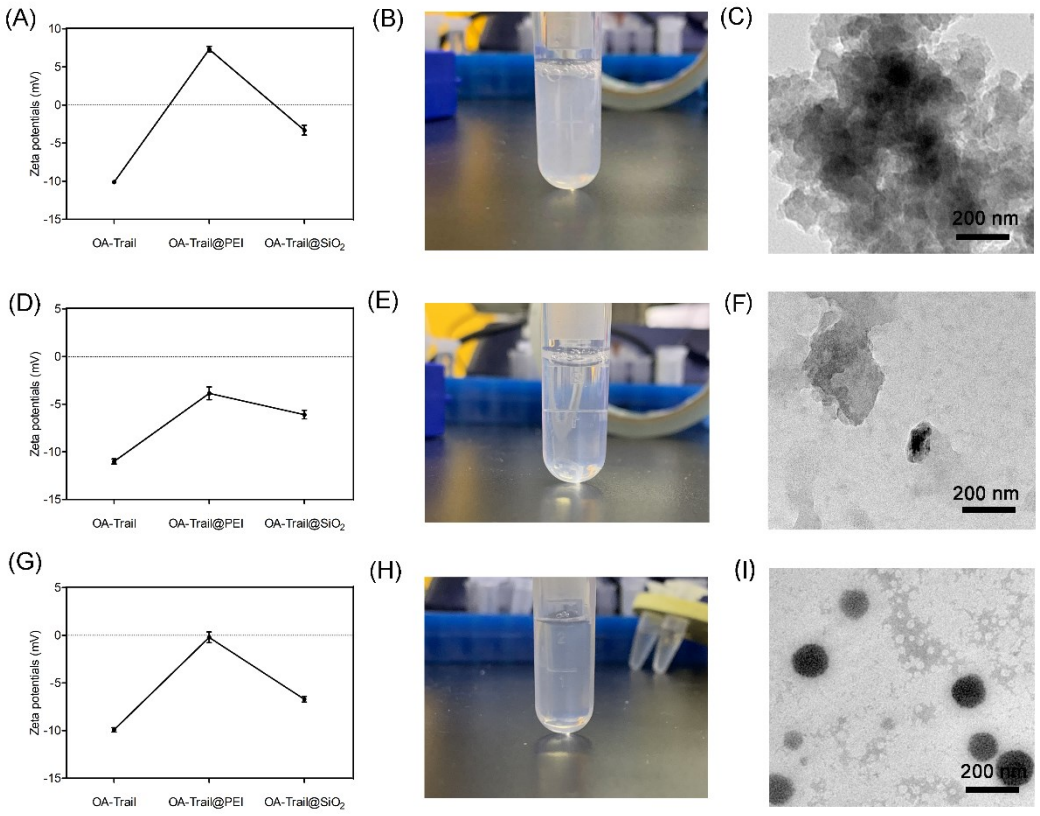

Figure S3. Zeta potential changes during OA-Trail biomineralization induced by 0.2 mg/mL PEI (A), 0.05 mg/mL PEI (D), and 0.1 mg/mL PEI (G). The turbidity of OA-Trail biomineralization solution induced by 0.2 mg/mL PEI (B), 0.05 mg/mL PEI (E), and 0.1 mg/mL PEI (H). TEM images of biomineralizated OA-Trail that induced by 0.2 mg/mL PEI (C), 0.05 mg/mL PEI (F), and 0.1 mg/mL PEI (I).

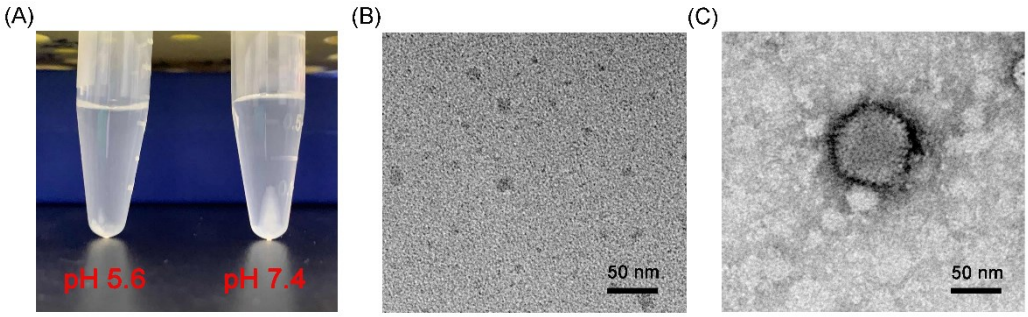

Figure S4. (A) Centrifugation results of silicified virus solution at pH 5.6 and pH 7.4. (B) TEM images of OA@SiO_2 without any staining at pH 5.6. (C) TEM images of OA@SiO_2 with negatively staining at pH 5.6.

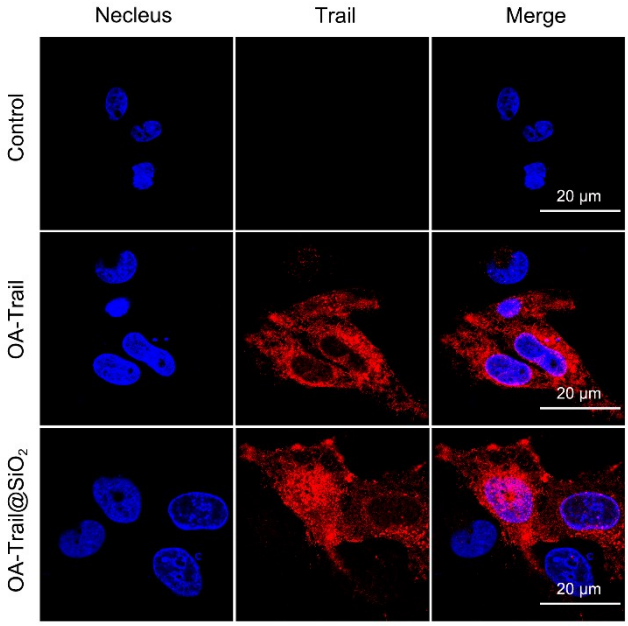

Figure S5. Immunofluorescence analysis of Trail expression in Hep-G2 cells. Cells were treated with DMEM, OA-Trail (200 VPs/cell) or OA-Trail@SiO_2 (200 VPs/cell) for 48 h. Nuclei (DAPI stain) are blue, and the Trail protein is red.

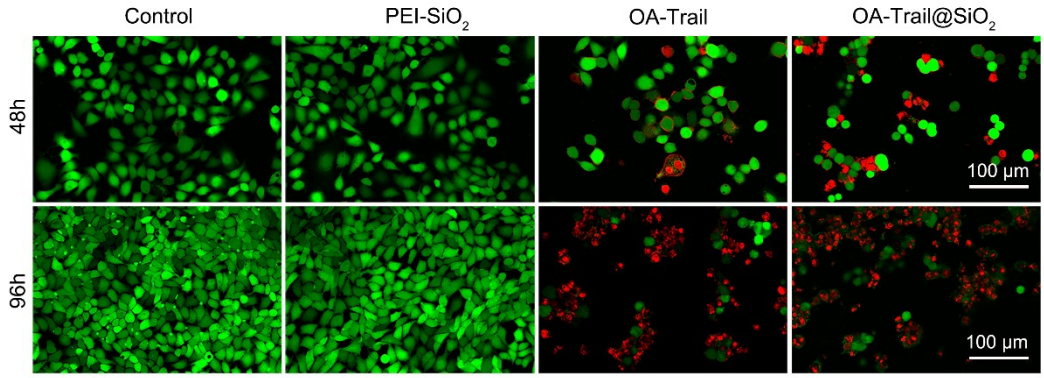

Figure S6. Live/dead analysis of Hep-3B cells. Cells were treated with DMEM (control), PEI-SiO_2, OA-Trail (200 VPs/cell) or OA-Trail@SiO_2 (200 VPs/cell) for 48 h or 96 h. Living cells are blue, and dead cells are red.

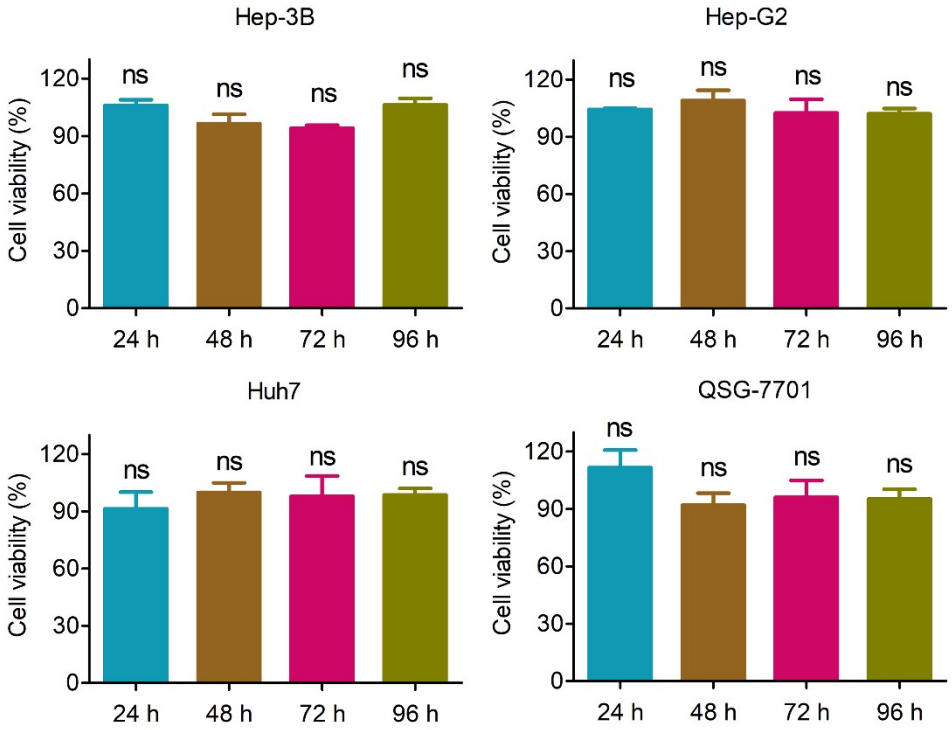

Figure S7. CCK-8 assay to detect cell viability. Human hepatocellular carcinoma cells (Huh-7, Hep-G2, and Hep-3B) and normal human liver cells (QSG-7701) were treated with PEI-SiO_2 (the concentration of PEI-SiO_2 is the same as OA-Trail@SiO_2 in **Figure 3A**). Data indicate the mean ± SD of four replicates. Normalized control group, statistical analysis of data: PEI-SiO_2 compared with control, ns indicates $P > 0.05$.

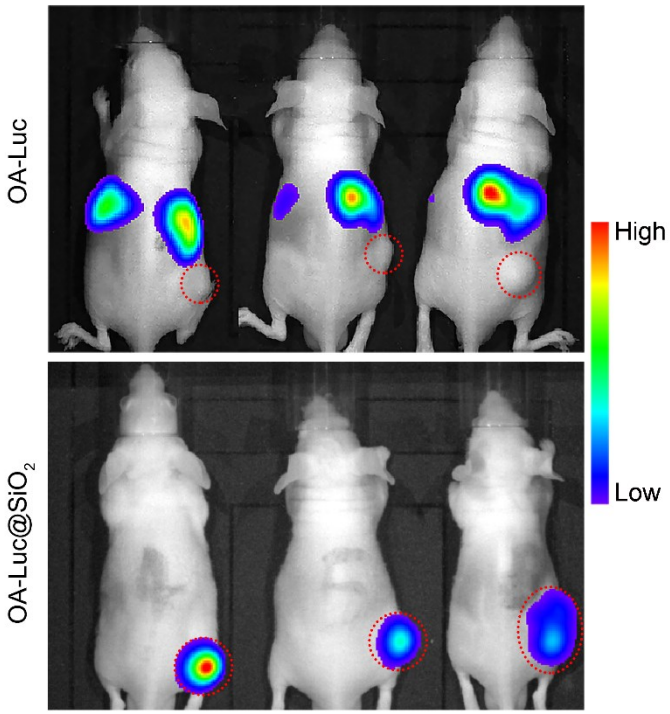

Figure S8. Fluorescence images of nude mice bearing Hep-G2 subcutaneous tumors at 2 days after treatment with OA-Luc or OA-Luc@SiO_2 administered by intravenous injection , and the area marked in the figure is the tumor location. (2×10^{10} VPs, once every day for three total injections).

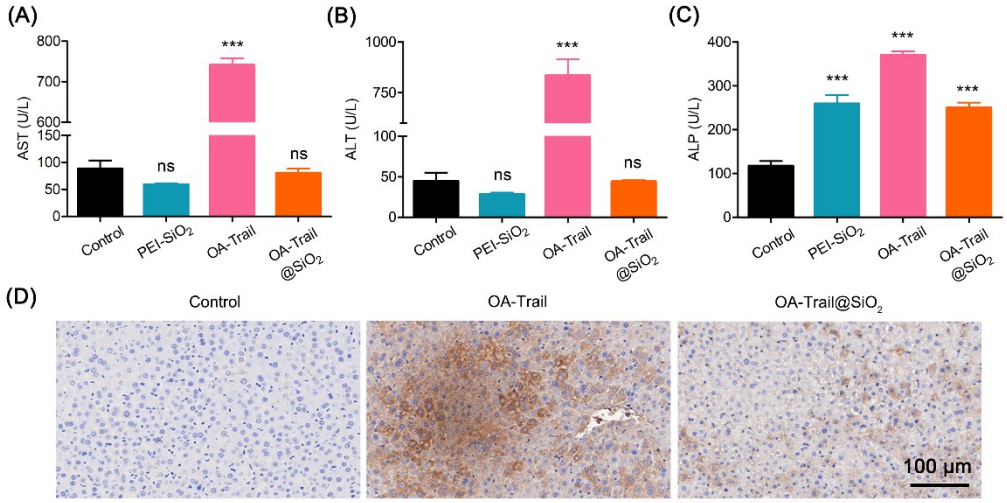

Figure S9. (A, B and C) Serum ALT, AST and ALP levels. ***$P < 0.001$; ns indicates $P > 0.05$. (D) Liver sections stained with hexon.

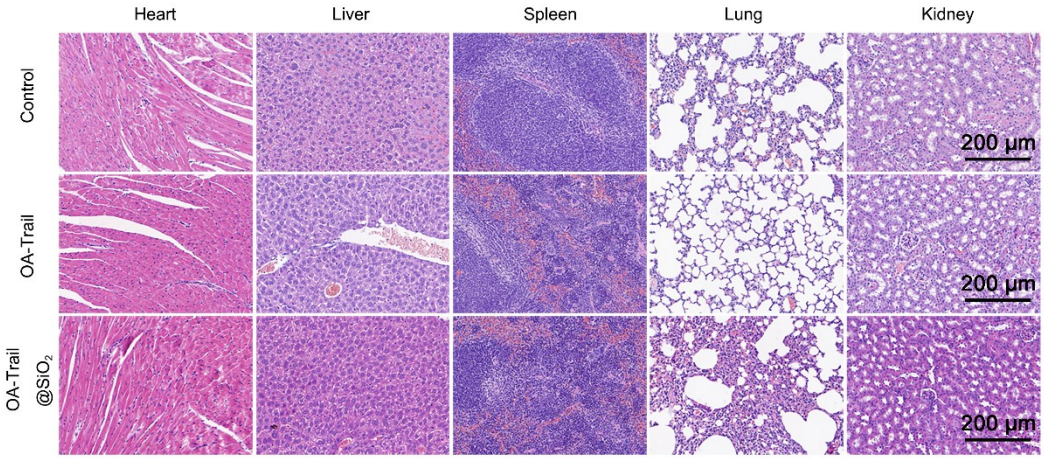

Figure S10. Hematoxylin and eosin (H&E) staining of organs in each group.

References

1. P. Hadipour, R. Mohammadpour and H. Ghandehari, *J. Control. Release*, 2019, **312**, 1-15.

2. E. Bindini, Z. Chehadi, M. Faustini, P. Albouy, D. Grosso, A. Cattoni, C. Chanéac, O. Azzaroni, C. Sanchez and C. Boissière, *ACS Appl. Mater. Interfaces*, 2020, **12**, 13598-13612.

3. G. Chen, Z. Teng, X. Su, Y. Liu and G. Lu, *J. Biomed. Nanotechnol.*, 2015, **11**, 722-729.