Supporting Information

Macrophages hitchhiked Arsenic/AB bionic-preparations for liver cancer

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1. Materials

Sodium arsenite (cat. no. S225I) was purchased from Ekeda Chemical Technology Co., Ltd (Chengdu, China). Manganese Chloride (cat. no. M813486) was purchased from Maclean Biochemicals (Shanghai, China). The BCA protein assay kit (cat. no. ZJ101) was purchased from EpiZyme Biotechnology Co., Ltd (Shanghai, China). Fluorescein isothiocyanate (FITC) (cat. no. F299199) was purchased from Aladdin Biochemical Technology (Shanghai, China). All chemicals are of analytical grade.

2. Experimental section and results

2.1. Methodological examination of access to ABs

Chemical treatment could stimulate the secretion of ABs. Different concentrations of sodium arsenite were examined to access more ABs. ABs were isolated using a differential-centrifugation strategy. The cell debris was first removed by centrifugation at 300 g for 5 min, followed by centrifugation at 3000 g for 20 min to separate the ABs from the supernatant. Then the ABs were washed with sterile water by centrifugation at 3000 g for 20 min and finally resuspended in PBS. All centrifugation steps were carried out at 4 ℃. Finally, the protein content was measured using the BCA protein quantification kit. The protein content was used to represent the amount of ABs secreted, and the optimum concentration of sodium arsenite was finally determined to be 150 μg/mL (**Figure S1**).

2.2. Determination of the preparation process for Arsenic/AB

The optimum preparation process for Arsenic/AB was determined using a single-factor experiment (**Table S1**). The main factors affecting the preparation were: sodium arsenite concentration, sonication time, and stirring time. The arsenic content was determined by inductively coupled plasma emission spectrometry (ICP-OES) (Thermo, USA) after preparation and the Arsenic/AB preparation process was determined by comparing the arsenic loadings.

Table S1 Single-factor examination table for Arsenic/AB

Levels /Factors	Sonication time (min)	Sodium arsenite concentration (mg/mL)	Stirring time (min)
1	5	5	15
2	10	10	30
3	15	15	45

The experimental results showed that the preparation process of Arsenic/AB was 1300 μg (amount of protein) of ABs first mixed with 3 mL of manganese chloride (30 mg/mL) and then sonicated in an ice water bath for 5 min using an ultrasonic cell crusher (JY92-IID; Ningbo Haishu Yiheng Instrument Co.; Ningbo, China) (2 s "on", 4 s "off " at 200 W). After centrifugation at 13,000 rpm for 10 min, the precipitate was washed with distilled water and centrifuged for another 10 min under the same conditions. The precipitate was resuspended with 3 mL of sodium arsenite (10 mg/mL) and stirred for 30 min in a 37 °C water bath. Arsenic/AB was obtained by centrifugation under the same conditions and then washed with distilled water, followed by resuspension in 1.2 mL PBS (**Figure S2**).

2.3. Preparation of FITC-AB

FITC-AB was obtained by incubating ABs and FITC solution with stirring at RT. Firstly, 2600 μg (amount of protein) of ABs were sonicated under an ice water bath for 5 min using an ultrasonic cell crusher (JY92-IID, Ningbo Haishu Yiheng Instrument Co., Ningbo, China) (2 s "on", 4 s "off " at 200 W). Then, the incubation was then carried out in a 37 ℃ water bath with stirring for 30 min. Next, the FITC solution (3 mg/mL) was added and incubated for 2 h at RT with stirring. After incubation for 2 h, centrifuge at 13,000 rpm for 10 min then wash with sterile water and finally resuspend in 1 mL PBS.

2.4. The stability examination of FITC-AB

To determine the stability of FITC-AB, five groups of FITC-AB were prepared as described in 2.3. The amount of FITC in one group was measured at 24-hour intervals over a period of five days (**Figure S3**).

2.5. Preparation of DIR-AB

DIR-AB was obtained by incubating ABs and DIR solution with stirring at RT. DIR was first dissolved in DMSO to configure 1 mg/mL of standard solution. 500 μL of DIR standard solution was diluted with 10 mL of PBS, and then mixed with 2600 μg (amount of protein) of ABs and stirred the reaction for 2h at RT. After reaction for 2 h, centrifuge at 13,000 rpm for 10 min then washed with sterile water and finally resuspend in 3 mL PBS.

2.6. Examination of the stability of macrophage uptake *in vitro*

To investigate the stability of macrophage uptake, RAW264.7 cells were divided into 12 groups and inoculated into 6-well plates at a density of $5x10^6$, followed by the addition of Arsenic/AB (the amount of arsenic was 100 μg). After incubation at 37°C

and 5% CO_2 for 1 h, the cells were collected and centrifuged immediately at 800 rpm for 5 min at 4 °C. For the first group, the supernatant was removed and washed, and the arsenic content was determined by ICP (Thermo, USA). For the other groups, the supernatant was removed, washed, and re-suspended into 6-well plates. Subsequently, the arsenic content of one group at each time interval was measured (30 min, 45 min, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, 7 h, and 8 h) after centrifuged and washed (**Figure S4**).

2.7. *In vivo* bio-distribution of Arsenic/AB at 24h

$1x10^6$ H22 cells were inoculated subcutaneously in male KM mice. When the tumor volume grew to ~100 mm^3, 0.1 mL of DIR-AB (200 μg/kg) or DIR solution (200 μg/kg) was injected. Then the mice were executed and dissected after 24 h, and the *in vivo* distribution of Arsenic/AB was examined by fluorescence imaging of *ex vivo* tissue using the AniView600 (Bo Luteng, China) in vivo imaging system (**Figure S5**).

3. Supplementary Figures

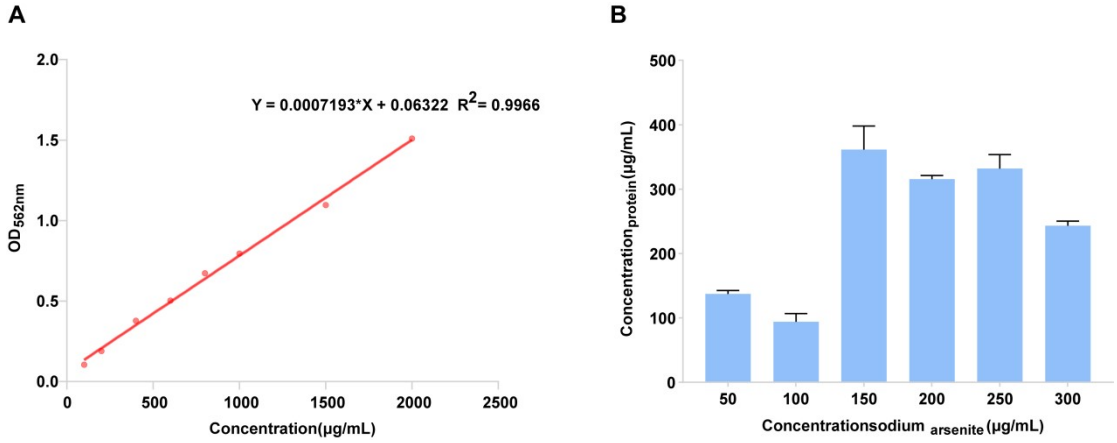

Figure S1 (A) BCA protein standard curve, (B) Protein content after stimulation with different sodium arsenite

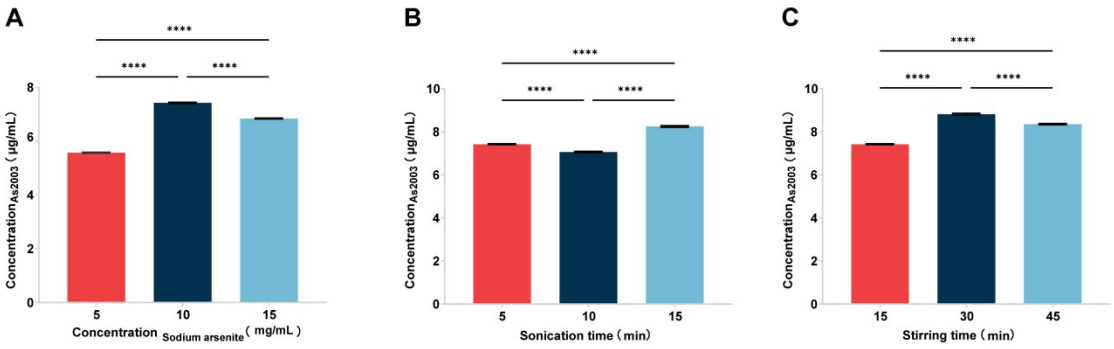

Figure S2 (A) Effect of sodium arsenite concentration on arsenic loading, (B) Effect of sonication time on arsenic loaded, (C) Effect of stirring time on arsenic loaded. Results are shown as group means ± SEM (standard error of the mean), ****p < 0.0001.

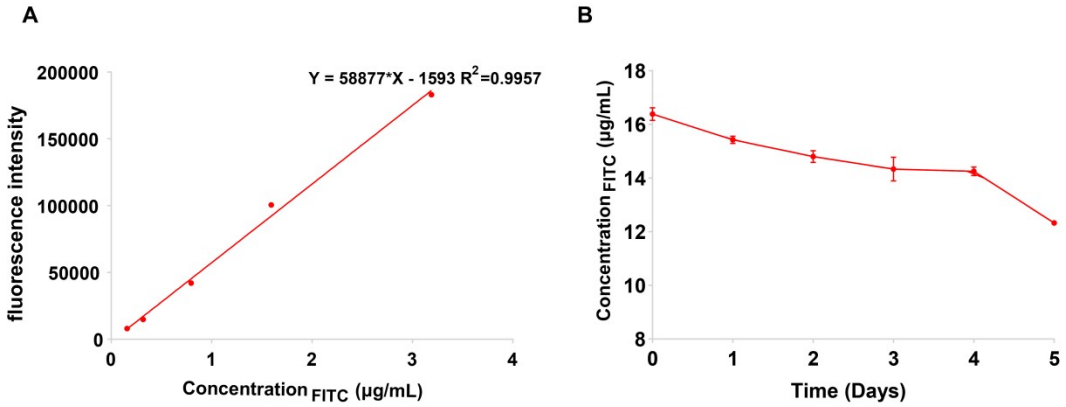

Figure S3 (A) Standard curve of FITC, (B) The stability examination of FITC-AB in five days

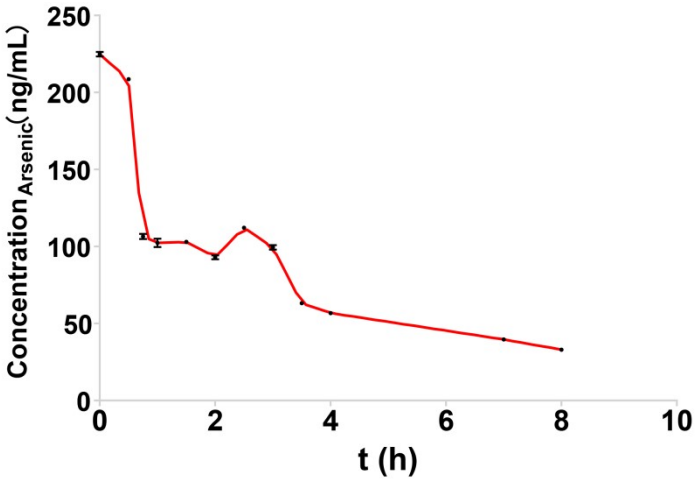

Figure S4 *In vitro* macrophages uptake stability

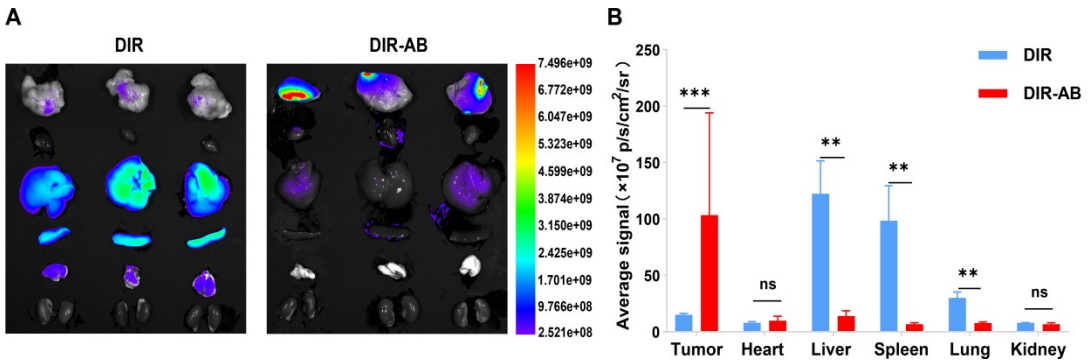

Figure S5 (A) *Ex vivo* fluorescent images of tissue samples collected 24 h after injection, (B) Quantitative fluorescence intensity and statistical analysis of tissue samples collected 24 h after injection. Results are shown as group means ± SEM (standard error of the mean), $p < 0.05$ was considered a statistically significant difference (ns: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.001$).