SERS diagnosis of liver fibrosis in early stage based on gold nanostars liver targeting tags

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1. Serological detection and HE staining

According to optimal accumulation time, GNSs and GLTTs were injected into the tail vein of normal group A_1, A_2 and model group B_1, B_2. The mice were sacrificed by overdose anesthesia, and blood was collected from heart using anticoagulant tube. Then centrifuge for 5 min at the speed of 3000 r/min, and the upper serum was collected. Refer to kit instructions, alanine aminotransfetase (ALT) and aspartate aminotransfetase (AST) indexes were tested, and the indexes of hyaluronic acid (HA), laminin (LN), type III procollagen (PC-III) and type IV collagen (C-IV) in liver tissue serum were also detected. The serum was prepared as follows: Left lobe of the mice liver was mixed with 0.9% saline to make 10% liver tissue homogenate. with a 2000 G centrifugation for 10 min, the upper serum was collected for subsequent detection.

Liver tissue was fixed with 10% formalin solution, paraffin-embedded and sectionalized. HE staining was used for histopathological diagnosis.

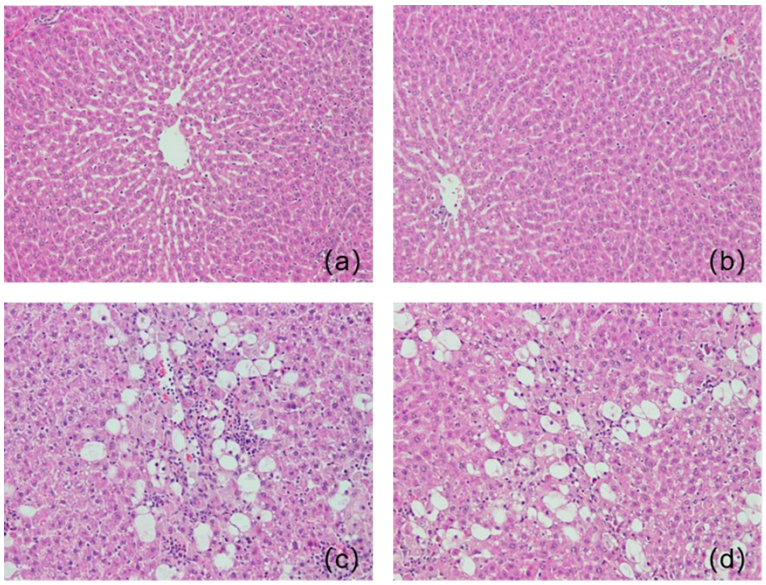

Figure S1. Micrographs of pathological sections of mice liver tissues in each group (HE dyed,×200) (a)normal tissue & GNSs; (b)normal tissue & GLTTs; (c)liver fibrosis tissue & GNSs; (d)liver fibrosis tissue & GLTTs.

Table S1. Serum liver fibrosis indexes in normal group (A_1, A_2) and model group (B_1, B_2) （ $\bar{x}\pm s$, ng/mL, n=15）

Group	HA	LN	C-IV	PC-III
Group A_1	2.26±0.36	1.24±0.20	129.70±7.38	55.16±4.84
Group B_1^*	4.94±0.63	1.94±0.16	279.67±16.30	94.43±5.23
Group $B_2^\#$	4.73±0.65	1.97±0.15	277.82±15.90	95.65±5.94
Group A_2	2.27±0.30	1.28±0.14	127.46±11.00	54.42±4.32
Group B_1^*	4.94±0.63	1.94±0.16	279.67±16.30	94.43±5.23
Group $B_2^\#$	4.73±0.65	1.97±0.15	277.82±15.90	95.65±5.94

Note: Compared with normal group A_1 and A_2, $^*P<0.01$, $^\# P<0.01$.

Normal Group A_1: normal tissue & GNSs; Normal Group A_2: normal tissue & GLTTs; Model group B_1: liver fibrosis tissue & GNSs; Model group B_2: liver fibrosis tissue& GLTTs.

Table S2. Serum liver function indexes in normal group (A_1, A_2) and model group (B_1, B_2) （$\bar{x}\pm s$, IU/L , n=15)

Group	AST (aspartate aminotransferase)	ALT (alanine aminotransferase)
Normal group A	63.64±4.59	47.23±5.97
Model group A^*	149.68±12.28	172.56±10.65
Model groups $B^\#$	152.67±11.74	174.38±9.30
Normal group B	60.60±4.04	47.85±5.88
Model group A^*	149.68±12.28	172.56±10.65
Model group $B^\#$	152.67±11.74	174.38±9.30

Note: Compared with normal group A_1 and A_2, $^{*}P < 0.01$; $^{\#}P < 0.01$.
Normal Group A_1: normal tissue & GNSs; Normal Group A_2: normal tissue & GLTTs; Model group B_1: liver fibrosis tissue & GNSs; Model group B_2: liver fibrosis tissue& GLTTs.

2. Studies on biosafety of GNSs and GLTTs

a. Cytotoxicity test

Carefully remove the frozen human liver cancer cell HepG2 from the liquid nitrogen tank, put them in a 37 ℃ water bath quickly, and transfer them to a sterile 4 mL EP tube after a complete dissolution. After centrifugation at 1500 G for 5 min, the supernatant was discarded, and the cells were dispersed with DMEM medium (10% fetal bovine serum, 100 IU/mL penicillin and 100 mg/mL streptomycin were added). With 5% CO_2 culture at 37 ℃, and after 2~3 generations, the cells in good condition were used to follow-up experiments.

The concentration of GNSs and GLTTs was fixed at 20 nmol/L, and the final concentrations were set to 0.00, 2.50, 5.00, 10.00 and 15.00 nmol/L respectively. Then they were incubated with HepG2 cells for 24, 48 and 72 h. 6 replicate wells at each time point and different blank controls were set. At each time point, 20 μL MTT (5 mg/mL) was added into each well and incubated for 4 hours. Next, add 200 μL DMSO and shake well to fully dissolve the crystals. Finally, the absorbance at 490 nm wavelength was measured, and the cell inhibition rate of each group was calculated. Cell inhibition rate = (control group-experimental group) / control group × 100%.

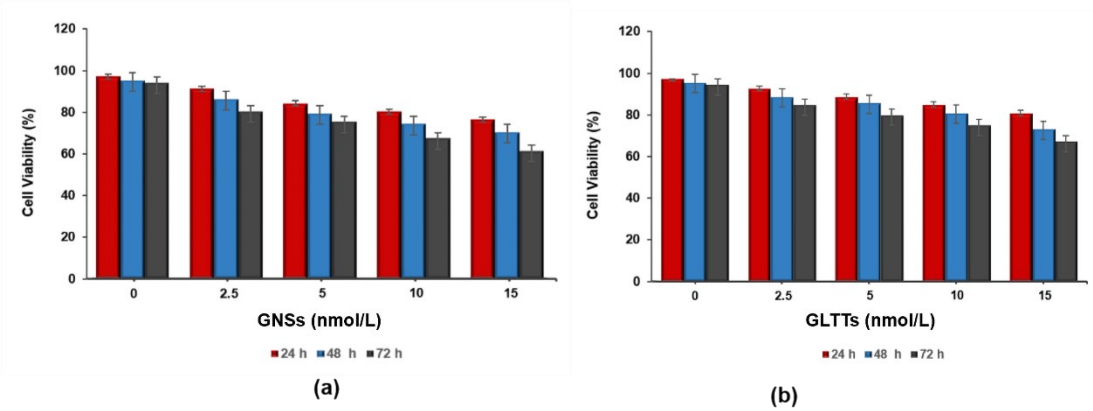

Figure S2. Cell viability of different concentrations of GNSs(a) and GLTTs(b) co-incubation with HepG2 cells at different times.

b. Survival analysis

30 mice in normal group were divided into 3 groups, namely the control group (A_3), GNSs group

(A_4), and GLTTs group (A_5). 200 μL GNSs and GLTTs with the concentration of 20 nmol/L were injected every day, and the control group was injected with the same volume of normal saline. The survival of the three groups within 45 days was recorded, and the survival rate was calculated.

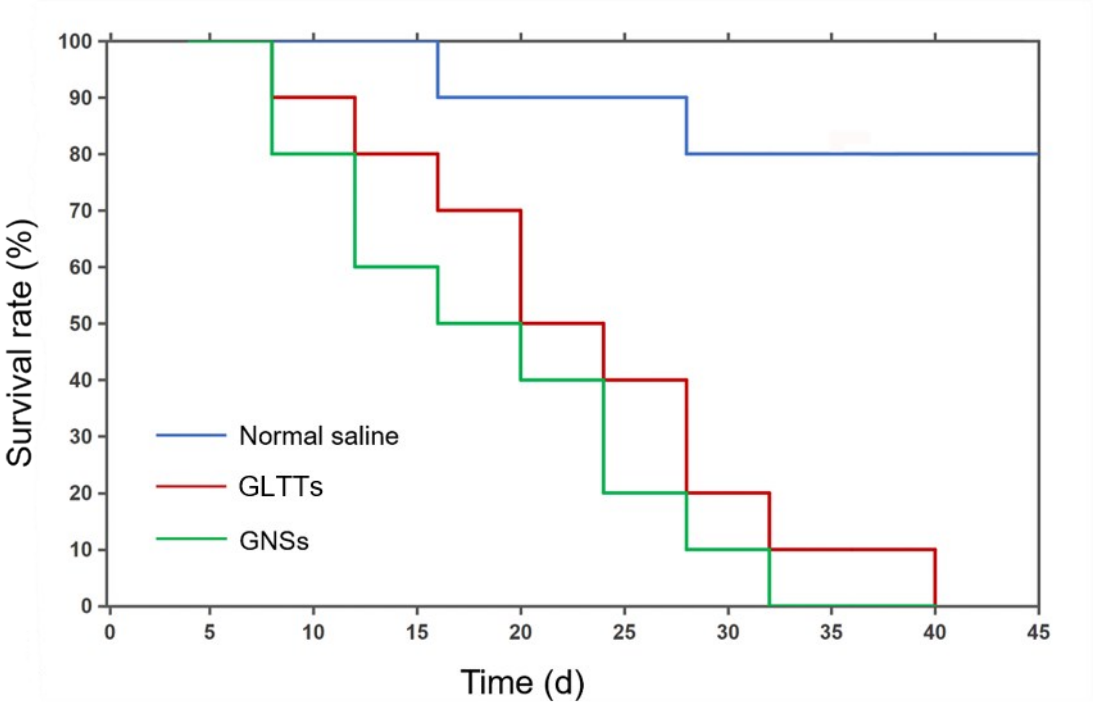

Figure S3. Survival curves of mice injected with saline, GNSs, and GLTTs through tail vein in 45 days.