Supplementary material

Label-free SERS ultrasensitivity and universality detection of low back pain fingerprint based on SERS substrated

Cai Wang[a1], Jixiang Chen[a1], Jingguo Wu[a], Huiyu Wan[a], Qianwen Yue[c], Baoliang Sun[a], Ying Wang[a], Qiang Xiao[a*], Jingyi Sun[b*]*

[a] *The Second Affiliated Hospital; Shandong First Medical University & Shandong Academy of Medical Sciences, Taian, Shandong, 271000, China*

[b] *Shandong Provincial Hospital Affiliated to Shandong First Medical University& Shandong Academy of Medical Sciences, Jinan, Shandong, 250021, China*

[c] *Taishan Vocational College of Nursing, Taian, Shandong, 271000, China*

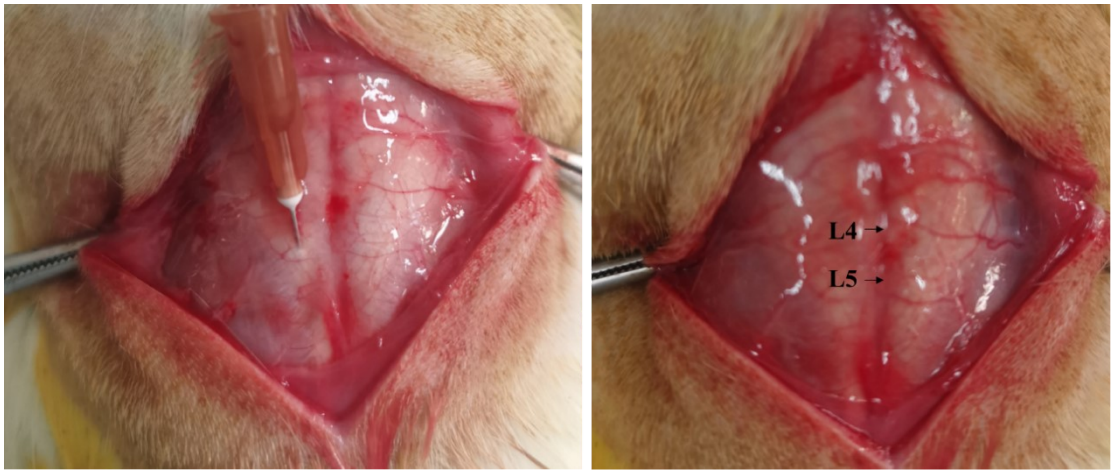

Figure S1. Fasciitis was induced by injection of CFA, and the arrows represented L4 and L5 spinous processes, respectively.

Evaluation of TLF model

We quantitatively analyzed the results of immunofluorescence by Image J software. The results showed that the expression of PGP 9.5 in the fascia tissue of fasciitis rats increased about 2 times compared with sham rats (Figure S2 D_1). PGP9.5 was a good structural marker of nerve axons[20]. The research results showed that in the process of fasciitis, the innervation had changed and the density of innervated nerves had increased, which was consistent with some previous research results. However, PGP9.5 was a pan neural marker, which could not well show the changes of functional nerves, and the changes in the innervation of sensory nerves and sympathetic nerves were not significant. The quantitative results of CGRP immunofluorescence (Figure S2 A_2-C_2) showed that the expression of CGRP of fasciitis rats was about twice higher than that of sham group (Figure S2 D_2). SP was a marker of sensory peptidergic nerve fibers, which had the effect of vasodilation and

enhancing the excitability of nociceptive neurons. According to the results of quantitative analysis of immunofluorescence by ImageJ, the expression of SP of fasciitis rats was about five times that of sham group (Figure S2 D_3). The results showed that the density of nerves was significantly increased, which may be the reason for the increase of excitability of nociceptive neurons after fasciitis, but the distribution and length of nerves at the horizontal level were also the potential reasons for the change of excitability. CGRP and SP synergistically promoted inflammation and sensitize nociceptors. In addition, CGRP effectively prolonged the painful behavior caused by SP, at the same time, CGRP also promoted the release of SP and inhibited its degradation, causing the pain of fasciitis [21]. Compared with sham group, the nerve density of CGRP and SP increased significantly. This indicated that the change of nerve density of CGRP and SP during fasciitis was most likely the cause of fasciitis pain. More importantly, the interaction mechanism between CGRP and SP needed further in-depth study, which may provide a suitable clinical solution for solving the chronic pain of fasciitis. TH was a marker of sympathetic nerve fibers, but it was not the only marker of sympathetic nerve. The results showed that the expression of fasciitis in rats was about four times that in sham group(Figure S2 D_4). This may be the reason for the change of lumbar sensation (other than pain) in patients with fasciitis clinically, but it was not unique. The causes of sensory changes may be multifaceted, which

needs further in-depth research[22,23]. In short, the abnormality of pain, sensation and motor function after fasciitis is related to the change of nerve innervation. In addition, this quantitative method of immunofluorescence could not effectively calculate the change of nerve length. The change of innervation may not only be reflected in the increase of density, but also in the change of length. Moreover, this study only focused on the research of fascia cross-section, and lacked the research of horizontal plane. In the future, we will further study the changes of nerve innervation in horizontal plane.

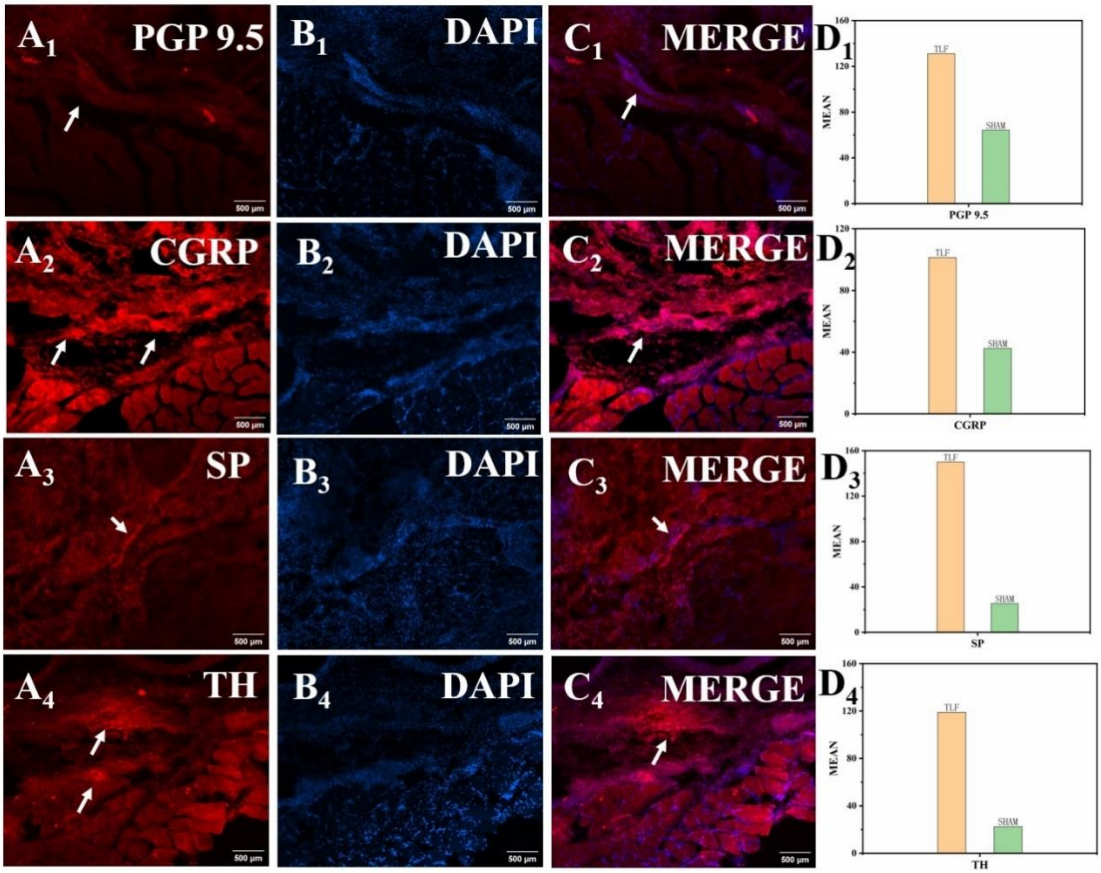

Figure S2. The results of immunofluorescence of neural markers and quantification data of neural markers positive area in fascia. (A_1-A_4) Immunofluorescence images showed PGP 9.5 (red), CGRP (red), SP (red), TH (red). The white arrow area refers to the positive nerve fibers. (B and

C) The immunofluorescence images showed DAPI and merge. (D) Quantitative calculation of neuronal markers in positive areas by conversion of gray value by Image J software(scale bar: 500um).

Rats have excellent swimming ability, but they hate the environment in the water very much. Moreover swimming is also a very strenuous exercise, so the rats will instinctively look for a place to rest in the water[24]. We performed Morris water maze test on 1 day, 3 days, 7 days and 12 days after injection of CFA in rats, the results showed that the difference was the most significant on the 3 days. Therefore, when we selected the more typical swimming trajectory map (Figure S3 A-D) in 3 days, we found that the trajectory of rats in High group was shorter (Figure S3 A) and tended to find a place to rest faster, while the trajectory map of rats in Low and Mid groups was relatively prolonged (Figure S3 B,C), while the trajectory of rats in sham group was more messy and long (Figure S3 D). Therefore, we speculate that fasciitis rats had a stronger desire to find a place to rest and survive because the inflammation and pain in the low back of fasciitis rats reduced their motor ability, which led them to find a place to rest faster. In addition, we also observed a phenomenon before and after the experiment that the rats preferred to accelerate the swimming speed by swinging the waist and back before the injection of CFA, while after the injection of CFA induced inflammation, this behavior decreased, which was most obvious at 3 days. We hypothesized that this pain-avoiding behavior was caused by the inflammatory pain in the low back. Although the Morris water maze experiment could not well verify the effectiveness of the TLF model, the

experimental results also reflected some differences, and further research was needed to verify it[25,26]. Perhaps the improvement of the Morris water maze experiment or the development of a new similar experimental method can well reflect the effectiveness of the fasciitis model.

The rotating rod fatigue test of rats was carried out at 1 day, 3 days, 7 days and 12 days after injection of CFA, and the falling time was recorded, and the average value was taken to represent the overall level. As shown in Figure S3 E, compared with the Low group and the Mid group, the Falling Time of the High group was the smallest, that was, the High group was the first to fall. In addition, the falling time of the Mid group was earlier than that of the Low group, which indicated that there was a positive correlation between the falling time and the concentration of injected CFA. We conducted a statistical rank sum test for the values of the SALINE group and the sham group. The results showed that $p > 0.05$, the difference was not statistically significant. It showed that there was no significant difference between saline group and sham group. The rotating rod fatigue test of rats was an experiment to study the exercise ability of rats. The larger the Falling Time, the better the athletic ability and coordination. The previous experiment did not have some behavioral evaluation of the rat fasciitis model, so we introduced the rotating rod fatigue test to study the exercise ability of rats. When fasciitis occured, the movement and sensation of the back of rats were impaired, and the motor ability of the back also

affected the function of the lower limbs. More importantly, the experimental results also showed that with the increase of concentration, the greater the Falling Time, showing a certain positive correlation between concentration and severity. In addition, the numerical value changed greatly in 1D and 3D, and the numerical change tended to be smooth with the increase of days. This indicated that the effect of fasciitis on the body tends to be stable in the late stage of fasciitis, while the effect on the body was obvious in the early stage of fasciitis. However, it had to be noted that in the process of fasciitis, the value of FallingTime showed a flat trend, which may be related to the learning adaptability of rats themselves. There was a similar trend in saline group and sham group.

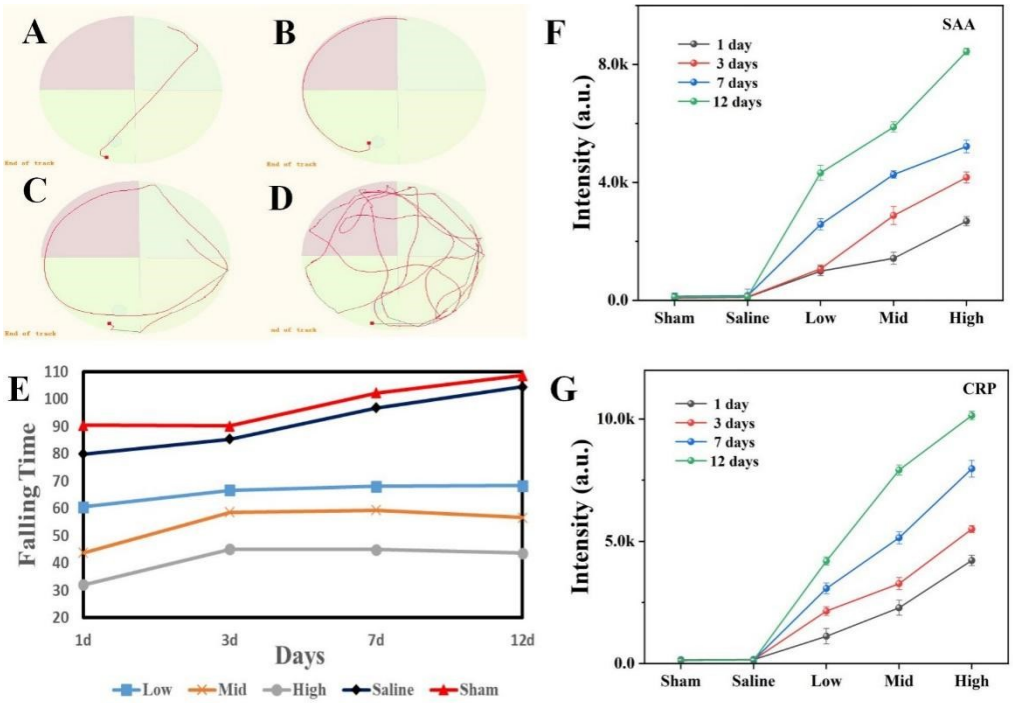

Figure S3. Trajectory map of rat water maze and rotating bar data line chart. (A)(B)(C) is the track map of the low, middle and high group respectively. (D) A track map of sham group. (E) Data line chart of rat rods. (F and G)Detection of SAA and CRP in rat fascia with different days and different concentrations.

We randomly detected CRP and SAA in the fascia of TLF rats using biosensors (Figure S4). Figure S3F and G the results showed that the expression of CRP and SAA in rat fascia increased with the increase of CFA concentration. Moreover, with the increased of the number of days, it was also accompanied by this upward trend. However, there was almost no difference between sham group and saline group, and the expression level was very low, close to 0. The results showed that injection of different concentrations of CFA effectively induced the occurrence of different degrees of TLF. This was consistented with the previous experimental results. Combined with the results of model evaluation, this showed that our fasciitis model had research value and objective authenticity.

Synthesis of biosensor

Hollow gold nanoparticles (HGNPs) were synthesized by slightly modifying the previously published preparation method. The diluted HGNPs was mixed with Nile blue A (NBA) and 4-mercaptobenzoic acid (4-MBA) with 300ul concentration of 10^{-3}mol/L for 1-2 hours, and then the same volume of 1-ethyl-(3-dimethylaminopropyl) carbodiimide hydrochloride /N-hydroxy succinimide (EDC/NHS) mixture was added for 1 hour. Then, combined with CRP and SAA labeled antibodies (10 μ L (10 μ g/mL)) incubated at room temperature for 1 hour, SERS labeled antibodies were prepared. In order to cover the positive sites of antibody, 100 μ L PBS containing 1% bovine serum albumin was used, and the CRP and SAA

antibody labeled HGNPs was finally prepared. Washed the original glass in an ultrasonic bath with soapy water, deionized water and anhydrous ethanol, repeated three times, 20min each time, and then dried the glass in the oven (37℃). In order to connect the glass substrate to the free radical Ohs, the glass substrate was immersed in a mixture of 2.5ml 3-mercaptopropyltriethoxysilane (APTES) and 250mL anhydrous ethanol at room temperature. APTES connected silver nanocube (AgNCs) and glass substrate by Si-OH bond and amine (NH_2) bond. Cleaned the glass again under ultrasonic to remove the excess APTES, and dried it at 70℃. It was put into AgNCs colloid suspension and stirred for 12 h to obtain AgNCs layer. The functional substrate was dried and rinsed for 5 times, then it was immersed in the mixed solution of PBS and DMSA (2mM) for 4 hours, and the DMSA@AgNCs modified substrate was washed with PBS once. Finally, the antibody solution of 10uL CRP and SAA (10 μ g/mL) was dripped into the AgNC layer of the modified substrate and incubated at room temperature for 3 hours. In order to remove excess antibodies, the anti-CRP/SAA@DMSA@AgNCs glass matrix was washed with deionized water. After that, the non-specific binding sites of CRP and SAA antibodies on the optimized SERS substrate were blocked with 1% BSA solution, and the optimized SERS substrate was prepared. Finally, the biosensor for the detection of CRP and SAA was completed.

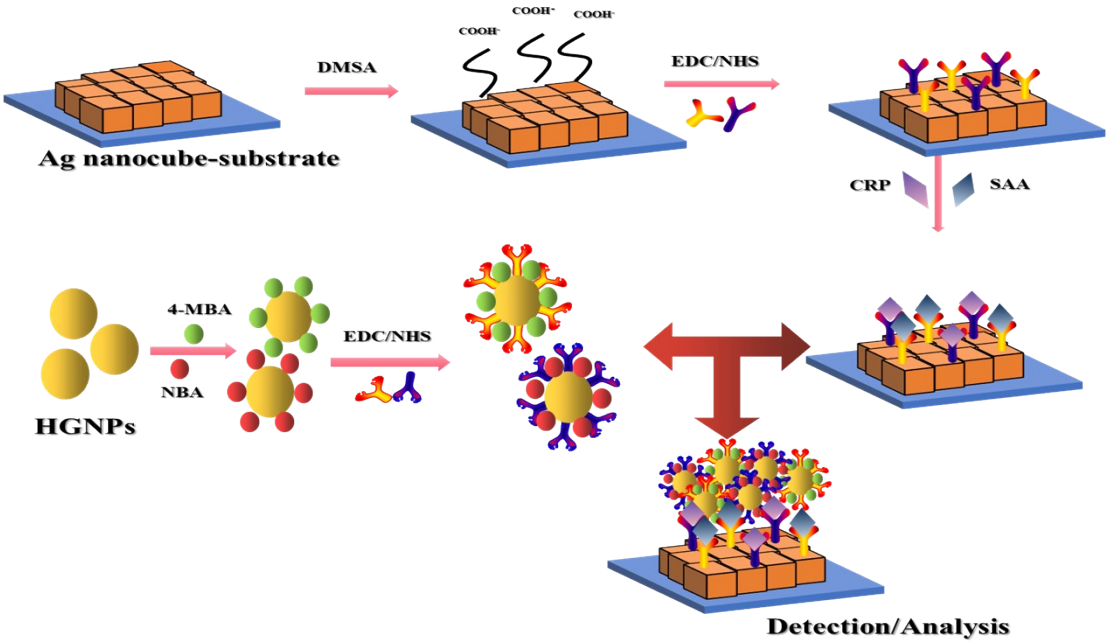

Figure S4. Schematic diagram of biosensor.

In order to better study and deeply understand the law of substance changes in the process of fasciitis, we obtained the difference spectrum through the map difference between different groups Table S1.

Table S1. Spectrum definition.

Same days	Same concentration
Low-Mid	1day-3days
Low-High	1day-7days
Mid-High	1day-12days
	3days-7days
	3days-12days
	7days-12days

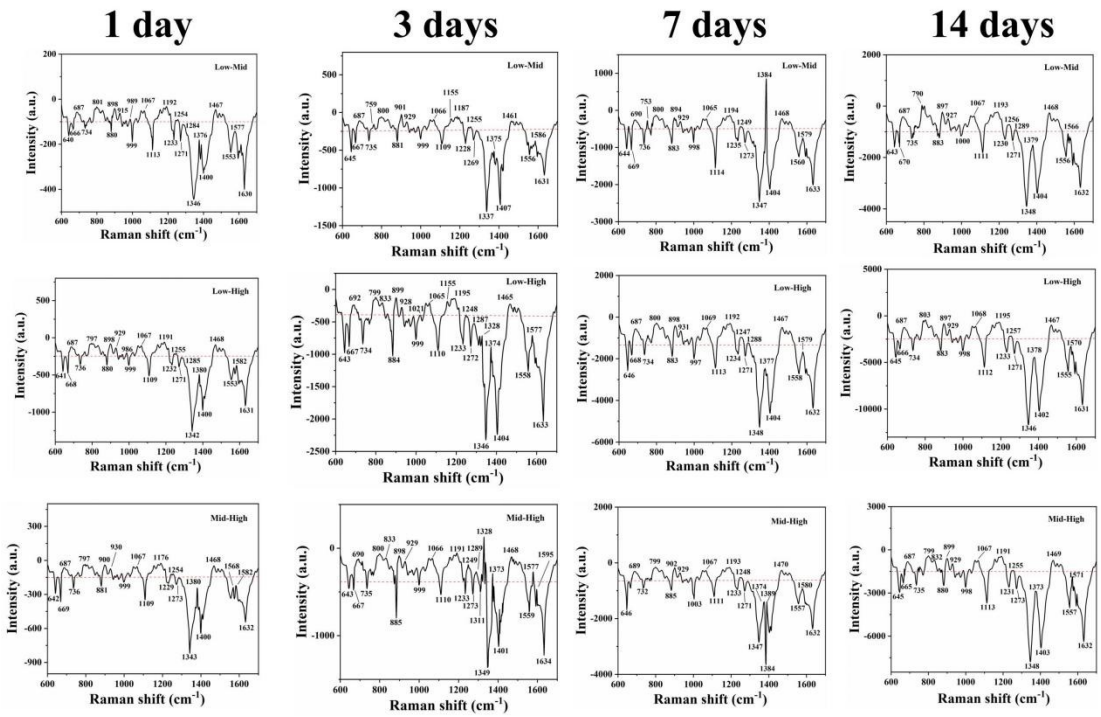

Figure S5. SERS spectrum analysis of substance differences of rats in different concentration groups on the same day.

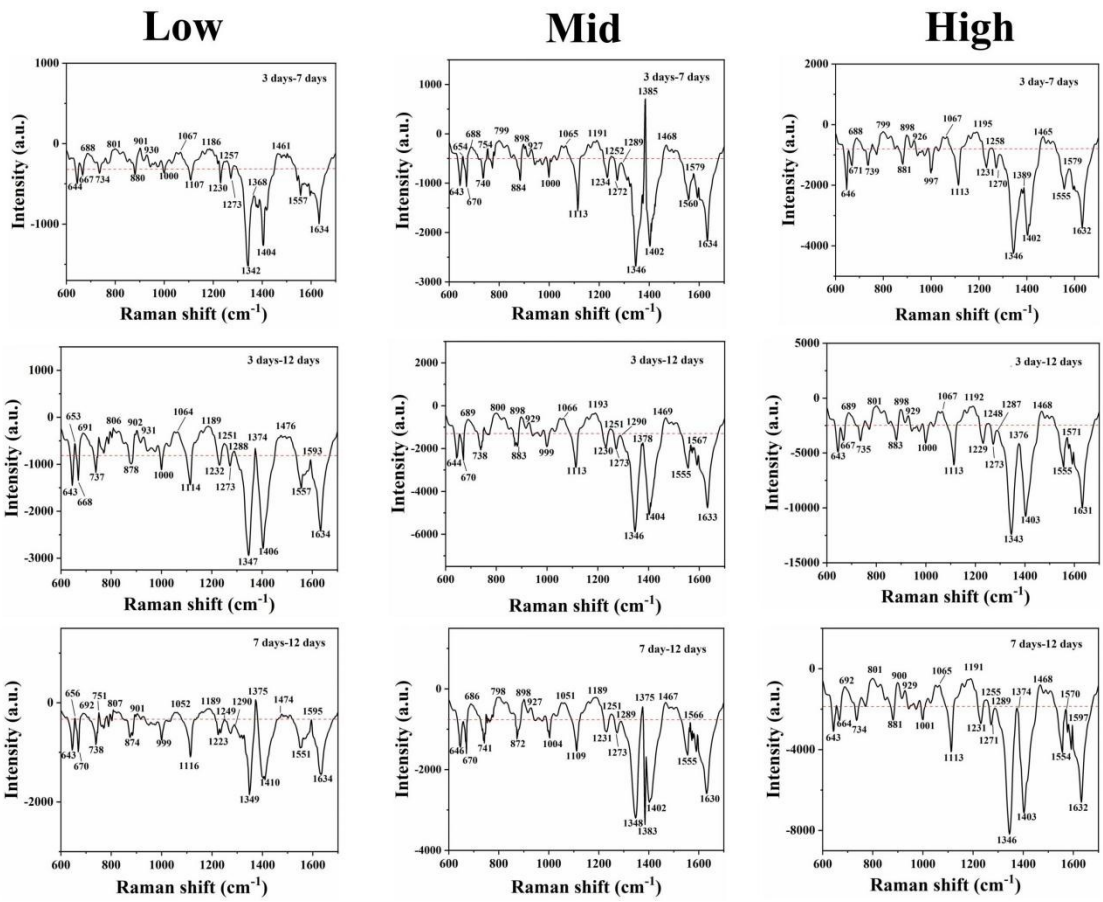

Figure S6. SERS spectrum analysis of substance differences in rats of the same concentration group at different days.