Supporting Information

Gold nanocages-based surface-enhanced Raman scattering probes for long-term monitoring intracellular microRNA during bone marrow stem cell differentiation

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1. Results and discussion

1.1 SERS analysis of miR-144a-3p expression in cell lysates

To increase the reliability of the results, the other two characteristic peaks in addition to 1368 cm^{-1} were used as a quantitative evaluation of the miR-144-3p levels. The peaks at 1130 cm^{-1} and 1597 cm^{-1} were assigned to aromatic CH deformation and C=N stretching modes of Cy5, respectively.[1,2] The calibration curve constructed by SERS intensity at 1130 cm^{-1} and logarithm of miR-144-3p concentration was shown in Fig. S1(A) and Fig. S1(B). The regression equation is y=930.1x+15694 and R^2=0.9806, and the limit of detection (LOD) was 13.38 aM. Fig. S1(C) and Fig. S1(D) showed the regression equation at the peak of 1597 cm^{-1}, which can be expressed as y=1092.9x+18447 with a correlation coefficient of 0.9750. In this case, the corresponding LOD was estimated to be 13.14 aM. The deviation of the calculated LOD at three different characteristic peaks is below 5%, showing the good reliability of the SERS strategy.

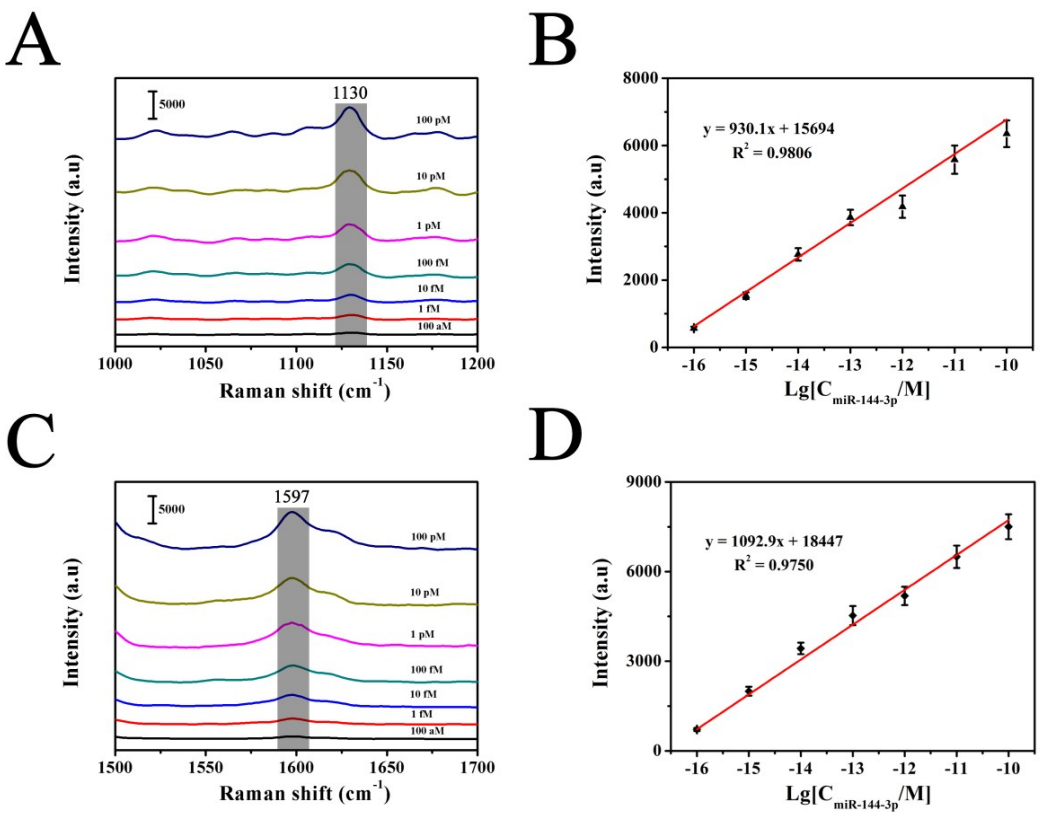

Fig. 1 (A) SERS spectra of the hpDNA-conjugated GNCs probes for detection of miR-144-3p with different concentrations. (B) The linear relationship between the SERS intensity at 1130 cm^{-1} and the logarithm of the miR-144-3p concentration. (C) SERS spectra of the hpDNA-conjugated GNCs probes for detection of miR-144-3p with different concentrations. (D) The linear relationship between the SERS intensity at 1597 cm^{-1} and the logarithm of the miR-144-3p concentration.

1.2 HpDNA assembly and dynamic behavior of SERS probes in living cells

The signal amplification ability of the GNC-hpDNA1-hpDNA2-GNC assembly in living cells was investigated. BMSCs were incubated with different nanostructures for 4 h, and then SERS imaging experiments were performed using a Renishaw Invia Raman microscope equipped with a 785 nm He-Ne laser. As shown in Fig. S2(A), SERS intensities of the characteristic peak at 1368 cm^{-1} for Cy5 were mapped at each grid point on a given cell, resulting in the distribution of miR-144-3p over the cellular area. The colors of the SERS images were used to display the intensity of the 1368

cm^{-1} characteristic peak at each grid point according to a color scheme ranging from blue (lowest intensity) through green, yellow, orange, and red (highest intensity). The target miRNA responses are mostly focused on the cytoplasm, whereas the SERS signals from the nuclear region are similar to the background. Compared to the control group without nanoparticle treatment, BMSCs incubated with GNCs-hpDNA1 exhibited a slight SERS signal of Cy5, while the cells incubated with GNCs-hpDNA1 and GNCs-hpDNA2 presented a stronger SERS signal of Cy5 associated with target miRNAs that induced the assembly of GNC-hpDNA1-hpDNA2-GNC and generated additional hot spots.

Fig. S2(B) showed the SERS spectra of BMSCs incubated with different nanostructures. Untreated BMSCs did not emit a SERS signal, and there was no obvious characteristic peak at 1368 cm^{-1}. For BMSCs incubated with GNCs-hpDNA1 and GNCs-hpDNA2, the SERS intensity at the 1368 cm^{-1} peak was approximately three times higher than that of GNCs-hpDNA1-treated BMSCs. These results demonstrated the highly catalytic amplification ability of GNC-hpDNA1-hpDNA2-GNC assembly for intracellular imaging of miRNA that was low in abundance. As shown in Fig. S3, a time-dependent decrease in SERS intensities at 1368 cm^{-1} was observed. The SERS intensities of the 1368 cm^{-1} characteristic peak increased with the prolongation of the culture time, and remained relatively stable after 4 h. Therefore, the optimized reaction time was determined to be 4 h.

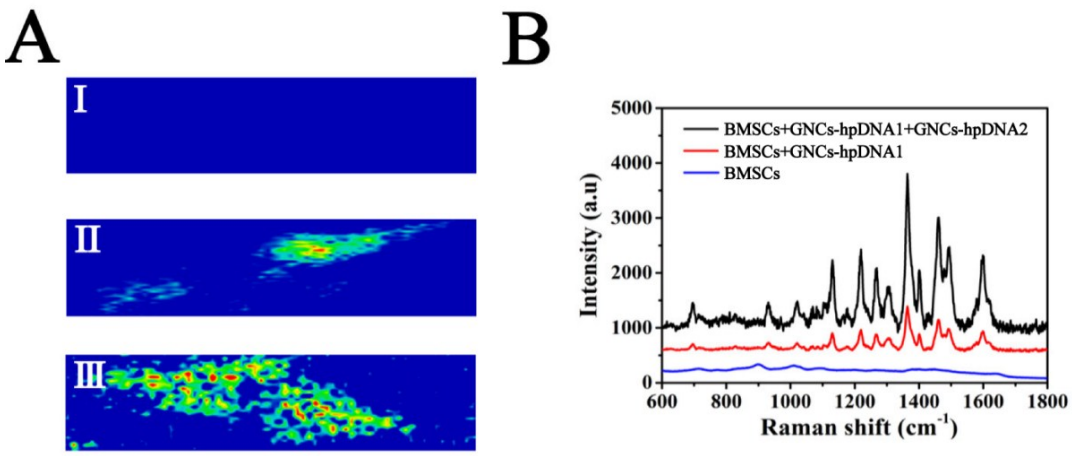

Fig. S2 (A) BMSCs were incubated with different nanostructures for 4 h. SERS images of (I) untreated BMSCs, (II) BMSCs incubated with GNCs-hpDNA1, and (III) BMSCs incubated with GNCs-hpDNA1 and GNCs-hpDNA1. (B) SERS spectra of BMSCs incubated with different nanostructures.

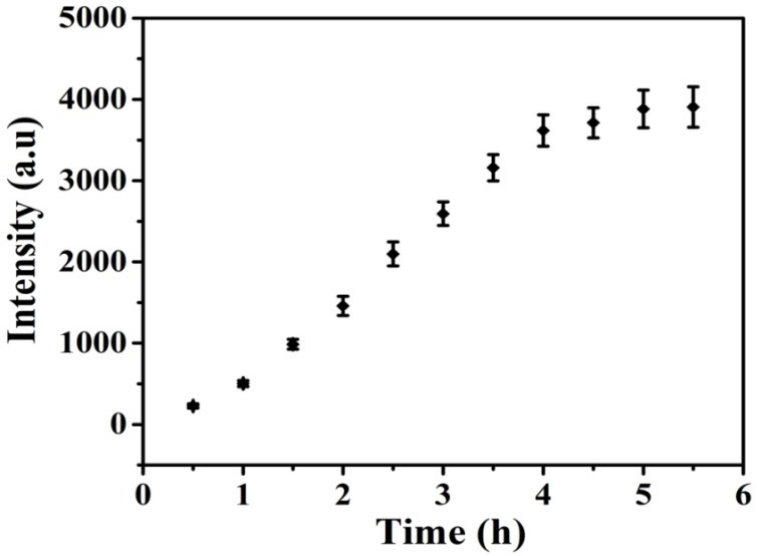

Fig. S3 SERS intensities at 1368 cm^{-1} for BMSCs cultured with hpDNA-conjugated GNCs probes at different times.

1.3 Cell uptake of hpDNA-conjugated GNCs probes during differentiation

The internalization of hpDNA-conjugated GNCs probes in BMSCs at different differentiation times were evaluated by TEM microscopy. As shown in the Fig. S4, the probes can be clearly visible and easily distinguished from cellular because GNCs

have high electron density. The hpDNA-conjugated GNCs probes were mainly located in the cytoplasm of differentiated BMSCs of 1 day as clusters of different sizes, which can be due to the target miR-144-3p-triggered numerous assemblies of GNC-hpDNA1-hpDNA2-GNC nanostructures through intramolecular hybridization. With the prolongation of differentiation time, the distribution of probes in cells was more uniform. When BMSCs were differentiated for 21 days, the surface morphology and size of GNCs were almost unchanged. We further observed only slight aggregation of probes that accumulated in cells. These changes were associated with the decrease of the expression level of intracellular miR-144-3p during osteogenic differentiation. Therefore, TEM results further proved SERS imaging, indicating that SERS can long-term monitoring of miRNA expression in BMSCs.

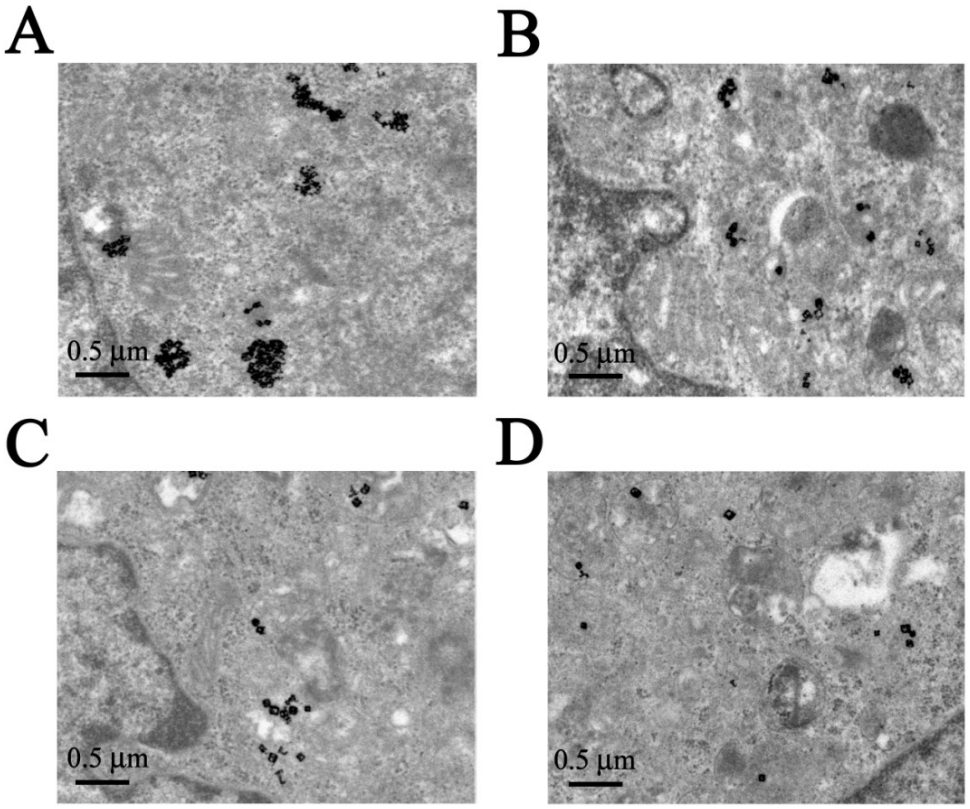

Fig. S4 TEM images of BMSCs loaded probes at (A) 1, (B) 7, (C) 14, and (D) 21 days of differentiation.

References

1. S. Y. Chen and A, A. Lazarides, *J. Phys. Chem. C*, 2009, **113**, 12167-12175.

2. M. Vendrell, K. K. Maiti, K. Dhaliwa and Y. T. Chang, *Trends. Biotechnol.*, 2013, **31**, 249-257.