Electronic Supplementary Information

Early Apoptosis Real-Time Detection by Label-free SERS Based on Externalized Phosphatidylserine

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EXPERIMENTAL SECTION

Chemicals, Biochemicals and Materials. Sodium hydroxide (NaOH), sodium choride (NaCl), hydroxylamine hydrochloride ($NH_2OH \cdot HCl$), silver nitrate ($AgNO_3$), phosphatidylserine (PS), HEPES buffer, L-Glutamine and calcium nitrate ($Ca(NO_3)_2$) were purchased from Sigma-Aldrich (Taufkirchen, Germany) and used without further purification. Glass slides (26 mm×76 mm×1 mm) were purchased from Carl Roth (Karlsruhe, Germany). MilliQ water (18.2 MΩ cm) was produced using a Millipore water purification system.

Cell culture. HL-60 cells (ATCC® CCL-240) were cultured in RPMI-1640 medium (Life Technologies, Germany) buffered with 25 mM HEPES, 0.1 % $NaHCO_3$ and supplemented with 10 % heat-inactivated fetal bovine serum (Sigma, Germany), and 2 mM L-Glutamine in a humidified 5 % CO_2 atmosphere at 37 °C. The cells were plated on 12 well plates with a density of 1×10^6 cells/mL the day before treatment.

Induction of cell apoptosis. In order to induce cell apoptosis, HL-60 cells were treated with 500 nM staurosporine for 3, 5 and 7 h in RPMI-1640 medium. Cells were collected and prepared for the Bright-field microscopy, Flow cytometry, Raman spectroscopy, and Dark-field microscopy analysis.

Flow cytometry. Suspended HL-60 cells were collected into a 1.5 mL Eppendorf tube by centrifugation at 2000 rpm at 4 °C for 3 min and prepared ready for analysis of apoptosis by flow cytometric analysis (Milteyi GmbH, Germany). The cell pellets were washed once with 1× PBS, then one time with 1× Binding Buffer. Cells were resuspended in 1× Binding Buffer at 1×10^6 cells/mL. Afterwards, 5 µL of APC-conjugated Annexin V were added to 100 µL of the cell suspension and incubated for 10 - 15 min at room temperature, protected from light. Cells were washed with 2 mL of 1× Binding Buffer and resuspended in 200 µL of 1× Binding Buffer, and were incubated with 5 µL of Propidium Iodide staining solution. Cells were analyzed by MACSQuant VYB (Miltenyi GmbH, Bergisch Gladbach, Germany).

In-situ coating method. A 1 mL of HL-60 cells suspension at 1 × 10^6 cells/mL (normal and apoptotic cells) was centrifuged at 2000 rpm, and then washed with PBS twice. The supernatant was discarded. Then 20 μL of 10 mM $AgNO_3$ solution was added, and the mixture was gentle vortexed and then 60 seconds of interaction time was allowed. Subsequently, 180 μL of $NH_2OH{\cdot}HCl$ (1.7 mM) solution was pipetted into the prepared mixture and the mixture was again gentle vortexed. Finally, we stored the suspension in the dark at 4 °C until it was analyzed. For Ca^{2+} and PS experiments, normal and apoptotic cells were harvested, and then washed with PBS twice. The supernatant was discarded. Subsequently added into 10 μL of 10 mM $Ca(NO_3)_2$ and 10 μL of 1 mM PS solution, respectively. Then the mixture was gentle vortexed and 2 min of interaction time was allowed. Finally, the suspension followed by in-situ coating method described above.

SERS Measurements. 3 μL of the sample suspension, already treated with in-situ coating method, was pipetted onto the glass slides. The recording of the Raman spectra was initiated right after this sample preparation, using the 633 nm line of a He-Ne with 14 mW power at the sample and a 10 × objective. The Raman spectra were continuously (one spectrum every 40 s) collected with the auto repeat function until the droplet was dried. If not explicitly stated, the exposure time was 1 s and the number of accumulations was 10, and the confocal slit width was 100 μm, detecting a m, spectral region of 50-3000 cm^{-1}.

Colloid diagnostics. SERS measurements were conducted with a Raman microscope (LabRAM HR, HORIBA Scientific, Japan). Cell morphology and AgNPs aggregates were examined by bright-field microscopy with 20× magnification (Axiovert 135, Carl Zeiss GmbH). The images and videos of samples were recorded using Dark field microscopy. The setup was based on an upright microscope (Axiotech 100, Carl Zeiss GmbH) equipped with an oil immersion dark-field condenser (Zeiss Axiotech 100, NA=1.25) and a 100× (NA=0.9) water immersion objective. A 100 W halogen lamp was used for illumination. The dark-field images were recorded using a digital SLR camera (Canon EOS 6D).

Table S1. The tentative band assignment of the SERS spectra of the HL-60 cells.[1, 2]

Raman Shift (cm^{-1})	**Assignment***
665	δ(COO-)
726	adenine, glycosidic ring mode
1332	$\upsilon(NH_2)$ adenine, polyadenine
1576	amide I, υ(CN), γ(NH)

* Approximate description of the modes (υ, stretch; δ and γ, bend).

Notes and references

1. X. X. Jiang, Z. Y. Jiang, T. T. Xu, S. Su, Y. L. Zhong, F. Peng, Y. Y. Su and Y. He, *Anal. Chem.*, 2013, 85, 2809-2816.

2. A. F. Chrimes, K. Khoshmanesh, S. Y. Tang, B. R. Wood, P. R. Stoddart, S. S. E. Collins, A. Mitchell and K. Kalantar-zadeh, *Biosens. Bioelectron.*, 2013, 49, 536-541.