Investigation on activating individual living monocytic U937 cell by interleukin-6 using Raman tweezers

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We investigate the activation of living monocytic U937 cells induced by interleukin-6 (IL-6) at the single cell level. We employ home-built Raman tweezers to measure the Raman spectra of living U937 cells with and without IL-6 at the single cell level. Raman peaks of amide III, amide I, DNA backbone, as well as guanine and adenine in U937 cells, change at 1312, 1652, 1090, and 1576 cm^{-1}, respectively, shortly after IL-6 is added in the medium. The change is a dynamic temporal process. In the activation process of U937 cells induced by IL-6, the protein signals recover in 20 min, while the nucleic acid signals continue to increase for 20 min. The results reveal that the biochemical cascades of activation in signal transduction induced by IL-6 can be investigated *in situ* at the single cell level.

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Interleukin-6 (IL-6) plays an important role in the immune system. It induces growth and differentiation or activation of several human cell lines by triggering a sequence of biochemical reactions from the cell membrane to the cell nuclear[1]. IL-6 may be actively involved in hypoxia-induced lung inflammation and pulmonary vascular remodeling[2]. It also modulates altered expression of several key hepatic genes in sepsis[3,4], and may be responsible for coronary heart disease by short acting[5]. IL-6 plays a crucial role in the biological behavior of blood-derived endothelial progenitor cells, which may help clarify the mechanism of IL-6 inflammatory-related diseases[6]. IL-6 also has an important role in skin development and maintenance[7]. However, as far as we know, there has been no study yet on the rapid and direct identification of IL-6 function on living cells, such as monocytic U937 cells. This is necessary for understanding the regulating mechanism of IL-6 on various cellular processes.

Raman spectroscopy is a practical technique for studying single living cells by enabling the detection of their spectral features[8−10] and their rapid response to structural changes at the molecular level[11]. Combining Raman spectroscopy with optical tweezers[12,13] has made non-destructive, non-invasive single cell detection and characterization possible without resorting to mechanical immobilization or introducing biochemical tags[14−16]. The Raman spectrum of optically trapped cells also has the advantage of eliminating the inference of cover-slips and confining cells from Brownian motion[17]. Recently, Raman tweezers have been used for study of the heat activation of single microbial cells[18], analysis of the effect of alcohol on single human red cells[19], and detection of hemoglobin-related blood disorders[20,21]. In addition, Harvey *et al.*[22] described the potential of Raman tweezers in cancer diagnosis.

In this letter, we employ home-built Raman tweezers to investigate real-time stimulation of IL-6 on biochemical reactions of monocytic U937 cells. By obtaining the Raman spectra of single monocytic U937 cells before and after adding IL-6, we hope to determine if there are differences between untreated and activated U937 cells, and how the spectra of the cells change during the signal transduction process induced by IL-6.

The Raman spectra of a single living cell were obtained using a home-built Raman tweezers system that integrated the capability of optical tweezers and Raman spectroscopy. Manipulation and spectra-measurement can be carried out simultaneously on a single cell with the apparatus. Using independent lasers can optimize laser parameters for separate purposes and modulate the Raman exciting beam position relative to the trapping beam[17].

Human monocytic U937 cell line was provided by the Third Hospital of Peking University. The U937 cell line is a human cell line from a diffused histiocytic lymphoma. Cells were cultured at 1×10^6 cells/ml in RPMI 1640 medium supplemented with 100 μg/ml penicillin/streptomycin and 10% fetal calf serum at 37 °C in humidified atmosphere (5% CO_2 in air). Cells were centrifuged (1 min, 4000 rpm) and harvested, and then washed twice in RPMI 1640 medium without phenol red and trapped by optical tweezers for Raman spectroscopy. Raman spectra ranging from 700 to 1800 cm^{-1} were recorded by a liquid nitrogen (LN) cooled spectroscopic charge-coupled device (CCD) (Spec-10, Princeton Instrument). The CCD had an integration time of 30 s and was excited by a 514.5-nm Ar^+-laser (Shanghai AiAo, ILT5500ASL) with power of 20 mW. The trapping power of 1064-nm Nd:YAG laser (Coherent, Compass 1064-2000N) was about 40 mW on each sample. Resultant Raman spectra were analyzed with SpectraSense software. In this study, the background spectra were subtracted. Data were averaged over 20 cells. Raman spectra were recorded with an ideal spectral resolution of 4 cm^{-1}.

The Raman spectra of individual living monocytic U937 cells are shown in Fig. 1. For the 20 averaged cells, normalized standard deviations of the intensities of Raman peaks at 1090, 1312, 1450, 1576, and 1652 cm^{-1} are listed in Table 1. Results show that the stronger the intensity, the more stable the peak. The normalized standard deviation of peak intensity at 1450 cm^{-1} is the smallest.

Peak positions and their tentative assignments are listed in Table 2. The feature is a large peak at ~1450 cm^{-1} (Fig. 1), corresponding to the CH_2-bending vibrations, mainly from lipids and long-chain amino acids[23]. The broad peak centered around 1312 cm^{-1} is assigned to amide III vibrations of the peptide backbone of proteins[23]. Another prominent feature of the Raman spectra of monocytic U937 cells is that the peak centered around 1652 cm^{-1} is assigned to amide I vibrations of the peptide backbone of proteins[23].

The peaks at 730, 787, and 896 cm^{-1} can be assigned to adenine, thymine, cytosine and the DNA backbone[8,24], respectively. The 1090 cm^{-1} peak is attributed to the symmetric PO_2 stretching vibration mode of DNA backbone[24]. The peaks at 1000 and 1126 cm^{-1} are assigned to a breathing mode of phenylalanine (aromatic ring) and C-N stretching vibration mode of proteins[8]. The peak at 1259 cm^{-1} can be attributed to the amide III vibrational modes[24]. The peak at 1340 cm^{-1} is assigned to adenine and guanine of DNA[23], and the peak around 1576 cm^{-1} to the nucleic acid ring stretches, especially to contributions from adenine and guanine[25].

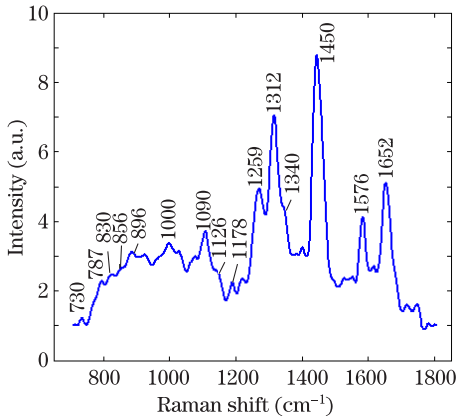

Fig. 1. Raman spectra of living monocytic U937 cells. The cell was optically trapped by 1064-nm infrared laser and excited by 514.5-nm Ar^+-laser with a power of 20 mW and integrating time of 30 s.

IL-6 plays a crucial role in the immune system and induces growth and differentiation or activation of several lines of human cells[1–7]. IL-6 exerted its biological function by binding a specific membrane receptor subunit (IL-6R$^{\alpha}$), then the dimeric complex dimerizes and binds to two signal-transducing molecules, gp-130, to form a functional hexameric complex[26]. However, direct rapid observation of the biological function of IL-6 at the individual cell level has never been shown. Here, we investigate real-time Raman spectra of trapped living single U937 cell after adding IL-6 into the solution.

Figure 2 presents the Raman spectra of single trapped U937 cells. The spectral line (a) is acquired from individual living U937 cells without IL-6, while spectral lines (b), (c), and (d) are acquired at different times after adding IL-6 in culture medium solution.

The peak at 896 cm^{-1} changes intensely (Fig. 2) due to the deformation of DNA backbone after adding IL-6 to the medium containing U937 cells[8,25]. The peak at 815 cm^{-1} in the spectral line (b) acquired after adding IL-6 in 5 min is assigned to DNA phosphodiester[27], the peak at 758 cm^{-1} may be assigned to thymine ring, the peak at 950 cm^{-1} to deoxyribose[28]. Data suggest that the interaction between IL-6 and IL-6 receptor on U937 cell surface may induce untwisting DNA within the living cell.

The peak at 1450 cm^{-1} can be treated as an internal intensity standard[27]. The intensity ratios of major Raman peaks for living U937 cells with and without IL-6 at different times are listed in Table 3. Results indicate that after adding IL-6 into the culture medium, the intensity ratios of peaks at 1259, 1312, and 1652 cm^{-1} to that of the band at 1450 cm^{-1} changed significantly in 5 min, then recovered in 20 min. This suggests that IL-6 may induce structure change of protein inside U937 cells in a short time, and the protein signals can recover in 20 min. After adding IL-6, the intensity ratios I_{1090}/I_{1450} and

Table 1. Normalized Standard Deviations of Raman Peak Intensities

I_{1090}	I_{1259}	I_{1312}	I_{1450}	I_{1576}	I_{1652}
0.186	0.188	0.146	0.035	0.188	0.155

Table 2. Raman Shift Observed in the Spectra of Single Living U937 Cell and Tentative Assignment

Peaks (cm^{-1})	Protein	Peaks (cm^{-1})	Nucleic Acids
830, 856	Tyrosine[8,25]	730	A (C-C, Ring Mode)[8,24]
1000	Phenylalanine[8,23]	787	C, T (Ring Mode)[8,24]
1126	C-N Stretching[8,24]	896	DNA:BK[8,24]
1178	Tyrosine, Phenylalanine[8,25]	1090	Backbone (P-O-P)[23–25]
1259, 1312	Amide III[8,25]	1340	A, G[8,24,25]
1450	CH_2 Deformation[8,23]	1576	A, G (Ring Stretch)[8,24,25]
1652	Amide I ν(C=O)[23,24]		

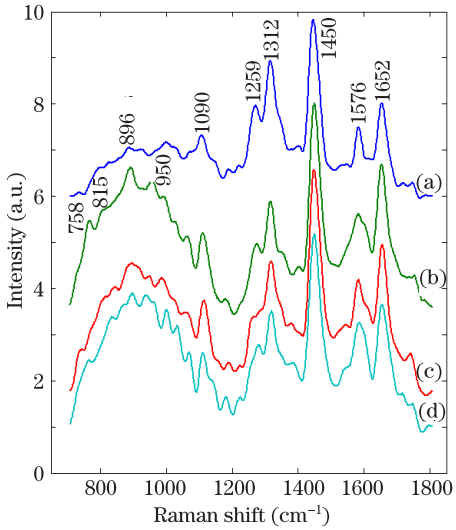

Fig. 2. Raman spectra of single trapped U937 cells with and without IL-6 at different times in the range of 700−1800 cm^{-1}. All spectra are averaged by 20 cells and the background subtracted. The spectrum line (a) shows the Raman spectra without IL-6, while lines (b), (c), and (d) indicate Raman spectra collected 5, 10, and 20 min after adding IL-6 to medium solution, respectively. Integrating time is 30 s.

I_{1576}/I_{1450} increase consistently with time, which means that the PO_2 stretching vibration mode of DNA backbone, guanine, and adenine ring stretches[25] change consistently with time. This suggests that nucleic acid signals continue to increase for 20 min. Raman spectroscopy results show that IL-6 could induce a sequence of biochemical reactions inside living U937 cells, which is consistent with the principles of IL-6 cytokine signaling and its regulation[1]. The Raman results also demonstrate that the response process of IL-6 on living U937 cells is acute, which makes sense in inflammation and immune response[1]. The results above suggest that biochemical reactions induced by IL-6 inside living U937 cells may be revealed *in situ* at the single cell level.

In conclusion, Raman spectra of living U937 cells are identified and investigated using a home-built Raman tweezers system. By obtaining the Raman spectra of single monocytic U937 cells before and after adding IL-6, we find that during the activation of U937 cells induced by IL-6, some protein signals recover in 20 min, while some nucleic acid signals continue to increase for 20 min. Results indicate that our approach enables the semi-quantitative real-time investigation of living cells *in situ*, including probing cellular dynamic processes at the single cell level.

Table 3. Ratios of the Intensities of Characteristic Raman Peaks of U937 Cells

	$I_{1090/1450}$	I_{1259}/I_{1450}	I_{1312}/I_{1450}	I_{1576}/I_{1450}	I_{1652}/I_{1450}
No IL-6	0.354	0.511	0.772	0.400	0.525
IL-6 in 5 min	0.389	0.311	0.537	0.475	0.711
IL-6 in 10 min	0.421	0.348	0.597	0.511	0.669
IL-6 in 20 min	0.401	0.382	0.613	0.554	0.645

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