Biophysical Chemistry 208 (2016) 48–53

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Raman micro-spectroscopy study of living SH-SY5Y cells adhering on different substrates

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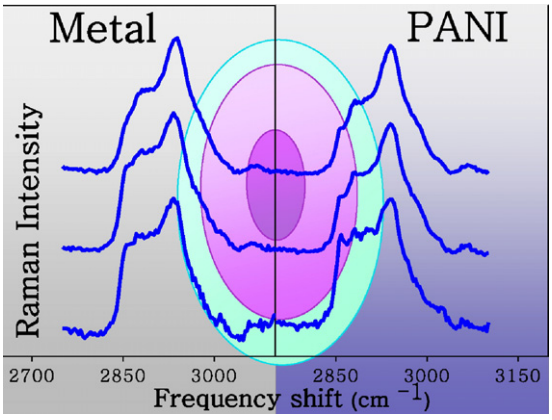
HIGHLIGHTS

- We produce a hybrid device: neuron-like cells adhering on organic memristor.
- The effect of different substrates on the cellular status is analysed.
- Raman mapping of single living cell is realized.
- Time evolution of Raman signals in stressed living cells is investigated.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form 16 July 2015
Accepted 29 July 2015
Available online 31 July 2015

Keywords:
Raman micro-spectroscopy
Cellular mapping
Neuron-like cells
Cellular stress
Cells adhering on different substrates

ABSTRACT

In this paper we test the ability of Raman micro-spectroscopy and Raman mapping to investigate the status of cells grown in adhesion on different substrates. The spectra of immortalized SH-SY5Y cells, grown on silicon and on metallic substrates are compared with those obtained for the same type of cells adhering on organic polyaniline (PANI), a memristive substrate chosen to achieve a living bio-hybrid system. Raman spectra give information on the status of the single cell, its local biochemical composition, and on the modifications induced by the substrate interaction. The good agreement between Raman spectra collected from cells adhering on different substrates confirms that the PANI, besides allowing the cell growth, doesn't strongly affect the general biochemical properties of the cell. The investigation of the cellular state in a label free condition is challenging and the obtained results confirm the Raman ability to achieve this information.

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http://dx.doi.org/10.1016/j.bpc.2015.07.009
0301-4622/

1. Introduction

Vibrational spectroscopies are largely used to investigate wide classes of materials ranging from glasses to photonic devices [1,2], from solutions to polymers [3–5]. In the last years, due to their non-invasive and label free nature and thanks to their easy applicability in physiological environments, vibrational spectroscopies have been increasingly used to investigate a larger and larger variety of properties and processes occurring in biological matter [6–11]. Raman scattering provides information on the chemical species, the composition, the amount of constituent molecules and their evolution in complex biochemical systems. Following specific markers in the spectra of living cells, Raman spectroscopy has proved to distinguish and identify different cellular states [7,12]. The drug internalization, the modification after the differentiation induction, the analysis of dynamic death events in single cells [13], and the pathological diagnosis of tissues [14] are only some of the latest challenges faced by Raman spectroscopy [15].

In the present paper, we exploit the potential of the Raman spectroscopy to characterize the status of single living cells grown on different artificial substrates. The chosen cell line is the human neuroblastoma cell line SH-SY5Y [16] growing as adherent monolayers and differentiating into neuron-like cells, which is nowadays considered a standard model for the in vitro study of neuronal growth and differentiation [17].

Aimed to study the biochemical variations induced in different cellular compartments by the interaction with several substrates, we selected silicon and metallic substrates, often used for the vibrational spectroscopy analysis [18,19]. They present low intensity Raman signals and they have a good bio-compatibility cause of their almost inert and non-interactive nature. In addition, we also used poly(aniline) (PANI) films. This substrate was chosen for its peculiar memristive properties: its electronic behaviour seems to effectively mimic some properties of neuronal synapses [20]. Many efforts are, indeed, addressed to prove and measure possible cross-talk between biological and polymeric samples to achieve a hybrid bio-electronic device mimicking neural activities [21]. For this purpose, memristive substrates have recently shown their strategic potentiality [22–24].

In this paper, following the intensity modification of characteristic Raman peaks and the shape of the CH stretching band, we monitor the status of cells grown on the different substrates. Combining spectroscopic data with a Raman mapping analysis we find that, though stress effects occur faster in cells grown on PANI than in those grown on the silicon substrate, the biochemical composition and the supermolecular organization seem to be rather unaffected by the substrate.

2. Materials and methods

2.1. Cell culture

Human neuroblastoma cell line (SH-SY5Y) cells were obtained from the American Type Culture Collection (ATCC). It is a well characterized human continuous cell line classified at low biohazard risk. All cell manipulations were carried out using mechanical pipetting devices in a vertical laminar flow biosafety cabinet. Contaminated material was disposed in biohazard waste according to national legislative requirements. Cells were grown in Dulbecco Modified Eagle's Medium (DMEM) containing 10% (v/v) heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, 100 U/mL streptomycin (SIGMA Aldrich, St. Louis) and were maintained at 37 °C in a 5% CO humidified atmosphere. The doubling time of SH-SY5Y cells was about 48 h until cells reach confluence. When cells reached 80% confluence, they were trypsinized and seeded at a density of 1×10^5 cells/cm^2 for 48 h onto the chosen supports sterilized either by ultraviolet (UV) irradiation or by ethanol immersion. Cells were grown in sterile conditions on three different substrates: on silicon, on stainless steel and finally on a coverslips covered with 20 multilayers of synthetic PANI. The PANI film was obtained by the deposition of Polyaniline (PANI) multilayers on glass substrates with the Langmuir–Schaefer (LS) technique (for details see ref. [25]). Cell growth on the three substrates was assessed by counting viable cells. The viability of cells was estimated by examining their ability to exclude Trypan blue (0.1% in 0.9% NaCl). Briefly, cell grown on different substrates were trypsinized, serially diluted up to a final 1:2 in Trypan blue solution and counted on a hemocytometer. This test assesses the number of viable cells in a cell suspension by selectively staining only dying cells without an intact cell membrane, unable to exclude the dye [26].

2.2. Raman experiments and data treatment

To perform Raman measurements, the culture medium was removed and the cells were washed twice and immersed in phosphate-buffer saline (PBS). Raman spectra were collected using a micro-Raman setup equipped with a solid state laser at $\lambda = 532$ nm whose power is reduced to less than 10 mW to decrease the photon damage of the cells during the acquisition time.

The Raman setup consists of iHR320 Triax Imaging Spectrometer of the HORIBA Jobin Yvon coupled in an home-made configuration with a CM1 microscope purchased form the JRS Scientific Instruments (Switzerland). The microscope is equipped with different objectives; for the measurements of the present study, a water immersion objective Olympus Mod. UPLSAPO 60XW was chosen in order to reach high quality images and spectra thanks to its high numerical aperture, N.A. = 1.2, its high transmission and chromatic aberration correction. In the chosen experimental configuration, the exciting source was focused onto the single SH-SY5Y cell through the objective directly immersed into the PBS solution. In this configuration, the achieved spatial resolution is about 2 μm.

The objective focalized the exciting light and collected the scattered radiation in a back scattering configuration and, thanks to the use of a polarizer filter, the polarized VV and the depolarized VH radiation can be selectively measured.

Analysing Raman signals in a selected polarization recently revealed its potentiality to increase the chemical contrast of the spectral imaging

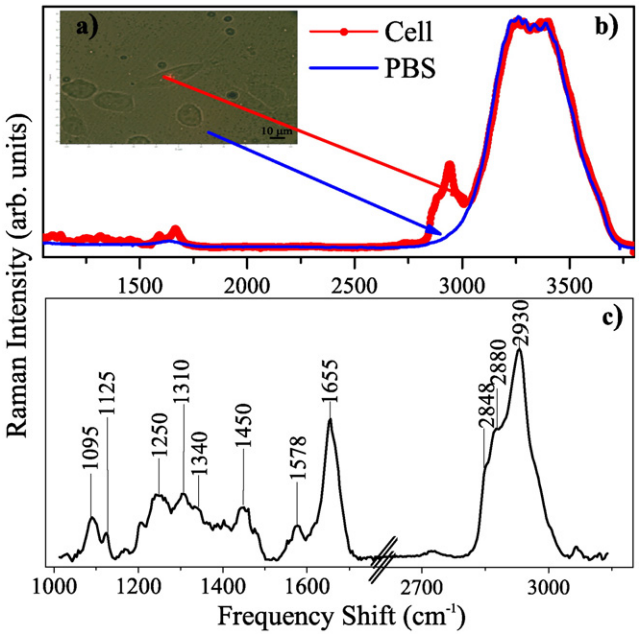

Fig. 1. a) Typical image of SH-SY5Y investigated by Raman Spectroscopy. The arrows indicate the characteristic points chosen to perform the measurements. b) Spectrum of the cell adherent on silicon substrate (circles) and of PBS (blue line). c) Spectrum of the cell with band assignments after subtraction of the buffer contribution.