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Monitoring X-rays exposed and unexposed cell culture media by means of Surface-Enhanced Raman Spectroscopy

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ABSTRACT

Monitoring cell culture media by traditional methods has high costs and requires significant analytical expertise and laboratory space. Surface-Enhanced Raman spectroscopy (SERS) can offer a method for a simple and fast analysis of cell culture media under different conditions. In particular, to examine cell culture media during cell exposure to ionizing radiation deserves particular attention. In this way, useful information on the complex processes occurring during the interaction between cells, cell culture media and radiation can be obtained. We report about a SERS study of the radiation-induced changes on cell culture media that were in contact or not with human cells. SERS measurements were performed by using commercial substrates and a conventional micro-Raman spectroscopy set-up. By employing a suitable data treatment based on "wavelet" denoising algorithm and background subtraction, spectra with clear Raman features were obtained for two cell culture media that were subject to different irradiation treatments. The obtained results evidence that SERS can be used to rapidly identify and monitor chemical changes in cell culture media.

Keywords: SERS, Cell culture media, X-ray irradiation effects, cells and cell culture media interaction, SERS spectrum data analysis

1. INTRODUCTION

Cell culture media (CCM) are an essential part of the cell culture processes guaranteeing optimal cell growth. They are composed by amino acids, glucose, salts, vitamins, and other nutrients, and are available either as a powder or as a liquid form. A main component of CCM is serum, a complex mixture of albumins, growth factors and growth inhibitors. Serum is a source for amino acids, proteins, vitamins, carbohydrates, lipids, hormones, growth factors, minerals, and trace elements [1]. The exact composition of the employed medium varies for the different cell lines and, for certain of them, because of the presence of additional components. These components are required to sustain proliferation and maintain normal cell metabolism. In addition, antibiotics are often used to control the growth of bacterial and fungal contaminants. It is also known that CCM are not stable and can modify their composition due to the occurrence of chemical reactions. Monitoring CCM is important for avoiding degradation that can negatively influence cellular processes [2] and also because it can give useful information during the interaction of cells with external agents. In this context, it is particularly interesting to examine cell media during cell exposure to ionizing radiation since in this way useful information on the complex processes occurring during the interaction between cells, cell culture media and radiation can be obtained.

Nowadays a wide spectrum of analytical techniques is available for monitoring variations in CCM composition. Among the others, mass spectroscopy has been shown to offer significantly high sensitivity, but its use for routine CCM testing is limited because this technique is expensive and also cumbersome to perform. Other alternatives include blotting, capillary electrophoresis, enzymatic methods, high-performance liquid chromatography, gas chromatography and SDS-PAGE [2]. Optical spectroscopic techniques and nuclear magnetic resonance (NMR) spectroscopy [3] have been demonstrated to be useful tools for identification and characterization of such variations. In particular, optical spectro-

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scopic techniques, that are continuously showing to be powerful methods for studies in the bio-medical field [4-12], offer new simple and fast methods for analyzing culture media. Raman, near-infrared, Fourier-transform infrared, fluorescence spectroscopy capabilities are well suited for such a kind of studies [3]. Surface enhanced Raman spectroscopy (SERS), a technique coupling the biochemical sensitivity of Raman techniques and the possibility to have an enhanced signal [13-15], has been already adopted for detection of trace melamine in CCM for protein pharmaceutical manufacturing [16], for monitoring CCM degradation [17, 18] and pH changes [19].

In the present work, we report about a SERS study of the radiation-induced changes on two different types of CCM that were in contact or not with human cells. The obtained results evidence that SERS can be used to rapidly identify and monitor chemical changes occurring in the investigated CCM.

2. MATERIALS AND METHODS

Two different kinds of Dulbecco's modified Eagle media were investigated as representative CCM, namely DMEM-A (from MicrotechTM Research Products, Italy) and DMEM-B (from GibcoTM, Thermo Fisher Sci. Inc., Waltham, MA, USA). In order to acquire significant Raman spectra from the cell culture media, commercial SERS substrates (Q-SERS, NanovaTM Inc., Columbia, Missouri, USA) were used. DMEM-A samples were exposed to different X-ray doses (0, 2, 4, 6 Gy). The DMEM-B samples were extracted from cultures of SH-SY5Y cell line irradiated at two X-ray doses (2 and 4 Gy). DMEM-B samples extracted from cultures of not-irradiated SH-SY5Y cell line were also investigated (control samples).

The SERS measurements were performed by using a Jobin-Yvon system from Horiba Scientific ISA (Edison, NJ, USA) described in details elsewhere [20]. Briefly, the system was equipped with a TriAx 180 monochromator, a liquid N_2-cooled CCD and an optical grating of 1800 grooves/mm, allowing a spectral resolution of 4 cm^{-1}. A He-Ne laser operating at a wavelength λ = 633 nm was used (maximum nominal power of 17 mW). The laser light was focused on the sample surface by means of a 100X (n.a. = 0.90) optical objective on an excitation area of about 1 μm in size. SERS measurements were performed by placing a volume of 1 μl for each DMEM samples on the SERS substrate.

SERS spectra collected from complex media typically show a smeared background signal. In order to enhance the signal readability and attenuate background and noise components of the signal, an automatic numerical treatment based on the wavelet algorithm was used [20]. In order to determine the vibrational modes that contribute to the Raman signal, the spectra were analyzed in terms of convoluted Lorentzian functions by using a best-fit peak-fitting routine of GRAMS/AI program (2001, Thermo Fisher Scientific, Waltham, MA, USA), based on the Levenberg-Marquardt nonlinear least-square method. Additional details about the fitting procedure are reported elsewhere [21,22].

3. RESULTS AND DISCUSSION

The light scattering spectra of DMEM samples typically feature a high fluorescence component that critically hampers the Raman analysis. In SERS measurements, the interaction between molecules and substrates strongly attenuates the intensity of the fluorescence signal. Nevertheless, a data treatment remains necessary in order to separate the SERS signal from unfeatured background and noise components. A numerical procedure based on wavelet algorithm was used at this aim. It was applied to SERS signals collected on DMEM samples, allowing to efficiently remove the background signal without affecting positions and relative intensities of Raman modes. A representative SERS spectrum of DMEM-A as obtained after this data treatment is reported in Figure 1(a). The large number of modes observed in the figure is related to the complex content of the DMEM and is due to contributes arising from different vibrational modes of proteins, lipids and other CCM components. It is not easy to discriminate the single components because of the large number of different substances involved. Nevertheless, some of the main components can be outlined by means of the deconvolution of the signal in terms of Lorentzian peaks obtained by fitting the experimental data. In Figure 1(b) a selection of the found modes are reported. According to literature, they can be thought to arise from some of the main components of CCM [17,21]. In fact, modes at 439, 863, 1071 and 1338 cm^{-1} can be assigned to glucose while those found at 917, 1282 and 1392 cm^{-1} to cysteine. Folic acid is instead responsible for the peaks at 682 and 1189 cm^{-1} and $NaCOH_3$ for the one located at 1080 cm^{-1}. The tentative mode assignments for glucose, cysteine and folic acid have been derived from Refs. [17,21]. A more complete list of the mode assignments is reported in Table 1.

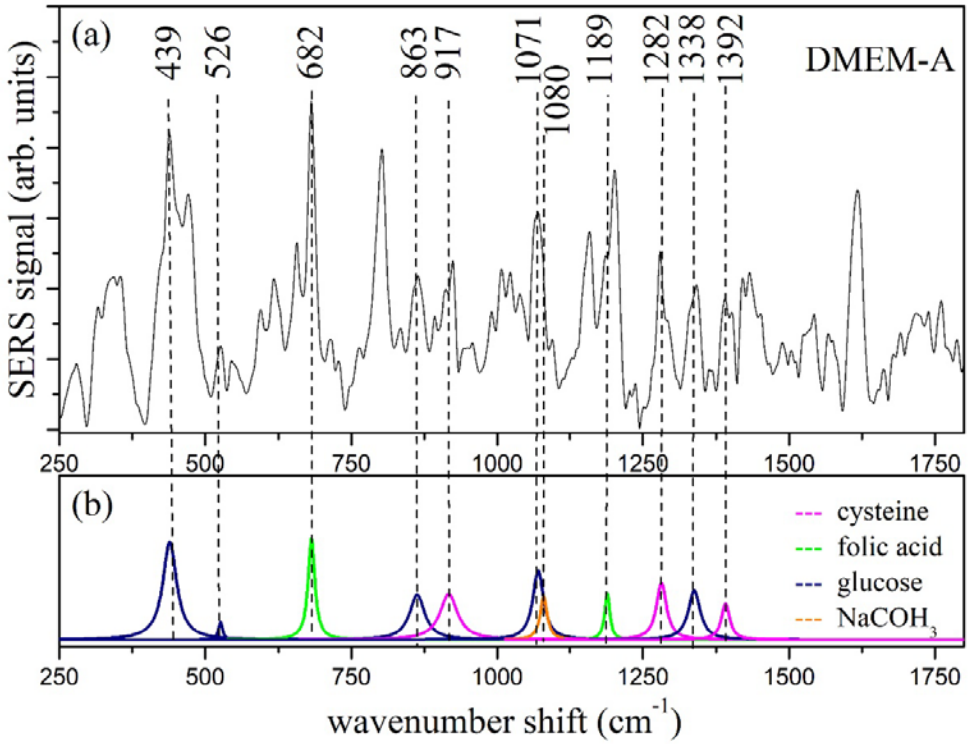

Figure 1. SERS spectrum of DMEM-A. (a) experimental data; (b) selected modes obtained by the deconvolution of the signal in terms of Lorentzian peaks and assigned to cystine, folic acid, glucose and $NaCOH_3$.

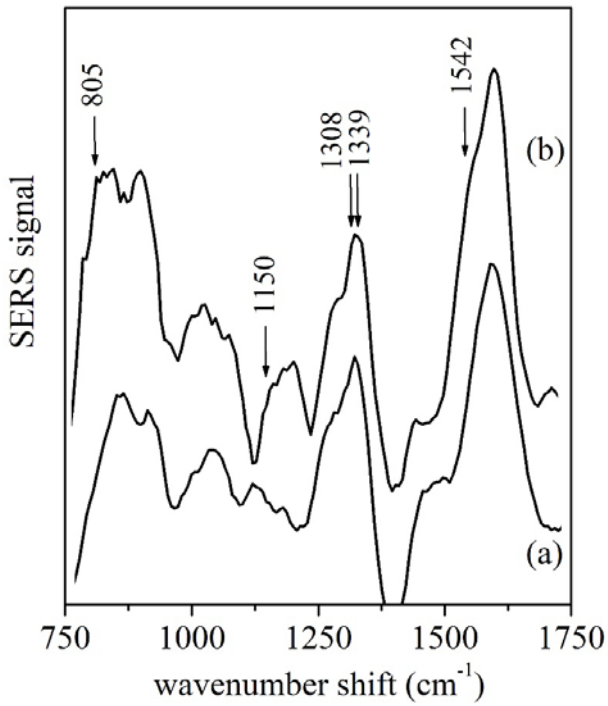

Figure 2. SERS spectra of DMEM-B. The SERS response of pure DMEM-B (a) is compared with the spectrum measured on sample that hosted cells (b).

Table 1. Tentative assignment of outlined DMEM-A SERS mode, in agreement with Refs. [17,18,21,23]. Bold characters are used for modes with high intensity.

Peak position (cm^{-1})	**Assignment**
339	-
439	Glucose
471	-
526	Glucose
633	-
682	Folic acid
716	-
800	-
863	Glucose
917	Cysteine, Glucose
1016	-
1071	Glucose
1080	$NaCOH_3$
1163	Acetoacetate
1189	Folic acid
1208	Phenylalanine
1282	Cysteine
1338	Proteins, Glucose
1392	Cysteine
1433	Adenine, Guanine
1494	Histidine
1529	Nucleic acids, Cytosine.
1543	-
1571	Proteins
1616	Proteins
1687	-

The SERS response of CCM is slightly modified by the presence of cells. In Figure 2, the SERS spectrum of DMEM-B sample before (plot a) and after cell culture process (plot b) are compared. Some slight differences are noticed between the two spectra. The analysis of the data by deconvolution of the spectra in terms of Lorentzian modes allows to locate some of the most significant differences at 805, 1150, 1308, 1339 and 1542 cm^{-1}. Tentative assignments of these modes are listed in Table 2 (data referred as "control" sample). The assignment has been mainly addressed to amino acid components in agreement to Ref. [23]. The same modes have been monitored in samples of CCM (DMEM-B) extracted from cultures of cells that have been exposed to X-ray irradiation at doses of 2 Gy and 4 Gy. The positions of the peaks as obtained by performing the mentioned deconvolution analysis are reported in Table 2 (data referred as "2-Gy dose" and "4-Gy dose" sample). The position of modes attributed to cell presence is affected by irradiation process. The relative intensity results changed as a consequence of the exposition to X-rays, too. By considering together the results reported in Table 2 and Figure 2, it comes out that some differences can be noticed in the spectral region of Amide I.

Table 2. Positions of peculiar SERS modes outlined by analysing the SERS spectra of DMEM-B in contact with cells and tentative assignments in agreement with Ref. [22]. Abbreviations: str. = stretching, sym. = symmetric, def. = deformation, wag. = wagging, tw. = twisting, ben. = bending.

Peak position Control	**Peak position 2-Gy dose**	**Peak position 4-Gy dose**	**Assignment**
805	801	779	C-C str. *Methionine* *Histidine*
1151	1152	-	NH_2^+ def. NH_3^+ def. *Alanine* *Methionine*
-	1193	1183	CH_2 wag. *Threonine*
1308	-	1310	CH_2 wag. *Phenylalanine* *Glutamine* *Glutamic acid* *Lysozime* *Alanine*
1339	-	1336	CH def. CH_3 sym. bend. *Tryptophan* *Threonine*
1543	1548	1546	C-C str. *Triptophan*

To better understand the information embedded in the Amide I region, the SERS spectra for DMEM-B detected in the region are shown in Figure 3. In particular, the spectrum for the pristine CCM is reported in Figure 3(a), SERS spectrum for the CCM that hosted the not-irradiated cells is displayed in panel (b) and the corresponding spectra detected for CCM after the contact with irradiated cells at doses of 2 Gy and 4 Gy are shown in panels (c) and (d), respectively. Beyond the shift of the position of the 1542 cm^{-1} mode, a change of the Amide I band is clearly noticed indicating a modifications of the protein secondary structure of the CCM components, that may be due to disorder increase [24].

In order to study this aspect, we have performed SERS measurement on the bare DMEM-A (without cells) irradiated with X-rays at different doses in the range of 2-6 Gy. The obtained spectra for control sample (0) and 2 Gy, 4 Gy and 6 Gy irradiation doses are reported in Figure 4. Even if there are not evident changes in the peak positions, the relative intensity of the modes seems to change reflecting modifications of the component structures. The occurrence of changes is also supported by the recently reported data on the acidity modification of CCM due to irradiation processes [19]. These aspects are important because the effects of cell damage depend on the acidity of environment [25].

A deeper investigation on these aspects is in progress.

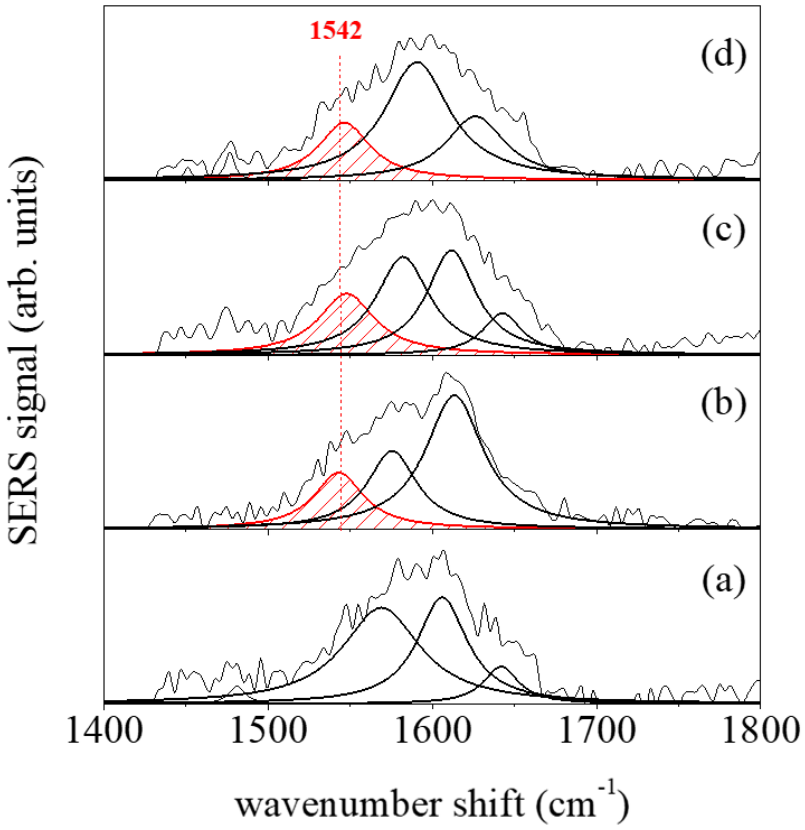

Figure 3. SERS spectra, in the Amide I region, of DMEM-B for (a) pristine CCM; (b) CCM hosting cells (control); CCM hosting cells irradiated by X-rays at (c) 2-Gy and (d) 4-Gy dose. The deconvolution of the signal in terms of Lorentzian functions is reported together the experimental data. Red shaded peaks refer to mode attributed to cell presence.

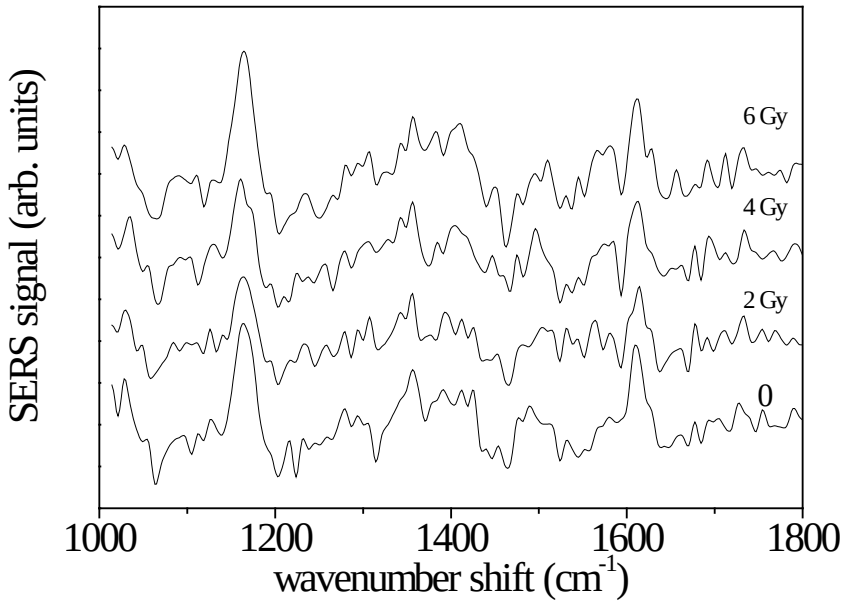

Figure 4. SERS spectra of non-irradiated DMEM-A sample (0 Gy) and for DMEM-A samples irradiated at the marked irradiation doses (2, 4 and 6 Gy).

4. CONCLUSIONS

The preliminary results of a SERS study of the radiation-induced changes on different cell culture media that have been in contact or not with human cells are here reported. The main contributions of the complex spectra were assigned in agreement with the literature.

Some of the evidenced contributions show changes that can be related to the different experimental conditions of the investigated samples. The results confirm the potentials of SERS as effective tools for cell culture media monitoring.

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