Conventional Raman and surface-enhanced Raman spectroscopy of ascitic fluid

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Abstract. The aim of this study is to analyze the spectral characteristics of ascitic fluid using Raman spectroscopy and surface-enhanced Raman spectroscopy (SERS). The utilization of SERS allows for detection the presence of ascitic fluid components that are not available for revealing when analyzed using conventional Raman spectroscopy. The potential to detect the spectral contribution of a number of pathogenic microorganisms utilizing the proposed SERS-based optical approach has been demonstrated.

1. Introduction
Currently, in experimental clinical studies, there is a growing interest in studying carcinogenesis and the state of the human body suffering from cancer using optical methods [1-3]. Pathologic processes during cancer formation starts a number of responses in the human body. One of the frequent complications accompanying cancer metastatic process is ascites, characterized by the accumulation of free fluid in the abdominal cavity. In order to analyze the content of the ascitic fluid bacterial swab test is widely used in the clinical practice. However, this method is time consuming and takes from two to ten days. Early treatment for ascites requires early detection and identification of pathogens in ascitic fluid [4]. The potential possibility of determining the component composition of the contents in ascites is allowed by the Raman spectroscopy (RS). The RS method is applicable to the study of ascitic fluid in vitro for several minutes. Improving the informativeness and the possibility of carrying out a more detailed analysis of the ascitic fluid component composition is possible with the utilization of surface-enhanced Raman spectroscopy (SERS). For instance, the use of surface-enhanced Raman spectroscopy in the study of blood allows for assessment of changes of hypoxanthine, whereas it is impossible to determine its presence from the conventional Raman spectrum [5]. Moreover, SERS is applicable to the identification and discrimination of yeast species from various taxonomic groups of fungi [6]. Therefore, in this study, ascitic fluid samples from cancer patients were investigated using RS and SERS in the near IR region; and the possibility of using SERS technology with the utilized experimental setup for further studies of the ascitic fluid component composition was analyzed.
2. Materials and methods

2.1. Colloidal gold nanoparticles solution

Considering that the spectra are recorded in the near-IR range, it is most expedient to use gold nanoparticles to create plasmon resonance and achieve the effect of surface enhancement [7]. The gold colloidal solution was prepared by the reduction of hydrochloric acid H\text{A}u\text{C}l\text{4} with sodium citrate Na\text{3}C\text{6}H\text{5}O\text{7} as described in [8]. In the absorption spectrum of the solution obtained, a maximum is observed at 525 nm, which, according to the [9], corresponds to a particle size of about 40 nm. The obtained colloidal solution of gold spherical nanoparticles is subjected to the formation of secondary particles due to aggregation. For this, hydrochloric acid (concentration 0.1 M) is added to the gold colloidal solution in a ratio of 7/40, respectively.

2.2. Samples preparation

The standardized collection of ascitic fluid samples from patients of Samara Regional Clinical Oncology Dispensary was performed. Collected samples were placed in sterile test-tubes and were stored at +2 ± 4°C before the analysis. Analysis of collected body fluids was performed within 24h after sample collection. Patients of Samara Regional Clinical Oncology Dispensary with malignant tumors were enrolled in this study. The study protocols were approved by the ethical committee of Samara State Medical University. To study the ascitic fluid samples using the RS method, the test samples were placed in an aluminum cuvette with a volume of 0.9 ml.

Preparation of solutions for research using the SERS method was performed according to the following algorithm: a ascitic fluid sample is diluted ten times with distilled water; the gold aggregated colloid is introduced into the obtained diluted sample in the ratio of 1/1; the solution in a volume of 6 \( \mu \)l is applied to the aluminum foil and dried for 30 minutes at room temperature.

2.3. Experimental setup

The excitation of collected ascitic fluid spectra was performed by the laser module LuxxMaster LML-785.0RB-04 (central wavelength 785 nm). The fiberoptic Raman probe RPB785 allows for focusing of the exiting radiation, collecting and filtering of the scattered radiation. The collected signal was decomposed into a spectrum using a high-resolution Shamrock SR-500i-D1-R spectrograph with integrated cooled up to -65°C digital camera ANDOR DU416A-LDC-DD. The utilized spectrograph with a grating of 600 slits/mm allows for recording the spectrum of the tested substance in 780-950 nm area divided by three spectral ranges; for each single spectral range the exposure time was 20 seconds. A sequential recording of three spectra for each tested sample was performed. The final spectrum was received from averaging of all three recorded spectra. The total time of the final spectrum recording was 3 minutes.

2.4. Spectra processing

The obtained spectra were processed by a polynomial method for the separation of autofluorescence and Raman [10]. The autofluorescence component was approximated by a tenth-order polynomial function. The Raman component of the spectrum was extracted by subtracting the autofluorescence component from the raw spectrum.

3. Results and discussion

Ascitic fluid may accumulate as a transudate or exudate. Ascites content is classified as infected contents, sterile contents or spontaneous bacterial peritonitis, depending on the presence of infection. In [11], the authors described an approach to the analysis of various strains of bacteria. The authors demonstrated the possibility of identifying the pathogen utilizing Raman microspectroscopy and chemometric analysis for subsequent use in the ascitic fluid analysis. In the present work, we analyzed the experimental data obtained using our experimental setup without a microscopic system. Figure 1 shows the raw spectral characteristics of ascitic fluid without subtracting the autofluorescence component.
Figure 1. The raw spectral characteristics of ascitic fluid: 1 — micro-dose of aggregated colloidal gold nanoparticles solution on the foil, 2 — ascitic fluid in a volume of 0.9 ml, 3 — micro-doses of a ascitic fluid solution using SERS technology.

Analysis of Figure 2 shows that with the application of SERS technology, the signal is amplified; and peaks appear on a number of spectral bands. It also follows from Figure 1 that the spectral contribution of the aggregated gold colloid to the tested sample characteristic is insignificant. Figure 2 presents the Raman spectra and analysis of the various biochemical components contribution to analyze the features of the Raman spectral characteristics of a bulk sample of ascitic fluid and SERS of a microdose sample [12-16].

Figure 2. Raman spectra of ascitic fluid.: 1 — ascitic fluid in a volume of 0.9 ml, 2 — micro-doses of a ascitic fluid solution using SERS technology.

The spectral characteristics of the tested samples, shown in Figure 3, allow to conclude that, in addition to the bands determined by Raman scattering, the utilize of SERS technology for analyzing the ascitic fluid composition also determines the biochemical changes associated with 718-730 cm$^{-1}$, 797-808 cm$^{-1}$, 904-915 cm$^{-1}$, 1030-1040 cm$^{-1}$, 1167-1175 cm$^{-1}$, 1220-1230 cm$^{-1}$ and 1665-1680 cm$^{-1}$ bands. Amplification of these bands demonstrates the potential to detect the spectral contribution of a number of pathogenic microorganisms using SERS technology with the used experimental setup [17, 11]. For instance spectral contribution to a number of these bands may be associated with bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Enterobacter aerogenes*, *Staphylococcus epidermidis*, *Streptococcus bovis*, etc. In addition, leukocytes and proteins contribute to the highlighted bands, the assessment of which allows for general analysis of the bacterial infection presence in ascitic fluid and differentiate the transudate and exudate.

4. Conclusion
The selected and analyzed spectral features of ascitic fluid samples may be the basis of the method for detection of the biochemical characteristics and analysis of the contents infection in ascites, which will
allow timely assign a chemotherapy, in particular, for treating patients with cancer. A comparative study of experimental data of conventional Raman and SERS demonstrated that using SERS allows determining components of ascitic fluid that are not available for detection when analyzed using conventional Raman spectroscopy, and also confirmed the potential to detect a number of pathogenic microorganisms using SERS technology with experimental setup for further research on the ascitic fluid composition.

5. References
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