DETECTION OF SINGLE BACTERIA – CAUSATIVE AGENTS OF MENINGITIS USING RAMAN MICROSCOPY

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Abstract.
Early diagnostics of meningitis is a very topical problem as it is a fulminant disease with a high level of mortality. The progress of this disease is, as a rule, accompanied by the appearance of bacteria in the cerebrospinal fluid (CSF) composition. The examination of the CSF is well known to be the only reliable approach to the identification of meningitis. However, the traditional biochemical analyses are time consuming and not always reliable, simple, and inexpensive, whereas the optical methods are poorly developed. This work is devoted to the study of Raman spectra of several bacterial cultures which are mainly present during meningitis. Raman microscopy is a prompt and noninvasive technique capable of providing reliable information about molecular-level alterations of biological objects at their minimal quantity and size. It was shown that there are characteristic lines in Raman spectra which can be the reliable markers for determination of bacterial form of meningitis at a level of a single bacterium.

Introduction

Bacteria invasion into the cerebral spinal fluids (CSF) is a serious problem leading to the development of meningitis – a dangerous bacterial form of the central nervous system (CNS) illness with a high level of mortality. The early diagnosis and the cure started of this illness are the actual problems of medicine. The examination of CSF has been well known to be the only reliable approach to the identification of meningitis [1, 2]. The current tools available for detecting the bacteria penetration into CSF are cytological, bacteriological, virological and serological analysis techniques. They are time-consuming and not always reliable, simple, and inexpensive. At present the optical methods of CFS analysis are poorly developed unfortunately. The Raman spectroscopy undoubtedly holds promise for CSF analysis, because it is a prompt and noninvasive technique capable of providing reliable information about molecular changes. In so doing the detection of pathological bodies impurities in CSF can be realized at a level of a single bacterium or cell [3-6]. It is very important as first bacterial identification is necessary for early diagnosis of bacterial meningitis. As it was shown earlier on [3, 5-7], a single bacterium is well detected in CSF by optical microscope. As regards the problem of different bacterial culture identification using Raman spectrometer the presence of the CSF matrix is not required. This work is devoted to the study of Raman spectra of several pure bacterial cultures which are mainly present during meningitis.

2. Materials and Methods

2.1. Sample preparation

Cultivation and preparation of in vitro cultured bacteria of four different species of Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus pyogenes and Enterococcus faecalis were performed by the bacteriological laboratory of the Moscow Infectious Clinical Hospital No 2. The different bacterial species were cultured in nutrition medium and their colonies suspended afterwards in phosphate buffered saline solution. About 1 ml of the suspension were spotted onto the clean polished surface of a substrate and dried at room temperature. 70% ethanol solution is used for bacteria inactivation.

2.2. Spectroscopic instrumentation

The spectral measurements were fulfilled with the help of Nicolet Almega XR spectrometer with a 532-nm laser excitation source (a Nd:YAG CW laser generating a 20-mW second-harmonic radiation). The spectrometer was equipped with a microscope capable of an X10 and X50 magnification. In microscopic investigations, the diameter of the probe laser beam spot was 1 mkm. The Stokes shift components in the range of 400–3100 cm^{-1} at a spectral resolution of 2 cm^{-1} were used to analysis. Wave number calibration was performed by using the characteristic silicon reference peak at 521 cm^{-1}. To reduce the effect of fluorescence the phenomenon of photobleaching for 30 seconds prior to the recording of the Raman spectra was used. The spectra obtained were computer processed using the OMNIK software.

3. Results nd Discussion

Our investigations showed that a bacterium sample in the form of a droplet dried at room temperature retains the main characteristic features of its Raman spectrum for at least one month. The sample in this form is convenient to store and transport. In so doing the computer processing of its Raman spectrum is simplified because of the absence of water. However the choice of the substrate can essentially influence on the detection quality of Raman spectrum as own substrate spectral lines and informative lines can be overlapped. In literature enough attention is not usually given to this problem. As was shown, the base informative lines of bacteria Raman spectra are concentrated in the range of 700–1700 cm^{-1}. About 20 substrates were examined in this work. Raman spectra of several substrates are presented in Fig. 1.

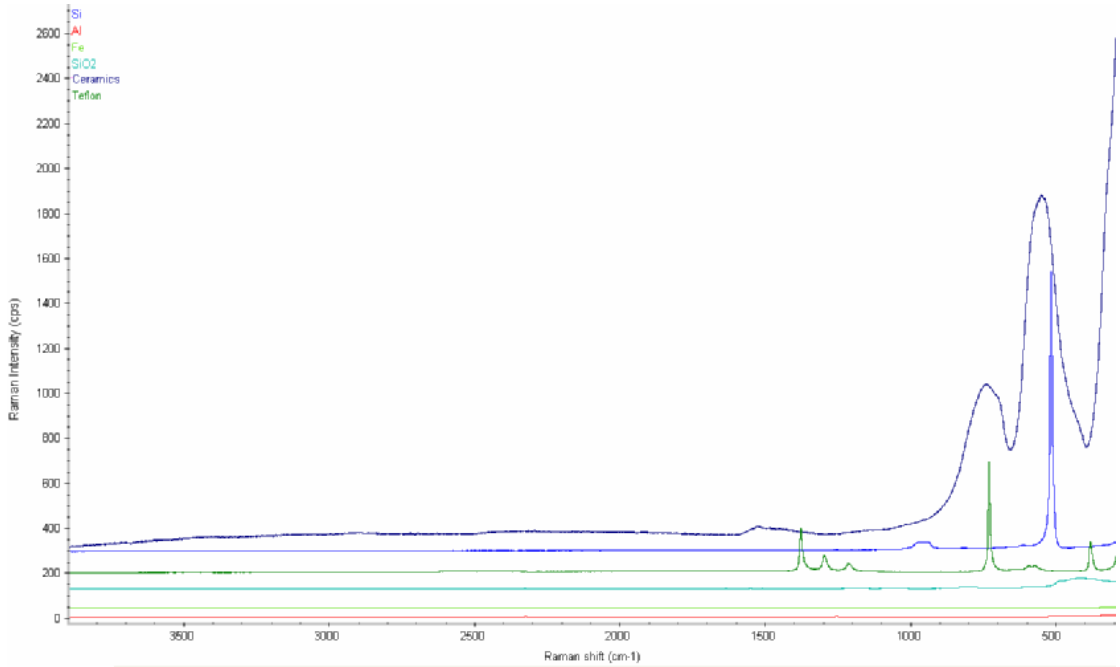

Figure 1. Raman spectra of six substrates. Bottom up: Al-mirror, steel, quartz, teflon, silicon, PZT ceramics. Excitation wavelength is equal to 532 nm.

It can be seen, that the substrates from metal, semiconductor, dielectric and even organic can be used for measurements in a large spectral range. However among them the metal substrates are more preferable for wide-range spectral investigations.

Raman spectra of four bacterial cultures spotted onto Al-mirror and fused quartz which are mainly present during meningitis are presented in Fig. 2. As it can be seen, there is the intensive signal around the wavenumber of 3000 cm^{-1}. This band is constituted by the closely spaced several lines associated with the CH_2 and CH_3 vibrations of proteins and lipids. This signal is peculiar to all bacteria and therefore can not be of interest for differential analysis. The fingerprint region between the wavenumbers of 700-1700 cm^{-1} can be used for the identification of different bacteria. The spectra contain the information on the molecular composition of the

whole bacteria, such as fatty acids, carbohydrates, proteins and nucleic acids [8, 9]. Raman spectra depend, obviously, on the measuring position within the bacteria along of different molecular composition [7]. However their basic features are retained for the bacterium as a whole. Characteristics lines of four investigated bacteria can be determined in Fig. 2.

As for Enterococcus faecalis spectral identification there are three lines at wavenumbers of 1000, 1360 and 1600 cm^{-1} which represent both proteins and Amide I bands. In Raman spectrum of Streptococcus dysgalactiae there are several strong lines in range of 1000-1700 cm^{-1}. Three lines at 1451, 1580 and 1662 cm^{-1} stand out against others. They correspond to the vibrations of CH groups in lipids, amino acids and carbohydrates. Two Raman lines at 1582 cm^{-1} and 1636 cm^{-1} which represent both C=C vibrations of phenylalanine and Amide I band can be used for Streptococcus pyogenes identification. As respects Staphylococcus aureus there are two strong lines at 1152 cm^{-1} and 1520 cm^{-1} for its identification. These lines in Raman spectrum indicate the presence of carotenoids in molecular structure of Staphylococcus aureus and can serve as the valid dominant of this bacterium detection.

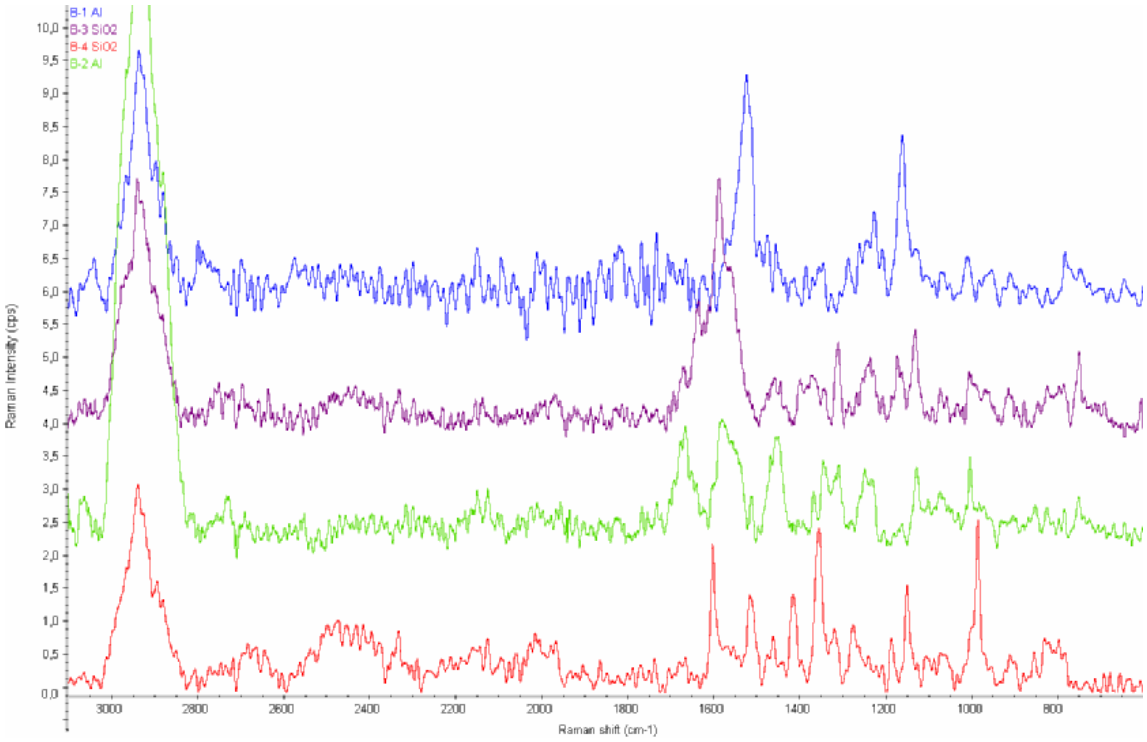

Figure 2. Raman spectra from (bottom up) Enterococcus faecalis, Streptococcus dysgalactiae, Streptococcus pyogenes and Staphylococcus aureus. Excitation wavelength is equal to 532 nm.

As it is known, the wide-spread carotenoids beta-carotene and lycopene strongly absorb in the green–blue area of the spectrum [10-12]. Therefore, using corresponding excitation wavelengths in this area, resonance Raman spectroscopy can take place and more intensive carotenoid lines can be acquired. As a result, noninvasive and fast detection of this bacterium can be realized. An argon laser operating at wavelengths of 488 and 514.5 nm could be a good excitation source in such a case.

4. Conclusion

In view of its potential for the fast and noninvasive analysis at a molecular level the Raman spectroscopy has been the method of precise research of biological objects for the last years. Unfortunately in the domain of meningitis diagnosis the Raman spectroscopy is poorly developed. In the frames of this work the Raman spectra of four pure bacterial cultures which are mainly present during meningitis were investigated. It was shown that a bacterium sample in the form of a droplet spotted onto metal mirror and dried at room temperature retains the main characteristic features of its Raman spectrum for at least one month. It was demonstrated that there are characteristic lines in Raman spectra which can be the reliable markers for the determination of a kind of bacteria – causative agents of meningitis. Further work in this direction has to lead to database forming for early bacteria identification.

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