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Research Article

Identification of Contaminated Cells with Viruses, Bacteria, or Fungi by Fourier Transform Infrared Microspectroscopy

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Fourier transform infrared microspectroscopy (FTIR-M) can detect small molecular changes in cells and therefore was previously applied for the identification of different biological samples. In the present study, FTIR spectroscopy was used for the identification and discrimination of Vero cells infected with herpes viruses or contaminated with bacteria or fungi in cell culture. Vero cells in culture were infected herpes simplex virus type 1 (HSV-1) or contaminated with *E. coli* bacteria or *Candida albicans* fungi and analyzed by FTIR microscopy at 24 h postinfection/contamination. Specific different spectral changes were observed according to the infecting or contaminating agent. For instance, both pure fungi and cell culture contaminated with this fungi showed specific peaks at 1030 cm^{-1} and at 1373 cm^{-1} regions, while pure *E. coli* and cell culture contaminated with this bacteria showed a specific and unique peak at 1657 cm^{-1}. These results support the potential of developing FTIR microspectroscopy as a simple, reagent free method for identification and discrimination between different tissue infection or contamination with various pathogens.

1. Introduction

Serious human diseases are caused by different pathogens such as viruses, bacteria, or fungi. In many cases, it is difficult to distinguish between these various infections by routine physician inspection, particularly at early stages of the infection. There are several routine assays for detection of the responsible pathogens of such infections:

(1) growing of the pathogen in culture and trying to identify it by microscopic observations. This assay is not always simple because each pathogen needs different growth conditions and takes relatively long time till getting the results depending on the pathogen (days to weeks). In addition, this assay is highly dependent on the physician qualification [1];

(2) immune assays using specific antibodies [2];

(3) molecular assays using specific primers.

Although these immune and molecular assays are highly specific, they are relatively expensive and not always available for all pathogens. Furthermore, in order to identify the cause of unclear infection, it might be necessary to examine the sample simultaneously by various assays because the conditions and techniques required for examining various pathogens are completely different.

The detection and identification of pathogen infections by spectroscopic techniques is highly promising due to their sensitivity, rapidity, low expenses, and simplicity. Fourier transform infrared (FTIR) microscopy is considered as a powerful tool for chemical analysis because of its ability to provide detailed information on the spatial distribution of chemical composition at the molecular level [3]. Its applications cover different disciplines including material science, forensics, biochemistry, biomedical science, and geochemistry [3–9]. This technique has been also proved to be sensitive for the identification of cancer cells [1, 10–13], stem cells [14, 15], virally infected cells [16–19], and microorganisms [20, 21].

Cell cultures are advantageous and more convenient for basic research, compared to "real" tissues due to their homogeneity and the ability to control important culture parameters [22, 23]. Thus, infection or contamination of cell cultures with viruses, bacteria, or fungi may be used to represent infected or contaminated tissues with these pathogens.

In the present study, we either infected Vero cells in culture with herpes simplex virus (HSV-1) or contaminated them with *E. coli* bacteria or *Candida albicans* fungi. All cell cultures were examined by light microscopy and by FTIR-microscopy technique trying to detect specific biomarkers for discrimination between the different pathogens at 24 h after the cells infection or contamination. The results showed significant spectral biomarkers which may be useful for detection or identification of different pathogens involved in tissue infection.

2. Experiments

2.1. Cells and Pathogens. African green monkey kidney cells (Vero cells) were grown at 37°C in RPMI medium supplemented with 10% new born calf serum (NBCS) and the antibiotics penicillin, streptomycin, and neomycin.

HSV-1 was obtained from the American Type Culture Collection (ATCC), Rockville, MD, USA. Stock of this virus with high titer was made and stored at −20°C. This stock was used for infecting the Vero cells.

E. coli bacteria and *Candida albicans* fungi were obtained from the microbiology department in our institute.

2.2. Cell Infection and Contamination

(1) Cell infection with HSV-1: monolayers of Vero cells grown in 9 cm^2 tissue culture plates were incubated at 37°C for 2 h with 1 multiplicity of infection (m.o.i.) of HSV-1 in RPMI medium containing 2% of NBCS. The unabsorbed virus particles were removed, fresh medium containing 2% NBCS was added, and the monolayers were incubated at 37°C. At 24 h post-infection (p.i.) the cells were examined as follows:

   (a) for the appearance of the cytopathic effect (CPE), defined as areas of complete destruction of cells or of morphologically modified cells in the inspected fields, using a light inverted microscope;

   (b) by FTIR microscopy.

(2) Cell contamination with bacteria and fungi: about 10^6 of *E. coli* cells or high amount of *Candida albicans* were added to monolayers of Vero cells grown in 9 cm^2 tissue culture plates and incubated for 24 h at 37°C. Then the cell culture was examined by light inverted microscope and by FTIR microscopy.

2.3. Sample Preparation for FTIR Microscopy. Normal cells, infected cells with HSV-1 or contaminated with either *E. coli* or fungi were picked up from the tissue culture. The cells were pelleted by centrifugation at 2000 rpm for 5 min. Each pellet was washed twice with saline and resuspended in 100 μL of saline. The number of cells was counted with hematocytometer, and all tested samples were pelted again and resuspended in an appropriate volume of saline to give a concentration of 1000 cells/μL. A drop of 1 μL of each sample was placed on the zinc selenide crystal, air dried, and examined by FTIR microscopy. The radius of such 1 μL drop was about 1 mm.

2.4. FTIR Spectra Measurement. FTIR measurements were performed in the transmission mode with a liquid-nitrogen-cooled MCT detector of the FTIR microscope (Bruker IRscope II) coupled to an FTIR spectrometer (BRUKER EQUINOX model 55/S, OPUS software). The spectra were obtained in the wavenumber range of 4000–800 cm^{-1}. Spectral resolution was set at 4 cm^{-1}. The spectra taken were an average of 128 scans to increase the signal-to-noise ratio. Rubber band baseline correction and vector normalization were applied for all the spectra by OPUS software [24]. Since the samples to be analyzed were sometimes heterogeneous, due to different thickness of the obtained cell layer at various regions over the prepared sample and due to possible presence of high amounts of salts particularly in areas with lower density of cells, appropriate regions were chosen by FTIR microscopy. The aperture used in this study was 100 μm (area 7.8×10^{-3} mm^2), since this aperture gave the best signal/noise ratio. Each experiment with each sample was repeated five times.

3. Results and Discussion

3.1. FTIR Spectra of Normal Vero Cells, Pure Bacteria, and Pure Fungi. As a first step in the present study we measured and compared the FTIR spectra of normal Vero cells, purified *E. coli*, and purified *Candida albicans* trying to identify significant spectral differences between them which might be used for the identification and the detection of the infecting or the contaminating pathogen in case of cell infection or contamination. The results presented in Figure 1(a) show a typical spectrum for each of the tested objects, although in general the FTIR spectra of the different tested objects are quite similar. The spectra are dominated by the absorbance bands at 1643 cm^{-1} and 1544 cm^{-1}, that is, the amide I and II bands, respectively. The amide I band arises from C=O hydrogen bonded stretching vibrations, and the amide II-from C–N stretching and CNH bending vibrations [25]. Amid III band at 1270 cm^{-1} is contributed by proteins arising from coupling of C–N stretching and N–H bending [26]. The bands at 900–1300 cm^{-1} were assigned to C–O bending modes of saccharides (glucose, lactose, and glycerol), the peaks at 1360–1430 cm^{-1} were attributed to COO^- of amino acids, and 1430–1480 cm^{-1} is attributed to fatty acids, phospholipids, and triglycerides [27, 28].

It can be noted that the absorbance intensity at 900–1200 cm^{-1} is significantly higher for fungi compared to bacteria and Vero cells. These results reflect the significant differences in the components content and in the composition of the tested objects. It is also worthwhile to mention that while

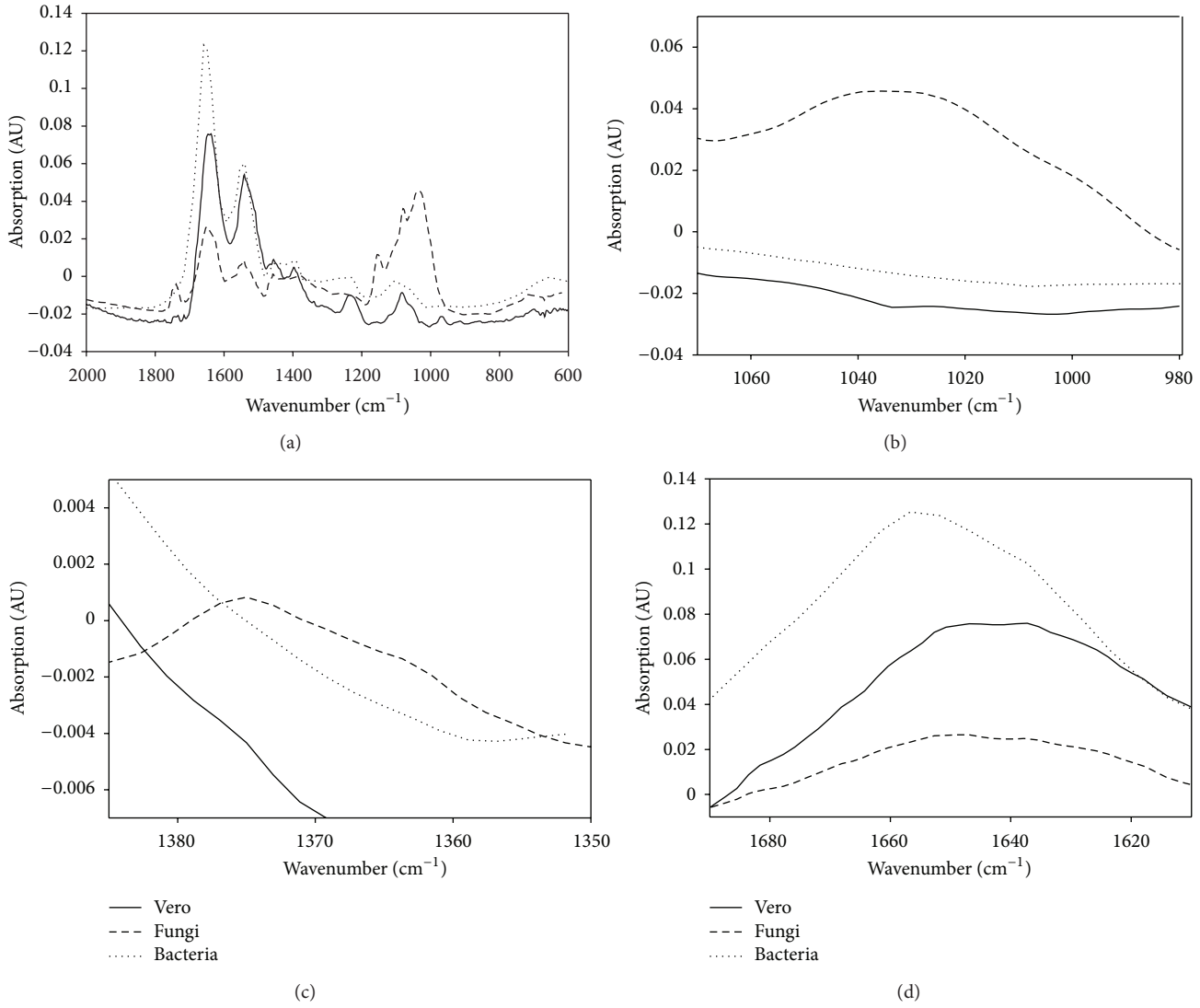

Figure 1: FTIR spectra of control Vero cells, pure *E. coli* bacteria, and pure *Candida albicans* fungi at the following regions: (a) 600–2000 cm^{-1}, (b) 1070–980 cm^{-1}, (c) 1390–1350 cm^{-1}, and (d) 1690–1610 cm^{-1}. Data are means of 10 different and separate experiments for each sample.

both bacteria and fungi are growing independently in the growth medium without penetrating into the contaminated cells, viruses are dependent on the host cells for their growth. They penetrate into the host cells, utilize all cellular resources for their advantage, and can cause death of the infected cells such as in the case of the tested virus (HSV-1).

By focusing on limited spectral regions the following can be seen.

(1) At the regions 1050–980 cm^{-1} (Figure 1(b)) and 1385–1350 cm^{-1} (Figure 1(c)) there are unique and significant peaks at 1030 cm^{-1} (Figure 1(b)) and at 1373 cm^{-1} (Figure 1(c)) belong to fungi sample which are completely missing in Vero and in *E. coli* samples (Figures 1(b) and 1(c)). These peaks can be attributed to carbohydrates and aliphatic compounds, respectively [26].

(2) At the region 1670–1630 cm^{-1} both Vero cells and *Candida albicans* show a unique peak at 1650 cm^{-1}. This peak was significantly shifted into 1657 cm^{-1} in the case of *E. coli* (Figure 1(d)). This peak is attributed to protein amid I band [26].

3.2. Spectral Biomarkers or the Identification of Cells Infected with HSV-1 or Contaminated with E. coli or Candida albicans. In the next step Vero cells in culture were infected with HSV-1 or contaminated with *E. coli* or fungi and at 24 h post-infection or contamination, the cell cultures were examined by FTIR microscopy trying to find specific spectral bands which might be useful for the identification of the infecting or contaminating pathogen.

Our results show significant peaks at 1030 and at 1373 cm^{-1} in all cell cultures contaminated with fungi (Figures 2(a) and 2(b), resp.).

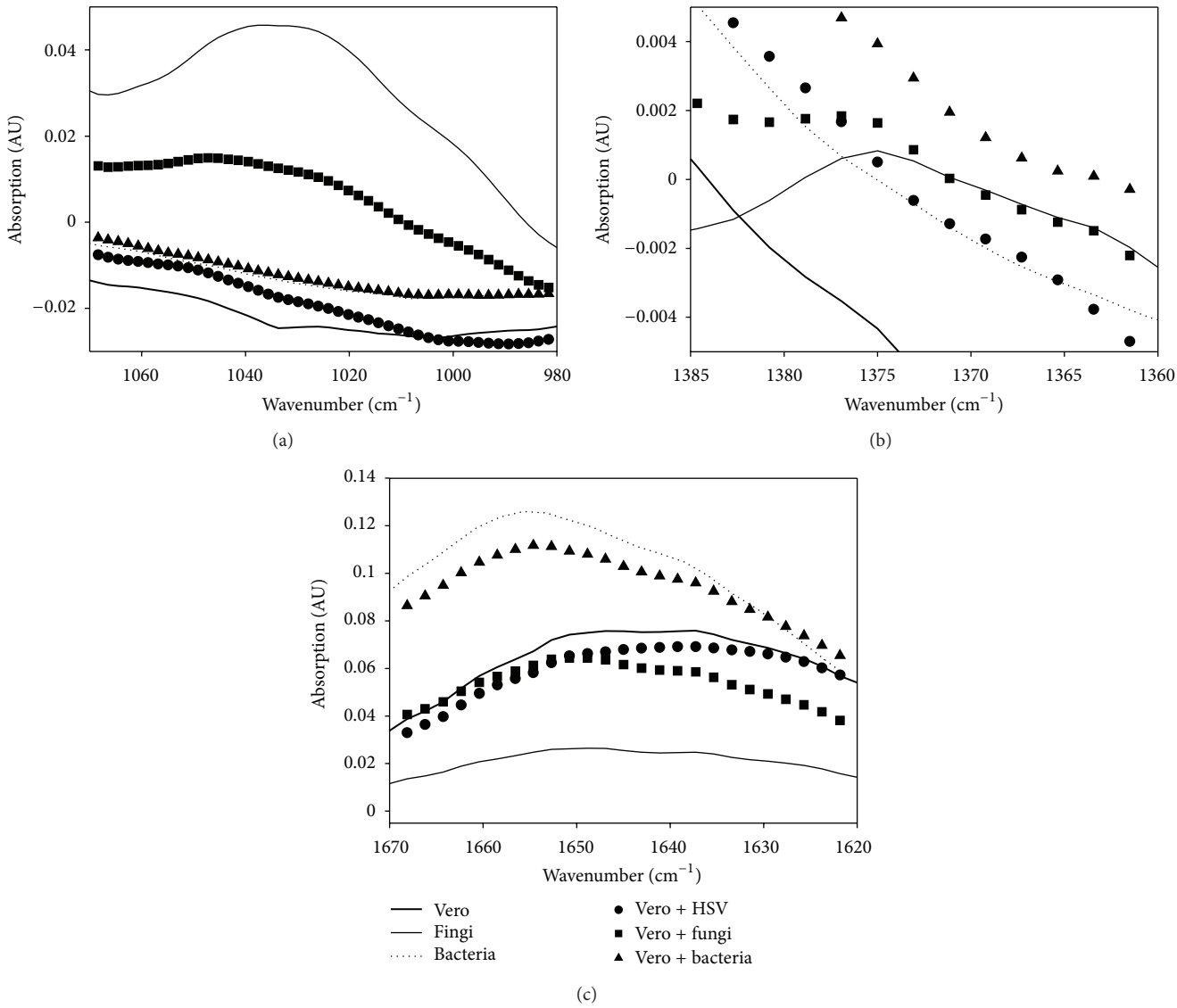

Figure 2: FTIR spectra of control uninfected Vero cells, infected Vero cells with HSV-1, pure *E. coli* bacteria, Vero cells contaminated with *E. coli*, pure *Candida albicans* fungi, and Vero cells contaminated with *Candida albicans* at the following regions: (a) 1070–980 cm^{-1}, (b) 1385–1360 cm^{-1}, and (c) 1670–1620 cm^{-1}. Data are means of 10 different and separate experiments for each sample.

These bands, which are typical to fungi as it can be seen in Figure 1, are completely missing in all cell cultures infected with HSV-1 or contaminated with *E. coli* (Figures 2(a) and 2(b)). However, at the region 1657 cm^{-1} there is a significant peak only for cell cultures contaminated with *E. coli* (Figure 2(c)). This peak as expected is typical for pure *E. coli* as it was shown in Figure 1(d) and Table 1.

In addition, it can be seen that the peak at 857 cm^{-1} in normal uninfected cells was significantly shifted into 853 cm^{-1} in all infected cultures with HSV-1 (Table 1). This spectral peak may be attributed to N-type sugars [29]. This result is in agreement with our previously published results [30]. This peak was not significantly affected as a result of contamination with either *E. coli* (peak at 856 cm^{-1}) or fungi (peak at 857 cm^{-1}) (Table 1). Also, both pure *E. coli* and pure fungi did not show any shift in this peak position (Table 1).

Thus, the results presented in this study show that there are specific spectral biomarkers, for each infection or contamination, which may be useful for reliable identification of the pathogen.

4. Conclusions

(1) Our results highlight the potential of FTIR microscopy for detection and identification of spectral changes related to cell culture infection or contamination with different pathogens.

(2) It is possible to differentiate between viral, bacterial, or fungal cell contaminations. Thus, FTIR may offer a promising technique for detection and identification of the pathogen causing tissue infection or inflammation. It is, therefore, worthwhile to continue

Table 1: Changes in peaks position at 857 and 1650 cm^{-1} as a result of Vero cells infection with HSV-1 or contamination with *E. coli* or fungi.

Sample	Wavenumber (cm^{-1})	
Vero	1650 ± 1.2	857 ± 0.6
Fungi	1651 ± 1.1	858 ± 1.1
Bacteria	**1657 ± 1.6**	857 ± 0.9
Vero + bacteria	**1656 ± 1.8**	856 ± 0.5
Vero + fungi	1651 ± 2.1	857 ± 0.8
Vero + HSV	1649 ± 1.2	**853 ± 0.5**

Vero cells were infected with 1 moi of HSV-1 or contaminated with *E. coli* or fungi. At 24 h later the different cell cultures were examined by FTIR microscopy. The results are means ± SD, ($n = 5$).

developing this technique as an efficient and reliable tool for the diagnosis of different pathogens.

(3) Furthermore, for each examination only a small amount of sample (1-2 μL) is required and the final results could be obtained during very short time (approximately 1 hr).

(4) Other viruses, bacteria, and fungi will be evaluated by this technique in the future in order to evaluate the specificity and reliability of this technique.

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