

Influence of Fixation Products Used in the Histological Processing in the FTIR Spectra of Lung Cells

Thiago Martini Pereira,¹ Maria Lucia Zaidan Dagle,² Grégory Mennequier,²
and Denise Maria Zezell¹

¹*Centro de Lasers e Aplicações, Instituto de Pesquisas Energéticas e Nucleares-CNEN/SP,
Avenida Professor Lineu Prestes 2242, Cid. Universitária, 05508-000 São Paulo, SP, Brazil*

²*Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia,
Universidade de São Paulo, Avenida Professor Dr. Orlando Marques de Paiva 87,
Cidade Universitária, 05508-900 São Paulo, SP, Brazil*

Correspondence should be addressed to Denise Maria Zezell, zezell@usp.br

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Abstract. The aim of the present study is to evaluate the differences on FTIR spectra of the normal lung cell (noncancerous mice lung epithelial cell line e10) due to different fixation protocols for histological processing. The results shown that formalin and methacarn (normally used in fixation) did cause many changes on the FTIR spectra of mice lung cells e10, mainly in the organic compounds ($800\text{--}1800\text{ cm}^{-1}$) in lipids, DNA, and proteins, and the alcohol 70% fixation protocol caused almost no changes on the FTIR spectra compared to unfixed cells spectra (in PBS). It can be concluded that histological processing with alcohol 70% fixation protocol can be used in the FTIR study of mice lung cell line e10.

Keywords: FTIR spectroscopy, cell model, lung, epithelial

1. Introduction

Among the infrared spectroscopy techniques, the ATR attenuated total reflectance infrared spectroscopy can offer high sensibility and accuracy to detect minimal chemical changes into the biological sample with minimal sample preparation [1]. There are several articles that showed that it is possible to identify many changes in the cells on the subcellular level [2] by FTIR-ATR and also that this is a powerful bioanalytical technique for the simultaneous analysis of lipids, proteins, and a variety of organic compounds within the cells [3]. Thus, infrared spectroscopy can be, in principle, used as a tool to study cell cycle and evaluate the drug effectiveness and the development of diagnostics methods.

There are many articles that report the fixation of cells after cell culture [3]. This procedure is used to preserve the structural biochemical constituents of cells in as close to *in vivo* conditions for long time [4]. During the fixation protocol, organic compounds such as formalin, alcohol, and methacarn are

used and all have strong absorption bands in mid-infrared region. Thus, the fixation protocol can change the infrared spectra of cells, as a consequence missing a lot of information that can be extracted from the infrared spectra.

The aim of the present study is to evaluate the differences on FTIR spectra of the normal cell due to different fixation protocols for histological processing.

2. Material and Methods

2.1. Cell Culture

Immortalized mice lung epithelial cell line e10 (American Type Culture Collection, Rockville, MD, USA) was maintained in complete e10 culture medium (1:1 mixture Dulbecco's modified Eagle's medium and Ham's F12 (DMEM/Ham's F12), supplemented with 100 ng/mL cholera enterotoxin, 10 μ g/mL insulin, 0.5 μ g/mL hydrocortisol, 20 ng/mL epidermal growth factor, and 5% horse serum) (Life Technologies, Rockville, MD, USA).

2.2. Sample Preparation

To carry out the experiments, the cells were removed from the bottles of the cell culture by using 0.2 mL trypsin-EDTA solution (2.5 g/L; Sigma-Aldrich, St. Louis, MO). They were washed at 1000 rpm/15 minutes with a 0.9% NaCl solution to remove the growth medium. The cultures were fixed with the following substances: 5% formalin in PBS, methacarn (60% methanol, 30% chloroform, and 10% acetyl acid), 70% alcohol, and also the unfixed cells, were cultured and maintained in PBS. The fixed cells were stored at 5°C during 48 hours before the FTIR measurement.

2.3. FTIR Measurements

The spectra were acquired on a Nicolet 6700 (Thermo Scientific Nicolet, Waltham, MA) spectrophotometer at a 4 cm^{-1} resolution, 32 scans, in the 4000–800 cm^{-1} spectral range. For the ATR-FTIR measurements, cells were deposited on the diamond crystal (approximately 10 μ L with 10^7 – 10^8 cells) and dried with air for 10 min before the measurements.

3. Results

Figures 1(a) and 1(b) show the mean of 9 infrared spectra of unfixed cells in PBS and 5% formalin fixed cells.

Figures 2(a) and 2(b) show the mean of 9 spectra of 70% alcohol and methacarn.

4. Discussion

It is possible to see in Figure 1(a) the spectra of unfixed cells (PBS). The principal biochemical compounds are such as amide I (1650 cm^{-1}), proteins and lipids (1456 cm^{-1} and 1395 cm^{-1}), DNA (1233 cm^{-1}). Figure 1(a) contains some of noise between 1350 cm^{-1} and 1800 cm^{-1} . Probably, it is the rotational vibrations due to water in the sample. This effect on the infrared spectra suggests that the dried procedure before the measurement was not sufficient to remove all water into the sample.

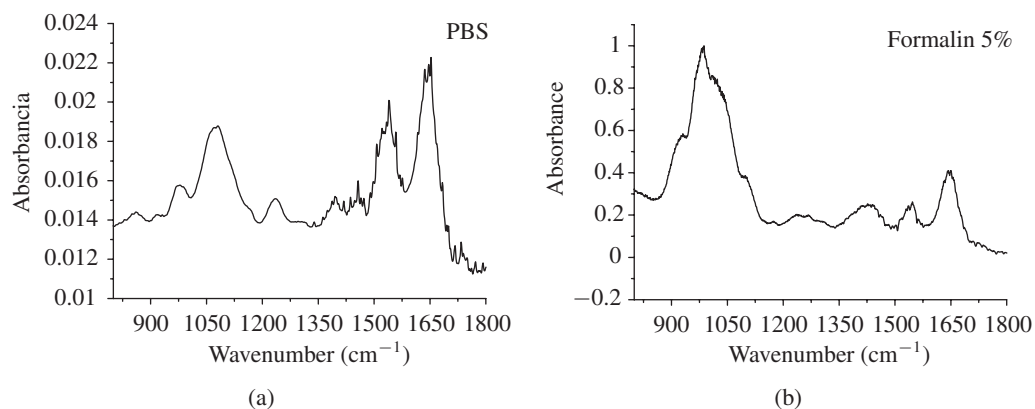


Figure 1: (a) Infrared spectra of unfixed cells. (b) Infrared spectra of 5% formalin fixed cells.

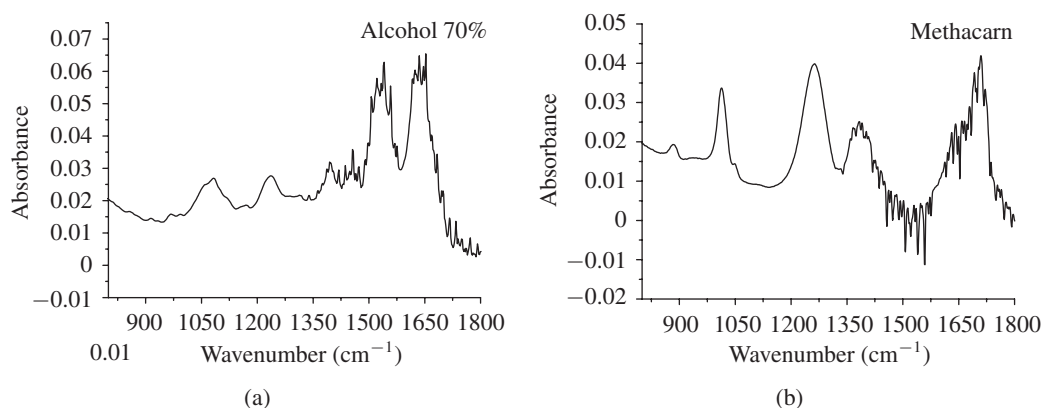


Figure 2: (a) Infrared spectra of alcohol 70% fixed cells. (b) Infrared spectra of methacarn fixed cells.

Figure 1(b) shows that 5% formalin and methacarn fixations protocols change the spectra in the mid infrared regions (800–1800 cm⁻¹) after 48 hours of fixation procedures. The formalin and methacarn are widely used for fixation of cells and tissue. The present results show that these organic compounds cause many changes on FTIR spectra (800–1800 cm⁻¹) in lipids, DNA, and proteins.

The alcohol 70% fixation protocol caused less change on the FTIR spectra than formalin and methacarn. The lipids, DNA, and proteins can be analyzed by FTIR ATR spectroscopy when these cells are fixed with alcohol 70%.

5. Conclusions

The results of the present study show that the alcohol 70% is the best protocol fixation among the 3 fixation protocols that were investigated in the present study.

Acknowledgments

The authors would like to acknowledge the financial support from the Grants FAPESP-CEPID (05/51689-2), CNPq-INFO (573916/2008), and CNPq (143166/2009-3).

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