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Research Article

Characterization of the breast cancer cell lines using fluorescence spectroscopy

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ABSTRACT

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Breast cancer is a widespread disease that affects individuals worldwide. The challenges lie in achieving timely diagnosis, accuracy, sensitivity, and reliable results from traditional diagnostic methods. Additionally, delayed diagnosis often leads to the metastasis of cancerous cells to the axillary lymph nodes, complicating treatment and frequently necessitating complete organ removal. Despite its limitations, fluorescence spectroscopy offers practical and applicable solutions. The Laser-Induced Fluorescence Spectroscopy (LIFS) technique is noteworthy for its high accuracy, sensitivity, and rapid capabilities in characterizing and detecting various cancerous areas and cultured cell samples.
Moreover, it boasts a low risk, cost-effectiveness, and minimal side effects. This research aims to analyze the various cancer cell lines (e.g., MCF10 cells), highlighting their distinct biological differences compared to human breast cells (MCF10 cells), referred to as the control. Due to the high accuracy and sensitivity of the proposed solution, this diagnostic tool can enhance breast cancer detection. The technique is ready for pre/post-clinical use as a non-invasive diagnostic tool, offering significant benefits in early diagnosis and patient evaluation before invasive procedures. Metabolic alterations in cancer cell lines result in substantial differences in the concentrations of key fluorophores, such as NADH and Flavins, compared to normal cells. Variations in fluorescence emission provide a foundation for the characterization and diagnosis of breast cancer through a fluorescence-guided approach. Cancer cells show intense spectral peaks, while normal cells have a continuous fluorescence range. These differences can aid in diagnostic applications, particularly in fluorescence spectroscopy.

KEYWORD

Fluorescence, Spectroscopy, Breast Cancer, Diagnosis, Fluorophores

I. INTRODUCTION

For breast cancer (BC) diagnosis, the main challenge is identifying critical factors for accurate and timely detection. High accuracy and sensitivity in early diagnosis are crucial, as they markedly diminish the risk of metastasis and the necessity for radical treatments. Consequently, this approach safeguards the patient's quality of life and averts the loss of body organs [1-3].

Traditional breast cancer diagnostic methods, such as X-ray mammography, MRI, and PET/CT, are widely used due to their ability to provide detailed anatomical images [4]. However, these techniques have notable limitations. While relatively cost-effective, X-ray mammography often lacks sensitivity and specificity, particularly in dense breast tissues. MRI provides high-resolution images but is expensive and time-consuming. PET/CT, although sensitive, involves high operational costs and exposure to ionizing radiation.

Laser-Induced Fluorescence Spectroscopy (LIFS) offers a non-invasive, cost-effective alternative with superior sensitivity and specificity [2, 4]. LIFS can detect biochemical changes at the cellular level, providing real-time results without expensive infrastructure or exposure to harmful radiation. Therefore, fluorescence spectroscopy can be a highly advantageous method for early and accurate breast cancer diagnosis, especially in resource-limited settings [4, 5].

In Laser-induced fluorescence spectroscopy (LIFS), the cell lines excite with a laser to emit fluorescence. The radiated light is then analyzed to detect biochemical changes at the cellular level. LIFS is particularly effective for detecting breast cancer at an early stage, as it can detect metabolic changes in vitro using cell lines [5-7].

Our previous study hypothesized that fluorescence-based approaches concerning photodynamic assessments hold significant potential for accurate discrimination and characterization of cancerous and normal breast cells. Using 5-aminolevulinic acid (5-ALA) as a probe to characterize biomarkers metabolic changes and provide valuable insights into functional and morphological alterations associated with breast cancer progression [2]. This study employs a dual approach by utilizing normal human (MCF10) and cancerous (MCF-7) breast cell lines under controlled laboratory conditions to enhance breast cancer diagnostic accuracy based on auto-fluorescence. This methodology facilitates a comprehensive analysis of cellular behaviors and responses, advancing breast cancer diagnostics [8, 9].

II. Materials and Methods

A. *Cell Lines*

The MCF-7 cell line was established in 1973 at the Michigan Cancer Foundation (the Barbara Ann Karmanos Cancer Institute). It was derived from the pleural effusion of a woman with metastatic breast cancer, a valuable model for studying hormone-responsive breast cancers [10]. The MCF10 cell line series was first described in 1990 by Soule and colleagues [10]. These cell lines were derived from normal human mammary epithelial cells obtained from a woman with fibrocystic breast disease. The MCF10 cell lines are precious for their ability to form three-dimensional structures that resemble normal breast tissue, making them an excellent model for studying breast cancer development and progression [10].

In the sample preparation phase, various cell densities were cultured in 1.5 mL of phosphate-buffered saline (PBS) under 5% CO2, 37°C, and 95% humidity. This approach facilitated the examination of different cell concentrations [11]. PBS was selected as the medium to avoid potential fluorescence interference from standard cell culture media and bovine serum, thereby minimizing background fluorescence and enhancing the accuracy of fluorescence spectroscopy measurements.

B. *Experimental Setup*

Fig. 1 shows the schematic optical array for cell line fluorescence characterizations. It employed a diode laser (CNI, PSU III LED) with a power density of 65 W/cm² over an area of less than 0.11 mm². It operated at a fixed wavelength of 405 nm. This laser was configured to excite key fluorophores within the cells (such as NADH, Flavin, etc.). This approach could lead to the development of an efficient and user-friendly system for discriminating samples based on their spectral features.

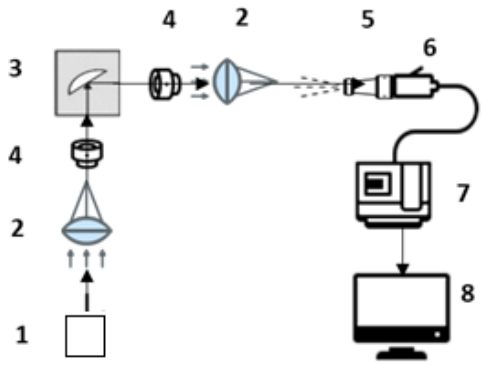

Fig. **1.** Schematic setup for cell lines fluorescence characterizations: (1) laser, (2) optical elements (lens, polarizer), (3) cell lines, (4) optical Objectives, (5) collective spectrophotometer, etc., (6) optical fiber cable, (7) spectrophotometer, (8) monitor.

This setup offers high efficiency and a longer lifetime than traditional lamp sources, with the laser capable of generating an output power ranging from 1 to 200 mW.

Despite the superior quality and stability of the laser beam, a set of filters, including two bandpass filters and lenses with 50 mm focal lengths, were used to direct the collimated beam to the center of the quartz cuvette at the center of the holder. The optical cable used for the UVS-2500 spectrometer is equipped with 905 SMA fiber connectors, facilitating connectivity with other equipment. The optical array also utilizes a lens with a 50 mm focal length.

We employed the UVS-250 miniature optical spectrometer from Poishtadikarane Fiztech (https://phystec.ir/en/), operating within the 190-850 nm spectral range, to detect fluorescence radiation from cell lines. This spectrometer features a 3648-pixel detector and employs visible and ultraviolet light sources. The device is compatible with optical fiber probes, enabling it to be used in various applications such as absorption, transmission, radiation, reflection, fluorescence, and phosphorescence measurements. The integrated diffraction grating enhances the clarity of the target spectrum.

Each experiment determining the optimal cell count was rigorously conducted twenty times to ensure the accuracy and reliability of our findings. Initial trials commenced with 50,000 cells, progressing to 500,000, 520,000, 1,055,000, 2,110,000, and 4,220,000 cells for the healthy cell line. We cultured 50,000 and 500,000 cells for cancer cells using the cell culture method and subsequently analyzed both types via non-invasive fluorescence induction.

III. Discussion

A. *key fluorophore*

In medical diagnostics, the measurement of quantum yield, polarization, and excited state lifetime is of considerable value. Quantum yield is a key parameter, defined as the ratio of photons emitted by a fluorophore to those absorbed. Polarization measurements provide insights into the dynamics of fluorophores, particularly their movement during the excited state lifetime, which is the period between light absorption and emission [4, 5].

Breast cells' intrinsic fluorescence emission spectrum spans the characteristic range of 400-800 nm [12, 13].

Cells contain molecules that fluoresce under specific UV/Vis wavelengths. This intrinsic fluorescence, from endogenous fluorophores, differs from signals from external markers. Cellular auto-fluorescence mainly comes from mitochondria and lysosomes. Key endogenous fluorophores include aromatic amino acids, lipopigments, pyridinic coenzymes (NADPH), and flavins. In cell lines, the extracellular matrix, especially collagen and elastin, often contributes more to autofluorescence due to their high quantum yields [2].

Physiological and pathological processes induce changes in the quantity and distribution of endogenous fluorophores and alter the chemical-physical properties of their microenvironment. Consequently, analytical techniques leveraging intrinsic fluorescence can provide valuable insights into cells' and Cellular morphological and physiological states. This technique offers real-time analysis without the need for sample fixation or staining. Recent advancements in spectroscopic and imaging methods have expanded their

applications in basic research and clinical diagnostics [14].

The metabolic alterations in cancerous cells result in distinct concentrations of key fluorophores compared to healthy tissues. This variation in fluorescence emission forms the foundation for characterizing and diagnosing breast cancer masses through fluorescence-guided strategies [15].

IV. RESULTS

A. *Characterization of Cell lines*

Characterization of Fluorescence Emission Spectrum in Healthy Breast Cells (MCF10)

Multiple normal and cancerous breast cell samples were cultured in the biomedical laboratory at Alzahra University. The fluorescence emission spectra of these cell lines were analyzed using laser-induced fluorescence spectroscopy (LIFS) in the photonics laboratory. The obtained data showed a high degree of consistency with previous findings. This experiment was conducted twenty times over six months on different days. Fig. 2 illustrates normal breast cells' fluorescence emission intensity versus wavelength (nm). The spectrum, spanning approximately 420 nm to 700 nm, exhibits prominent peaks at 429 nm, 470 nm, 595 nm, 624 nm, 632 nm, and 674 nm, indicating significant fluorescence emission at these wavelengths [12, 13].

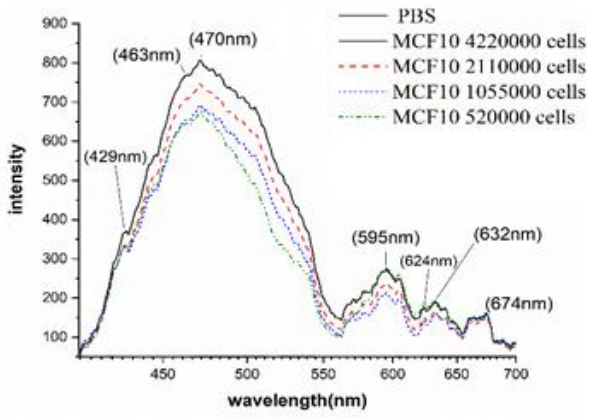

Fig. **2**. Fluorescence emission spectrum of normal human breast cells

As can be seen, the fluorescence spectrum of the phosphate-buffered saline (PBS) used as a cell substrate exhibited no emission. The experiment was conducted under optimal configuration, with specific exposure times (450 and 500 ms). Notably, the optimal cell line concentration was determined before characterization. All samples were diluted in 1 ml of PBS. The primary fluorescence emission range, from 410 to 700 nm, was observed with significant intensity without markers [12, 13].

The well-known intrinsic cellular fluorescence emission range spans from 410 to 540 nm (covering flavins, NADH, NADPH, and elastin) and from 570 to 700 nm (covering vitamins and lipids). Within these ranges, prominent fluorophores have been observed. Specifically, at 463 nm, Ceroid, a member of the lipid family, is detected. A distinct peak at 470 nm corresponds to NADPH, while at 595 nm, a type of lipid known as Phospholipid is observed. A different kind of this lipid is also seen at 540 nm [2, 5, 13].

B. *Characterization of Fluorescence Emission Spectrum in Normal Breast Cells (MCF7)*

Fig. 3 presents the fluorescence emission spectrum of breast cancer cells, spanning from 450 to 700 nm. Notably, there is a significant decrease in intensity within the interval typically associated with normal breast cells [16].

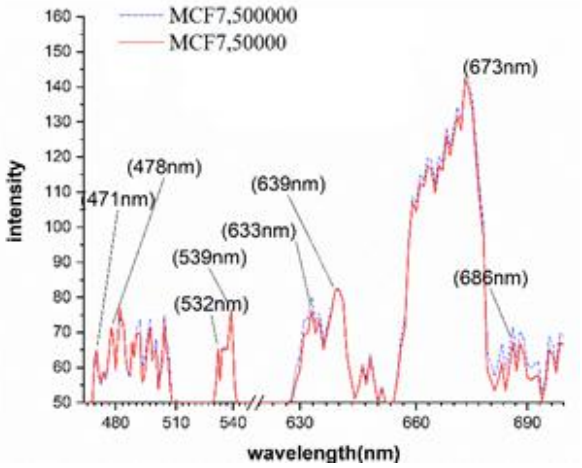

Fig. **3.** The intrinsic fluorescence emission spectrum of MCF7 cancer cells

The optimal cell numbers were identified after conducting multiple experiments with cell counts of 50,000 and 500,000. The optimal counts were determined to be 50,000 and 500,000, with these two samples serving as reference points for comparison and detailed analysis. As observed in the above spectrum, the spectral range of the cancerous cell line, between 600 to 700 nm, exhibits higher intensity. In contrast, the range of 450 nm to 580 nm, which corresponds to the spectral range of normal breast cells, shows significantly weaker fluorescence.

Additionally, the 630 to 700 nm range, near the IR region and characteristic of the breast cancer cell line, displays evident changes in porphyrin metabolism [12, 16].

The most prominent distinction in the spectral characterization of cancerous versus healthy cells is the emergence of sharp, intense peaks. Each lipid and protein is distinctly separated, highlighting a significant deviation in cellular composition.

The comparison of fluorescence emission spectra between normal (MCF10) and cancerous (MCF7) breast cell lines provides valuable insights into the changes in fluorophores. The fluorescence emission of normal cells begins at a wavelength of 410 nm, whereas for cancerous cells, it starts at 465 nm. The primary fluorescence range of healthy cells is more intense and continuous, in contrast to the more compressed and discrete spectrum observed in cancerous cells.

V. Conclusion

The characteristic wavelength range for healthy cells spans from 410 nm to 550 nm, with a secondary range from 570 nm to 700 nm. The continuous spectrum from 400 nm to 700 nm, which includes numerous fluorophores, behaves smoothly in healthy cells. The key segment of this spectrum, from 420 nm to 550 nm, exhibits high fluorescence intensity in healthy cells. The well-known intrinsic fluorescence emission range of cells extends from 420 nm to 540 nm (covering flavins, NADH, NADPH, and elastin) and from 570 nm to 700 nm (covering vitamins and lipids), where notable fluorophores are observed.

At the 425 nm peak, pyridoxic acid from the vitamin B complex group is detected, which shifts to 471 nm in cancerous cells, indicating a wavelength shift of approximately 50 nm. In healthy cells, clear peaks are observed at 470 nm for NADPH, 595 nm for a type of lipid known as Phospholipid, and 540 nm for another kind of the same lipid. These peaks exhibit significantly lower intensity in cancerous cells, with a wavelength shift of 21 nm.

References

[1] Institute, N.C., cancer.gov, National Cancer Institute, http://www.cancer.gov/publications/ /patient/patient-education/understanding-breast-changes, 2024.

[2] M. Ghezelbash, B. Sajad, and S. Hojatizadeh, "Fluorescence-Enhanced Assessments for Human Breast Cancer Cell Characterizations," Photonics, vol.11, pp. 746, 2024.

[3] WHO, https://www.cancer.gov/about-cancer/understanding/what-is-cancer. 2024.

[4] He Z. et al. "A review on methods for diagnosis of breast cancer cells and tissues," Cell Prolif, vol. 53, pp. e12822, 2020.

[5] Dramićanin T. and M.D. Dramićanin. *Using Fluorescence Spectroscopy to Diagnose Breast Cancer*. 2016.

[6] Bernard Valeur M.N.B.-S. *Molecular Fluorescence: Principles and Applications*, 2nd Edition. 2012.

[7] Adhikari S., et al. "Effect of Invitro Zinc (II) supplementation on Normal and Cancer Cell lines," International Journal for Research in Applied Science & Engineering Technology (IJRASET), Vol. 3 ,pp. 2321-9653, 2015.

[8] Holliday D.L. and V. Speirs, "Choosing the right cell line for breast cancer research," Breast Cancer Research, vol. 13, pp. 215, 2011.

[9] Welsh J. *Chapter 40 - Animal Models for Studying Prevention and Treatment of Breast Cancer, in Animal Models for the Study of*

Human Disease, P.M. Conn, Editor. Academic Press: Boston, 2013,

[10] Lee A.V. S. Oesterreich, and N.E. Davidson, "MCF-7 cells--changing the course of breast cancer research and care for 45 years," J Natl Cancer Inst,vol. 107, pp. 1-4, 2015.

[11] Segeritz C.-P. and L. Vallier, *Chapter 9 - Cell Culture: Growing Cells as Model Systems In Vitro, in Basic Science Methods for Clinical Researchers, M. Jalali, F.Y.L. Saldanha, and M. Jalali, Editors.* 2017, Academic Press: Boston.

[12] Croce A.C. and G. Bottiroli, "Autofluorescence spectroscopy and imaging: a tool for biomedical research and diagnosis," Eur J Histochem, vol. 58, pp. 2461, 2014.

[13] Tatjana D. and M. Dramicanin, *Using Fluorescence Spectroscopy to Diagnose Breast Cancer. Chapter metrics overview*, 2016.

[14] Monici M. "Cell and tissue autofluorescence research and diagnostic applications, in Biotechnology Annual Review," Elsevier. vol. 11, pp. 227-256, 2005.

[15] Martínez-Reyes I. and N.S. Chandel, "Cancer metabolism: looking forward," Nature Reviews Cancer, vol. 21, pp. 669-680, 2021.

[16] Yang Y.L., et al. "Characteristic autofluor-rescence for cancer diagnosis and its origin," Lasers Surg Med, vol. 7, pp. 528-532, 1987.