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Nonlinear optical investigation of normal ovarian cells of animal and cancerous ovarian cells of human in-vitro

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ABSTRACT

Laser-based study of two types of ovarian cells has been reported. For both normal and cancerous ovarian cells, growth and reproduction of cells formed to transparent and fresh substrates. Normal and cancerous cells fixed on glass substrate and nonlinear behavior of this microsize biolayer investigated by Z-scan technique using CW He–Ne laser. The magnitude and sign of nonlinear refractive index of normal and cancerous ovarian cells have been reported. Results show that nonlinear optical behavior of normal animal (Wistar rat) and cancerous human cells is different. Nonlinear refractive index (n_2) of all studied normal biolayers has negative sign and vice versa for cancerous biolayers. Additionally magnitude of n_2 for all samples was in order of 10^{-8} (cm^2/w). So optical apparatus like Z-scan can be used for discriminate normal and cancerous ovarian cells and may be considered for optical diagnostic methods in medicine later.

1. Introduction

The supply of laser-light actions on biomaterial has its absorption and light-action spectra. Laser light can supply energy of any magnitude necessary, and its irradiation intensity can be varied over a wide range by varying the energy and duration of the laser pulse [1]. These unique capabilities make laser a popular and efficient biomedical tool.

Stained histopathology is currently the accepted standard for cancer diagnosis but remains a subjective practice on processed tissue taking from hours to days. There are several optical techniques that can be used to identify cancerous from non-cancerous states of a tissue such as fluorescence, Raman and light scattering. Changing the state of a cell from normal to cancerous will affect the fluorescence, Raman spectra and light scattering of cell [2–4].

Light scattering techniques have been widely used in medical diagnostics. Several techniques have introduced to observe laser scattering and to measure the optical properties of tissues [5–7]. One common method has been to measure the attenuation of a CW laser beam incident on a tissue at various distances [8]. Using of laser light, is very consistent method to detect, diagnose, and treat diseases noninvasively [5] and one of the most promising methods to study tissue and blood, in-vitro or in-vivo.

Laser-based diagnostic methods have some advantages, increasing accuracy and decreasing time of diagnostic process are two main advantages that come from special properties of laser beam and optical setup. Optical diagnostic methods include laser and tissue interaction. Optical characterization of tissue can be used for cancer diagnosis and therapy [9].

Chemical and morphological change can help to discriminate cancer from normal cells [10]. Morphological changes include cellular size difference, cellular surface changes, nucleus expansion, cytoplasm contraction, etc. [11]. Cancer cells usually do not function in a useful way and their shapes are often distorted. Unlike normal cells that tend to have the same size and shape, cancer cells often vary in their sizes and shapes [12,13]. The size and shape of the nucleus as the center of the cell's DNA is often abnormal. Typically, the nucleus of a cancer cell is larger and darker than that of a normal cell and its size can vary greatly. The nucleus of a cancer cell is larger and darker because it often contains too much DNA [13].

Changing size and chemical components of cell can affect the optical properties. some laser-based techniques can detect and report these changes. One of the useful methods to detect optical properties of matter is Z-scan technique. Measuring both nonlinear absorption and refraction is possible and simple by this technique. The Z-scan technique has been used to measure the nonlinear optical response of wide range of materials [14–19]. This technique can be used for determination of biomaterials concentration like protein and bovine serum [20], urea and uric acid [21], etc.

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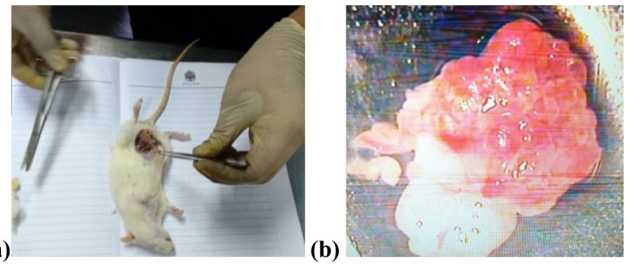

Fig. 1. (a, b) Surgery of mature female Wistar rat and ovary separation to obtain normal ovarian cells. Appropriate ethical committee approval was obtained for experiments on rats.

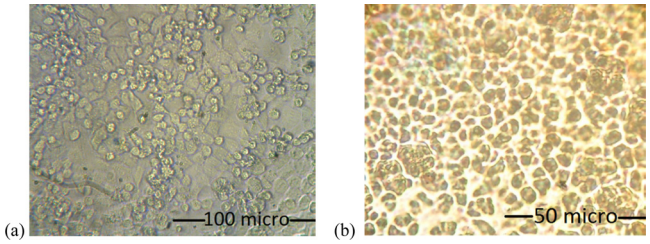

Fig. 2. (a) Cancerous ovarian cells before fixation process. Free cells removed and remaining cells fixed on glass slide (lamella) as biolayer. (b) Normal ovarian cells fixed on glass slide as a biolayer.

2. Materials and methods

2.1. Cell culture

Normal cells obtained from mature female Wistar rat (Fig. 1). Rats were weighed and anesthetized with chloroform. Ovaries were separated from the twisted oviduct tubes, fixed in a dish containing PBS solution. Fatty tissue was separated under a loop microscope then we scratched ovary by use of scalpel in 50 μl physiological serum. We took 20 μl of this serum and put on lamella (glass slide) then lamellas incubated them for drying and fixation. All procedures were carried out according to the guide for the care and use of laboratory animals. National research council, guide for the care and use of laboratory animals.

We used cancerous human ovarian cells (cell line: A2780cp) in this work. Cells are provided with growth medium comprising the essential nutrients required for proliferation. Cells cultured in RPMI1640 enriched by FBS (Fetal Bovine Serum) 10% and 1% antibiotic (Penicillin/Streptomycin) within the purified plates including transparent substrate (glass slide). Antibiotics maintained in a humidified atmosphere of 5% CO_2 in air at 37 °C. Logarithmically growing cells were used for all experiments.

2.2. Preparing biolayer

Cultured cells were trypsinized from the original. After that, cells were washed twice in saline, suspended and centrifuged at 1500 rpm for 5 min, cells counted with methylene blue technique. Cells seeded on 1 cm^2 lamella that putted in standard 6 wells plate. Finally sample of cells prepared with one biolayer on lamella (Fig. 2).

2.3. Optical method

This new method is based on investigation of optical behavior of biolayers using CW He–Ne laser. Sample is translated through the focal region of a focused Gaussian beam in a typical Z-scan setup and the transmitted signal is recorded (Figs. 3–5). By signal analysis, nonlinear behavior of biolayer can be traced.

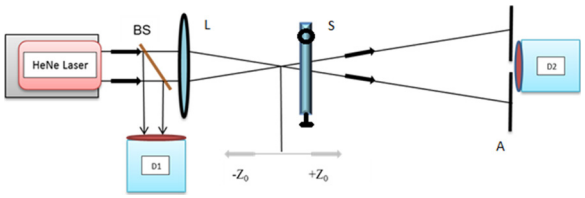

Fig. 3. Typical Z-scan setup to investigate nonlinear behavior of biolayers. D1 = detector one, D2 = detector two, BS = beam splitter, L = lens,A = aperture and S = sample with adjustable holder.

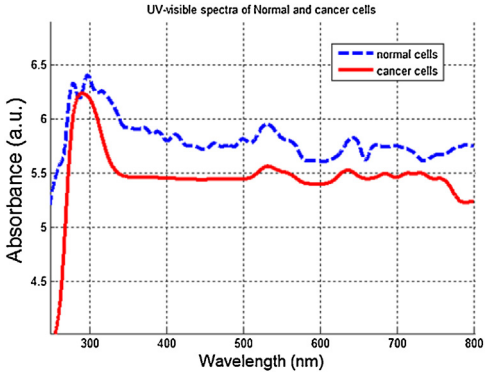

Fig. 4. UV–visible absorbance spectra of normal and cancer cells.

Nonlinear refractive index (NLR) of biolayer can be calculated from Eq. (1) [18,22]:

$$n_2 = \frac{\lambda \Delta T_{P-V}}{\left(2\pi L_{eff}\,(0.406)(1-S)^{0.25} I_0\right)}, \tag{1}$$

In this equation, λ is laser wavelength, ΔT_{P-V} is peak to valley normalized transmittance difference and I_0 is peak on-axis irradiance at the focus that comes from laser power and Gaussian beam spot radius at focus (half-width at $1/e^2$ of maximum of the irradiance): $I_0 = \frac{2P}{\pi w_0^2}$.

Fraction of laser beam transmitted by the aperture in the Z-scan setup gains from radius of aperture (r) and radius of beam on aperture plate (w) perpendicular to optical axis: $S = 1 - e^{-2\left(\frac{r}{w}\right)^2}$ and finally effective length of medium (biolayer in this work) has discussed by length of medium (L) and absorption coefficient (α): $L_{eff} = \frac{1-e^{-\alpha L}}{\alpha}$ [23,24].

Absorption coefficient has been measured by Beer–Lambert relation in linear regime. Proportion of input and output power for all samples remains fixed in low irradiance.

3. Results and discussion

UV–visible spectra pattern is released in Fig. 4. Peaks and valleys of pattern for two samples are approximately in similar points especially in wavelength of laser irradiation (632.8 nm). This is because of main components similarity in normal and cancerous cells. Normal ovarian cells have smaller size but more concentration in same area that interrogated by laser light. Minimum amount of interrogation area of the laser lies on Gaussian beam waste position with 40 μm diameter. Hence this area includes between 20-40 cancer cells or 40-80 normal cells in average and transmitted light reflects information of these cells. We tried to irradiate laser light on group of disposed cells by adjustment of sample using micrometer through x–y directions in optical setup. This trick is used to confidence of reaching repeatable results and accurate measurements.