medicinska revija
medical review

UDK: 616-006.6-085.849.11 ;
537.531:577.1

Peidaee P. et al. G. MD-Medical Data 2013;5(1): 007-013 *MEDICAL DATA / Vol. 5. No 1 /* III *2013.*

Originalni članci/
Original articles

IN VITRO EVALUATION OF THE EFFECTS OF VISIBLE, NEAR - AND FAR INFRARED LIGHT RADIATION ON CANCER AND NORMAL CELLS*

IN VITRO EVALUACIJA DEJSTVA VIDLJIVOG, INFRACRVENOG I SKORO - INFRACRVENOG SVETLOSNOG ZRAČENJA NA KANCERSKE I NORMALNE ĆELIJE *

Pantea Peidaee*[1], Nahlah Almansour[2], Ravi Shukla[3,4,] and Elena Pirogova[1,4]

[1] School of Electrical and Computer Engineering, RMIT University, Melbourne, Victoria, Australia
[2] Department of Biotechnology and Environmental Biology, School of Applied Sciences, RMIT University, Bundoora, Victoria, Australia
[3] Department of Applied Chemistry, School of Applied Sciences, RMIT University, Melbourne, Victoria, Australia
[4] Health Innovation Research Institute, RMIT University, Australia

*Invited paper/Rad po pozivu

Correspondence to:
Elena Pirogova (PhD Biomed Eng, BEng Hons Chem Eng) Senior Lecturer
Program Director, Biomedical Engineering Undergraduate
School of Electrical and Computer Engineering
Health Innovations Research Institute
RMIT University City Campus
GPO Box 2476 Melbourne VIC 3001
ph: 61 3 9925 3015 fax: 61 3 9925 2007
Bld 10 Level 11 Room 6
E-mail: elena.pirogova@rmit.edu.au
Cc.:Author's email:
s3121511@student.rmit.edu.au

Key words
electromagnetic radiation, Resonant Recognition Model, low intensity light exposures, cancer cells

Ključne reči
elektromagnetsko zračenje, rezonantni model, izlaganje svetlosti niskog intenziteta, kancerske ćelije

Abstract

There is strong evidence that the changes in energy states of bio-molecules, induced by applied electromagnetic radiation (EMR), can lead to changes in particular biological processes [1, 2]. In this study we investigated experimentally the hypothesis of the Resonant Recognition Model (RRM) that the selectivity of protein activities is based on specific resonant electromagnetic interactions [3]. The RRM theory proposes that an external electromagnetic field at a particular activation frequency would produce resonant effects on the biological activity of a particular protein, and this activation frequency can be determined computationally [3]. In our previous study [1], it was proposed that the wavelengths of the EMR in the range of 3500-4200nm are expected to affect biological activity of proto-oncogenic proteins [1, 4]. Thus, an exposure system based on Infrared Light Emitting Diodes (IR-LEDs) was constructed and used to irradiate mouse melanoma (B16F0) and Chinese Hamster Ovary (CHO) cell lines with the computationally determined far infrared wavelengths of 3400nm, 3600nm, 3800nm, 3900nm, 4100nm and 4300nm specific to mouse cells. In addition, the same cells, B16F10 and CHO, were exposed to visible and near infrared light radiation at the wavelengths of 466nm, 585nm, 626nm, 810nm, 850nm and 950nm. The results discussed here are obtained from lactate hydrogenate (LDH) cytotoxicity assay for all twelve studied wavelengths of visible and infrared light. A qualitative analysis has also been conducted using Light microscopy and the results from this study are presented and discussed.

INTRODUCTION

The complexity of controlling and monitoring biological functions that lead to cancer development has baffled scientists while cancer maintains its position as one of the highest mortality rate diseases around the globe. In fact, cancer is among the top ten causes of death and is the second highest cause of disease related death in developed countries [5-7]. The primary reason for cancer development is permutations in the DNA of a cell that leads to uncontrolled growth of mutated cells that contribute to tumor growth. Oncogenes are a specific group of growth effectors that promote uncontrolled cell growth and proliferation. These proteins are derived from normal cellular growth factors (so-called proto-oncogenes) by a limited number of modifications: mutations, insertions, or deletions. Because proto-oncogenes

control the cell cycle, it is obvious that should a proto-oncogene be mutated, the potential for an unregulated cell cycle results. An unregulated cell cycle is the essence of cancer. In order to control the biological function of a cell, correlation between its coding sequences along with its biological function need to be unravelled.

The resonant recognition model (RRM) is a novel physico-mathematical approach established to analyse the interaction between a protein and its target. The RRM assumes that the specificities of protein interactions are based on the resonant electromagnetic energy transfer at the specific frequency for each interaction [1, 3, 4, 8-10]. The RRM presents an efficient tool for computation of frequencies which have resonant effects on a proteins' biological activity [4, 8]. Protein interactions are highly selective, and this selectivity is defined within a protein's structure. In our previous work [2], a relationship between the RRM spectra of some protein groups and their interaction with visible light was established. In our previous study, the RRM was used to predict the activation frequency of EMR that would modulate the function of proto-oncogene proteins. We have designed and presented the exposure system that can emit light at the selected frequencies [11].

A number of studies presented and discussed the effects of induced visible light in living organisms [12, 13] for homogenous and nonhomogenous light which demonstrated that absorption of light by biological media is non homogenous in nature [14]. Also, among these published *in vitro* and *in vivo* studies for therapeutic effects of light exposures [13, 15, 16], there are few attempts to study the effects of visible light on cancer cells [17]. Thus, this study investigates the effect of non-coherent low intensity light exposures on B16F0 mouse melanoma cancer and CHO (a non-cancer control cell line) cells. We aimed at investigating the cytotoxic effects of far infrared light (3400nm, 3600nm, 3800nm, 3900nm, 4100nm and 4300nm), blue, red and yellow visible light (466nm, 585nm, 626nm) as well three other wavelengths in the near infrared range (810nm, 850nm, 950nm) on the selected cancer and non-cancer cells. This comprehensive experimental study investigate the biological effects induced by a LED-based exposure system on M16F0 and CHO quantitatively and qualitatively."

MATERIAL AND METHODS

Resonant Recognition Model (RRM)

It was shown in our previous studies that all protein sequences with a common biological function have a common frequency component in the free energy distribution of electrons along the protein backbone. This characteristic frequency was shown to be related to protein biological function [1, 3, 4, 8, 10] . It was also shown that proteins and their targets share the same characteristic frequency. Thus, it can be postulated that RRM frequencies characterise not only a general function but also a recognition/interaction between the particular proteins and their target at a distance. Thus, protein interactions can be viewed as a resonant energy transfer between the interacting molecules. This energy can be transferred through oscillations of a physical field, possibly electromagnetic in nature [18, 19]. Since there is evidence that proteins have certain conducting or semi-conducting properties, a charge moving through the protein backbone and passing different energy stages caused by different amino acid side groups can produce sufficient conditions for specific electromagnetic radiation or absorption[10]. A strong linear correlation exists between the predicted and experimentally determined frequencies corresponding to the absorption of electromagnetic radiation of such proteins [2, 18]. It is inferred that approximate wavelengths in real frequency space can be calculated from the RRM characteristic frequencies for each biologically related group of sequences. These calculations can be used to predict the wavelength of the light irradiation, which can affect the biological activity of exposed proteins. The frequency range predicted for protein interactions is from 10^{13}Hz to 10^{15}Hz. This estimated range includes IR, visible and UV light. These computational predictions were confirmed by comparison of: (i) absorption characteristics of light absorbing proteins and their characteristic RRM frequencies [18, 20]; (ii) frequency selective light effects on cell growth and characteristic RRM frequencies of growth factors [10, 18]; and (iii) activation of enzymes by laser radiation [2, 18, 19]. These results indicate that the specificity of protein interaction is based on a resonant electromagnetic energy transfer at the frequency specific for each interaction. A linear correlation between the absorption spectra of proteins and their RRM spectra with a regression coefficient of K=201 was established.

Using the RRM postulates, a computationally identified characteristic frequency for a protein functional group can be used to calculate the wavelength of applied irradiation, λ, defined as $\lambda=201/f_{RRM}$, which could activate this protein sequence and modify its bioactivity [18, 21].The RRM was used to analyse oncogene and proto-oncogene proteins and determine their corresponding characteristic frequencies [3, 21]. These computationally defined RRM frequencies for oncogene (f_1) and proto-oncogene (f_2) proteins (f1=0.0302 and f_2 =0.0576) can be converted to real space frequencies of applied radiation using the ratio $\lambda=201/f_{RRM}$. Thus, the computationally predicted wavelength for oncogene activation is 6656nm, and for proto-oncogene is 3490nm.

Of particular interest to this study was irradiation of selected mouse cancer B16F10 and non-cancerous normal CHO cells by light in the vicinity of the mouse specific proto-oncogene activation frequency of 3490nm. This formed a basis for selection of different LEDs for use in the exposure system. As mentioned above, the selected LEDs cover the range of visible, near infrared and far infrared light exposures. A comprehensive quantitative analysis of the exposed and sham-exposed B16F10 and CHO cells has been carried out. The results obtained from a LDH cytotoxicity test of B16F10 and CHO cells exposed to the selected wavelengths of light are discussed below. Qualitative analysis of the effects of applied irradiation on cancer and normal cells was performed using light microscopy. Light microscopy images were taken immediately upon exposure as well as after 24 hours incubation of the exposed cells.

Exposure System

1. Adaptor of 12V and 1A current has been used.

2. The selected irradiating wavelengths: 3400nm, 3600nm, 3800nm, 3900nm, 4100nm, 4300nm, 466nm, 585nm, 626nm, 810nm, 850nm, 950nm.

3. To have these LEDs work at their optimal characteristics, the input signal of 250mA, 2 kHz and 50% duty cycle has been fed into the system.

4. To have the minimum dispersion, the exposure system was designed to have the narrowest possible irradiation angle and the gap between the system and an exposed sample was set at less than 1mm.

5. All the LEDs used had the irradiation angle of less than 40o for minimum power dissipation from the energy source.

6. The system was designed in a way to avoid any cross talk between different LEDs. Each exposed well was surrounded with one empty well to avoid any cross talk between frequencies.

7. To make sure that there are no heating effects on irradiated cells, a heat shield material was used between the wells in order to prevent heat dissipation and absorption produced by LEDs irradiating at the selected wavelengths.

The intensity of each LED was constant (between 15μW to 30μW) and was not changed during the experiments. The relevance conversion factor between radiant intensity and luminous intensity can be calculated as follows:

$K(\lambda) = I_v$ (Luminous Intensity)/ I_e(Radiant Intensity)

Cell cultures

Three different passages of B16F10and CHO cells were obtained from the School of Applied Sciences, RMIT University. Cells were cultivated in no phenol-red- DMEM medium that was obtained from Invitrogen, Australia. Each bottle of 500ml medium had 10ml of HEPES buffer with 10% of Fetal Bovine Serum and 1% of Antibiotics (streptomycin). Cells were dislodged with Trypsin-EDTA solution and washed with Phosphate Buffered Saline (PBS) for routine maintenance of cell line during the cell culture. The experiments were conducted in tan incubator at the temperature of 37^{O}C with 5% CO_2 and at a relative humidity > 90%.

Experimental Procedures

Cells were seeded at the density of $1x10^4$ per mL in 96-well plate and incubated overnight. Then, they were treated in triplicate in the same plate. Three variations of exposure and post-exposure regimes were tested on each cell line. Each combination of different exposure and incubation times were repeated 3 times in order to determine statistically significant results and reveal whether post-exposure incubation or irradiation duration (dose) have any specific effect on cell cytotoxicity.

The first regime: cells were irradiated for 1.5 hours and immediately after the exposure were tested using LDH assay without any incubation. The second regime: cells were irradiated for 1.5 hours and incubated for 24 hours afterwards. The third regime: cells were irradiated for 3 hours and incubated for 24 hours afterwards.

To eliminate any effects from the heat generated by the IR-LEDs used in the exposure device, we introduced a heat shield gel. The gel was purchased from Inventables, USA. The gel was placed around the well from outside the gaps. Before seeding, plates with the gel inside of them were placed in a UV camera two times for 30 min. More importantly, to eliminate any cross talk between the LEDs and the effect of two frequencies on the same well, we had empty wells around each well where the experiments were run.

Lactate Dehydrogenase (LDH) Assay

LDH Assay has been used to reveal the induced cytotoxic effect in cells applied response to light irradiation. The LDH Activity Assay Kit was purchased from Roche Diagnostics, Australia. The following equation was used for calculation of cell cytotoxicity. The background control (media without cells) was subtracted from all of values in the equation.

Cytotoxicity = 100*[(experimental value – low control)/ (high control – low control)]

- Low control is the mean absorbance of sham exposed cells
- High control is the mean absorbance from lysis cells

Light Microscopy

Light microscopy was used to conduct qualitative assessment of the effect of light exposures on cancer and control cells. The images were acquired at a 10X magnification for the sake of better quality and a relatively large coverage area to observe a general trend in the cellular morphology. The images were taken immediately after the exposures as well as after 24 hours incubation post- exposure. For control, the unexposed cells were also studied using light microscopy.

RESULT AND DISCUSSION

Quantitative Analysis

Figure 1 represents data on cell viability,%, (Cell viability = 100 – Cytotoxicity), as well as their respective standard errors of B16F10 and CHO cells exposed with three different regimes of exposure and incubation times.

(*Figure 1 on the next page*)

As can be seen from Figure 1, the cell viability of CHO cells with different exposure and incubation times has not changed significantly. The untreated control cell viability was considered 100%, as it is observed that CHO cells do not show any significant changes in cell viability when compared to untreated cells. In contrast, the irradiated B16F10 cells clearly exhibited changes in cell viability when compared with untreated cells. Moreover, change between the variation of exposure and incubation times is presented in Figure 2.

(*Figure 2 on the next page)*

It can be observed from the results shown in Figure 2 that by increasing incubation and irradiation duration, cell viability of cancer cells is decreased when compared with the treated/irradiated normal CHO cells.

(*Figure 3 on the next page*)

Figure 3 demonstrates the 3D plane of all results obtained from LDH assay for the exposed and non-exposed B16F10 and CHO cells. From Figure 3, we can see that there is a clear reduction in cell viability of B16F10 cancer cells when compared to normal CHO cells.

In general, B16F10 melanoma cells were found to be susceptible to all types of irradiation. However, at infrared radiation exposures, at the computationally predicted FIR wavelengths, significant toxicity was observed in cancer

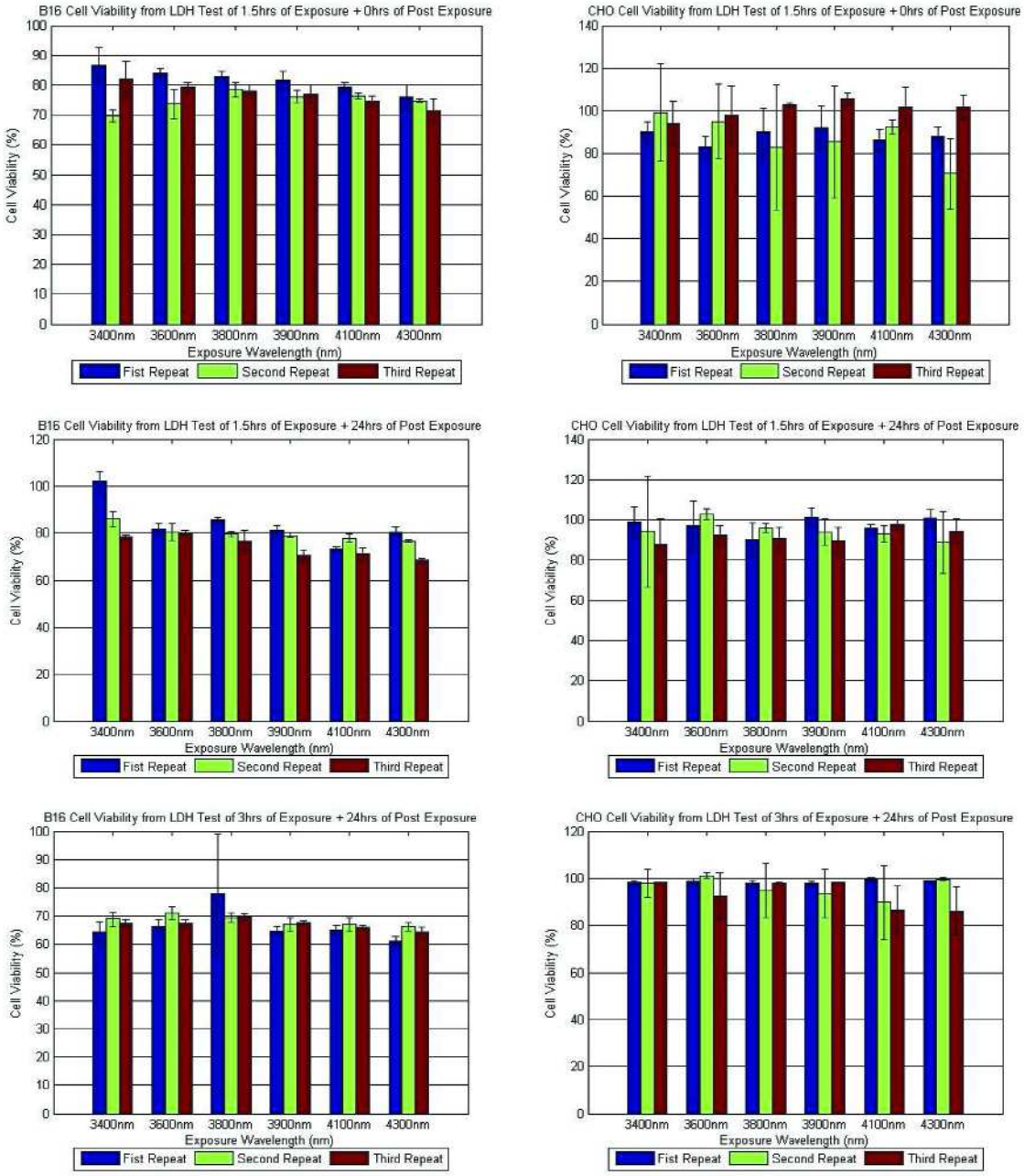
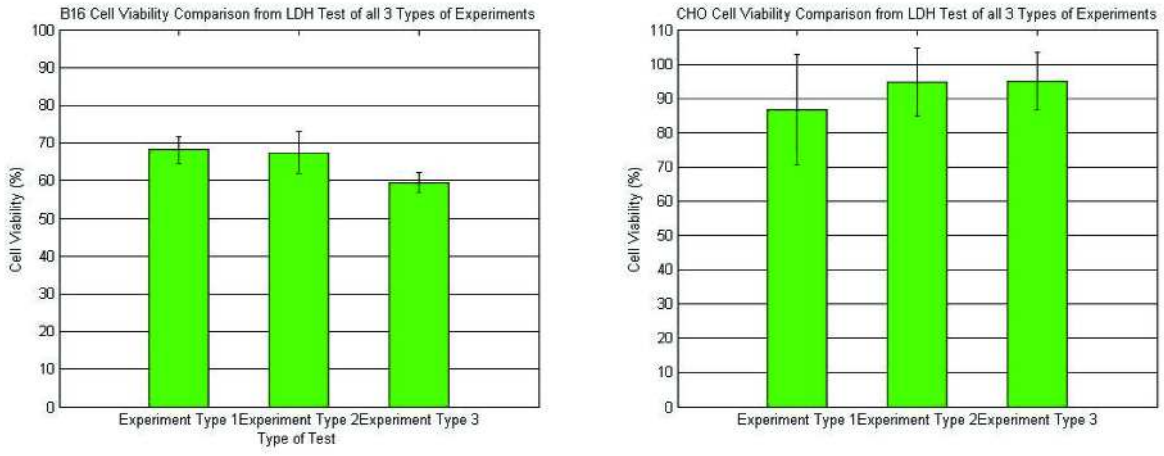
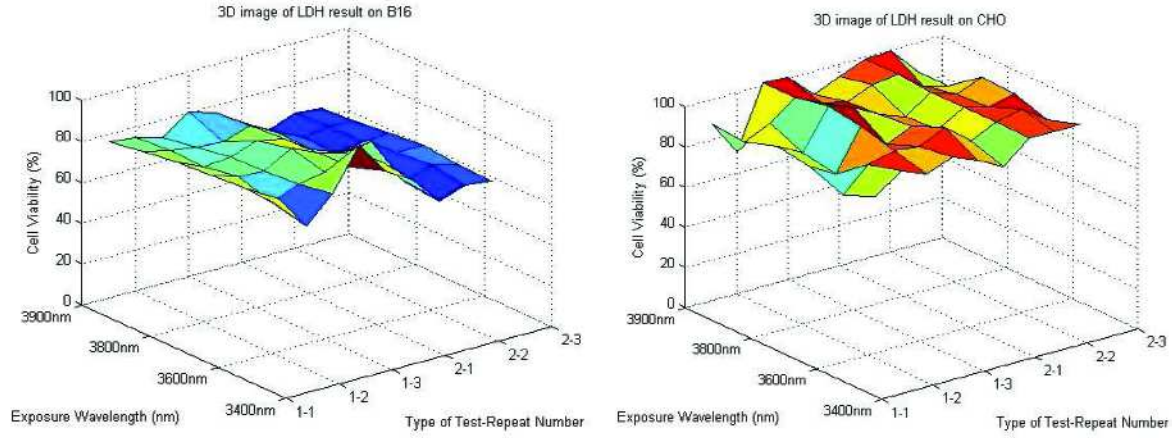


Figure 1. Cell viability of B16F10 and CHO for three different regimes of exposure and incubation times



(a) (b)

Figure 2. Comparison between three different experimental regimes of irradiation and incubation times: a) B16F10 cells and b) CHO cells.



(a) (b)

Figure 3. Plot of all results obtained from LDH assay for three types of experimental regimes performed in triplicate

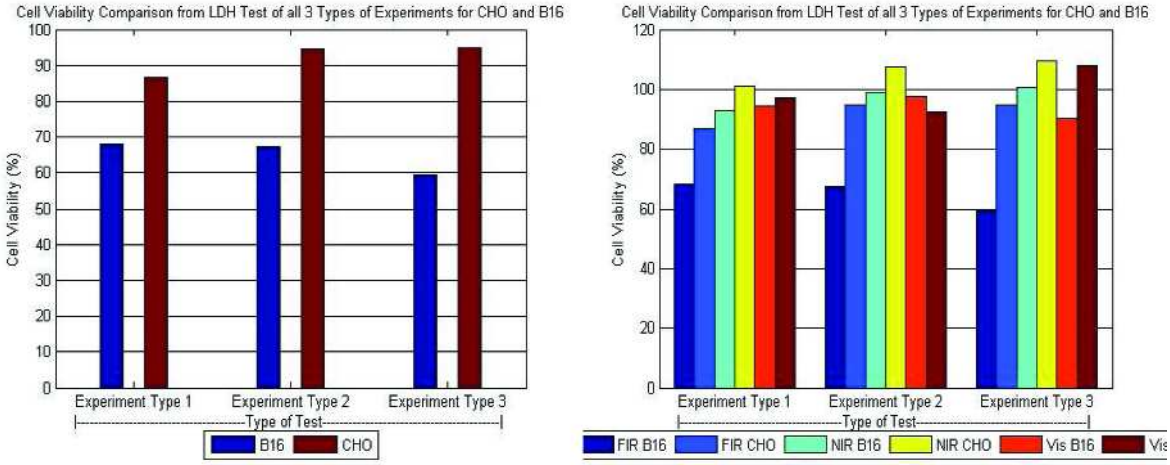


Figure 4. a) Far infrared irradiation effect on B16F10 and CHO cells b) comparison of far infrared (FIR), near infrared (NIR) and visible (VIS) light Irradiation effect on both CHO and B16F10.

cells compared to CHO normal cells. The effect of far infrared wavelength computed by the RRM (range of 3400nm to 4300nm) is clearly visible and distinguishable. These findings were further analysed using light microscopy.

Qualitative Analysis

In this section, we present and discuss the images obtained from light microscopy immediately after the exposures as well as after 24 hours of incubation (post exposure). Figure 5 shows the images taken just after 3 hours of exposures with the wavelengths of 3400nm, 3600nm, 3800nm, 3900nm, 4100nm, 4300nm.

The next set of images from light microscopy shows cell detachment resulted from cell death. In fact, Figure 6 corroborates the findings obtained from the quantitative analysis using the LDH assay. The results demonstrate that 24 hours incubation of the irradiated B16F10 cancer cells has decreased cell viability. Figure 6 shows the effects of all far infrared wavelengths that were tested in this study in ascending orders. The last image presents the untreated B16F10 cells well, which does not show any noticeable changes in cell morphology.

CONCLUSION

Low intensity light radiation has been studied extensively for its beneficial therapeutic effects on molecules and cells. However, use of low intensity light therapy for cancer treatment is still a relatively new idea but it has attracted attention in scientific community in recent years. The main concept of the RRM that biological functions of a cell can be controlled or modulated by the external source of irradiation was studied here. Based on the findings obtained we can infer that amongst all studied wavelengths of light radiation the far infrared wavelengths (3400nm, 3600nm, 3800nm, 3900nm, 4100nm, 4300nm) have induced effects on cell viability in studied B16F10 cancer cells while the non-cancer CHO cells were not significantly affected. Light microscopy results confirmed findings obtained using LDH assay. The results reveal that exposures of cancer B16F10 cells to different wavelengths of far infrared light decrease their cell viability. While our research demonstrated the effect of far infrared radiation on mouse melanoma and normal cell, more experiments on human cancer and normal cell line are required to establish and generalize this concept.

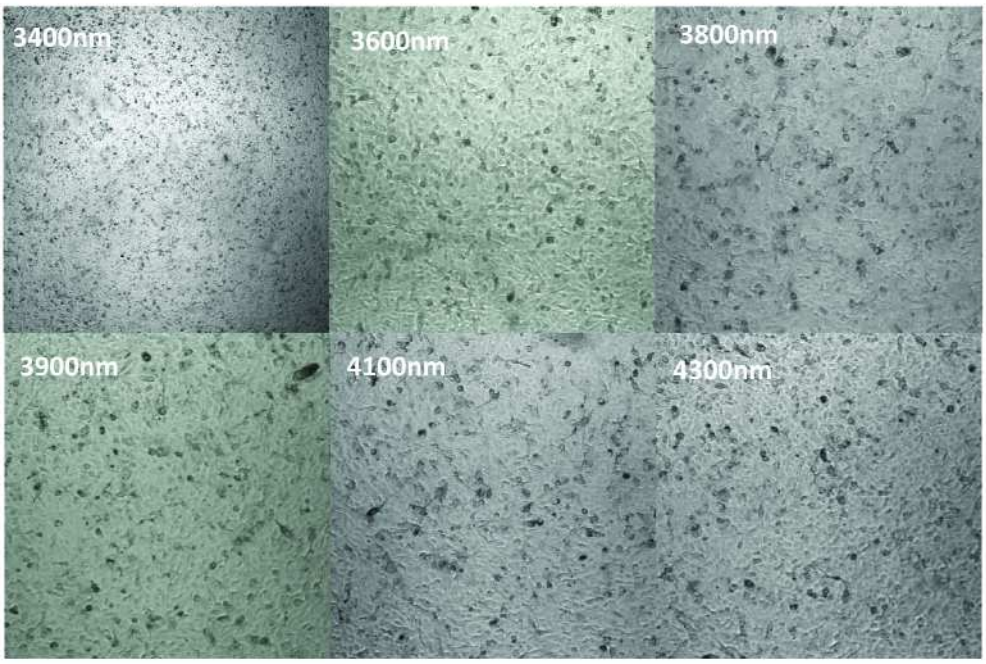


Figure 5. Light microscopy images of B16F10 cells exposed for 3 h to far infrared light

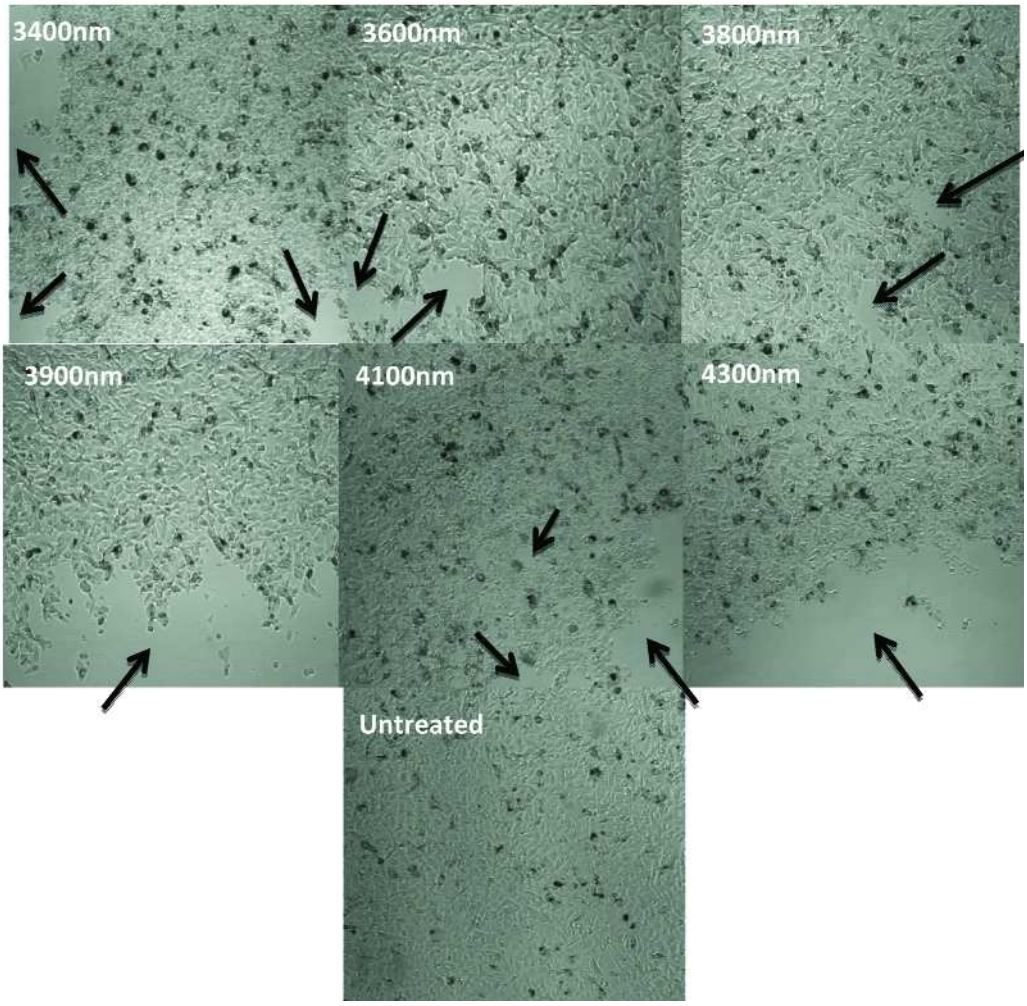


Figure 6. Light microscopy images of B16F10 cells untreated and treated for 3 hours irradiation with far infrared wavelengths followed by 24 hour incubation (post-exposure)

Apstrakt

Postoje jaki dokazi da promene energetskih stanja biomolekula koje su indukovane elektromagnetskim zračenjem (EMR) mogu dovesti do promena u odredjenim biološkim procesima. [1, 2]. U ovoj studiji smo eksperimentalno ispitivali hipotezu rezonantnog modela (RRM) da je selektivnost proteinskih aktivnosti zasnovana na specifičnim rezonantnim elektromagnetnim interakcijama. [3]. RRM teorija polazi od pretpostavke da spoljašnje elektromagnetno polje na odredjenoj aktivacionoj frekvenciji proizvodi rezonantne efekte na biološku aktivnost određenih protein it a se aktivaciona frekvencija može odrediti računskim putem. [3]. U našoj prethodnoj studiji [1], predloženo je da se može očekivati da talasne dužine EMR u opsegu od (3500-4200)nm utiču na biološku aktivnost proto – onkogenskih proteina.[1, 4]. Stoga je dizajniran sistem baziran na infracrvenim diodama (IR-LEDs) koji je korišćen za ozračivanje ćelija melanoma kod miševa (B16F0) i ovarijuma kineskih hrčaka (CHO) talasnim dužinama od 400 nm, 3600 nm, 3800 nm, 3900 nm, 4100 nm i 4300 nm. Iste ćelije, B16F10 i CHO, su izložene vidljivoj svetlosti i skoro infracrvenom svetlosnom zračenju talasnih dužina od 466 nm, 585 nm, 626 nm, 810 nm, 850 nm i 950 nm. Ovde prikazani rezultati dobijeni su ispitivanjem laktalne hidrogenetske citotoksičnosti primenom svih dvanaest talasnih dužina. Kvalitativne analize izvršene su primenom svetlosnog mikroskopa i ti rezultati su ovde prikazani i diskutovani.

REFERENCES

1. E. Pirogova, Q. Fang, M. Akay, I. Cosic, *Investigation of the structural and functional relationships of oncogene proteins.* Proc. of IEEE, 90(12), 1859-1867 ISSN: 0018-9219.

2. V. Vojisavljevic, E. Pirogova, I. Cosic, *The effect of Electromagnetic Radiation (550nm-850nm) on l-Lactate Dehydrogenase Kinetics.* Int. J. Rad. Biol., 83(4), 221-230 ISSN: 0955-3002

3. E. Pirogova, M. Akay, I. Cosic, *Investigating the interaction between oncogene and tumor suppressor protein.* IEEE Trans. on Info. Tech. in Biomed., 13(1), 10-15 ISSN: 1089-7771.

4. E. Pirogova, V.Vojisavljevic, I. Cosic, *Biological effects of electromagnetic radiation, in Biomedical Engineering*, In-Tech, Vukovar, Croatia, pp. 87-106 ISBN: 9789533070131.

5. Karp, G., *Cell and Molecular Biology: Concepts and Experiments,* ed. T. Edition. 1996, 1999, 2001: John Wiley & Sons.

6. Society, A.C. *Cancer Facts and Figures.* 2008.

7. Organisation, W.H. The top 10 causes of death. 2011.

8. E. Pirogova, V.Vojisavljevic, T. Istivan, P. Coloe, I. Cosic, Review Study: *Influence of electromagnetic radiation on enzyme activity and effects of synthetic peptides on cell transformation.* MD-Medical data, 2010, 2(4), 317-324.

9. I. Cosic, E. Pirogova, *Bioactive peptide design using the Resonant Recognition Model.* Nonlinear Biomedical Physics, 1(7), 1-17. ISSN: 1753-4631.

10. I. Cosic, V. Vojisavljevic, M. Pavlovic, *The relationship of the resonant recognition model to effects of low-inensity light on cell growth*. Int. J. Radiat. Biol., 1998. 56(2), 179-191.

11. P. Peidaee, I. Cosic, E. Pirogova, *Low Intensity Light Therapy Exposure System. in Proc. World Congress of Medical Physics and Biomedical Engineering*. 2012. Beijing, China.

12. J. P. M. Wood, A. J. Bron, N. N. Osborne, *The influence of visible light exposure on cultured RGC-5 cells*. Molecular Vision, 2008. 14, 334-344.

13. Mitchell, U.H., *Use of near-infrared light to reduce symptoms associated with restless legs syndrome in a woman: a case report*. Journal of Medical Case Reports, 2010.

14. R. Lubart, H. Friedmann, S. Rochkind, *Photochemistry and Photobiology of Light Absorption by Living Cells Photomedicine and Laser Surgery*, 24(20), 2006.

15. D. Yang, X.W., W. Hou, X. Zheng, J. Zheng, Y. Jiang, *Assessing the Therapeutic Effect of 630 nm Light-Emitting Diodes Irradiation on the Recovery of Exercise-Induced Hand Muscle Fatigue with Surface Electromyogram*. Int. J. Photoenergy, 4, 2012.

16. P. H Desan, E. E. Michalak, E. M. Tam, Y. Meesters, M. J Ruiter, E. Horn, J. Telner, H. Iskandar, D. B. Boivin and R. W. Lam, *A controlled trial of the Litebook light-emitting diode (LED) light therapy device for treatment of Seasonal Affective Disorder* (SAD). BMC Psychiatry, 7, 2007.

17. A. Sparsa, V. Sol, H. Durox, S. Boulingues, V. Doffoel-Hantz, C. Calliste, J. Cook-Moreau, P. Krausz, F. G. Sturtz, C. O. Bedane, M. Jauberteau-Marchan, M. Ratinaud and J. Bonnetblanc, *Blue Light is Phototoxic for B16F10 Murine Melanoma and Bovine Endothelial Cell Lines by Direct Cytocidal Effect*. Anticancer Research, 2010. 30(1), 143-147.

18. Cosic, I., *The Resonant Recognition Model of Macromolecular Bioactivity*. Biomethods. Vol. 8. 1997: Birkhauser Verlag.

19. Biscar, G., *Photon Enzyme Activation.* Bull. Math. Biology, 1986. 38, 29-38.

20. I. Cosic, S. Birch, *Photoreceptors Having Similar Structure but Different Absorptions Can be Distinguished using the Resonant Recognition Model*. Proc. IEEE EMBS, 1994. 16, 256-266.

21. V. Vojisavljevic, E. Pirogova, I. Cosic. *Influence of electromagnetic radiation on enzyme kinetics. in IEEE Proc. of Inter. of EMBS*. August 2007. Lyon, France.

■ The Invited paper was received and accepted on 15.02.2013.