

Laser Light Induced Cytotoxicity on Normal and Cancer Cell Lines

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Abstract-Laser lights with photosensitizers can be used to destroy various cancer in photodynamic therapy. However, solely laser irradiation can also cause photochemical interactions resulting in cytotoxicity depending on the wavelength, energy dose and the cell source. Fibroblast and glioblastoma cells were irradiated with laser light at 635 nm and 1071 nm to deliver energy doses of 15, 30, and 60 J/cm². MTT was used to determine viability of cell lines. Statistical analysis was performed using ANOVA. The viability of glioblastoma was decreased significantly by laser exposure at both wavelengths. The viability of fibroblast was not affected by laser irradiation with the same parameters. While lasers were applied, temperature rise was not observed. In photodynamic therapy, photochemical or photothermal effects occur if laser is combined with a photosensitizer. On the other hand, our preliminary results show that when only laser, without a photosensitizer, is used, it has cytotoxic effect on cancer cells, not on normal cell, especially at lower energy densities of 635 nm and 1071 nm. These data contain valuable outcomes especially for photodynamic therapy which is used to treat cancer patients. Effect of laser exposure has to be taken into consideration when conducting PDT, depending on cell type.

Key-words: 635 nm, 1071 nm, Laser, Cell viability, Glioblastoma, Fibroblast

1 Introduction

Laser technology has been used in many fields such as dentistry, biology, and medicine because of its outstanding characteristics. In biological tissues, each wavelength has different influence levels. Best practices for the optimal condition-specific laser parameters (e.g. irradiance, wavelength, total dose) for different laser applications are yet to be established [1]. One of the advances in laser technology has been the presence of diode lasers, as a light source, which are small, portable, very reliable, and inexpensive for using in photodynamic therapy (PDT). Diode lasers are ideal for routine use as clinical tools and require little technical expertise for operation. PDT was used in several studies as an adjuvant therapy in glioblastoma treatment after surgical resection. However, the trial sizes were small, reports were published about advances in both quality of life and life span [2].

Glioblastomas are grade IV astrocytoma. They are the most aggressive malignant brain tumor type seen in adults. The prognosis is poor for most glioblastoma patients. For glioblastoma therapy, PDT has been used as an adjuvant treatment throughout the last 15–20 years. The effects of PDT using lasers are, however, still controversial. These varied results may be due to

many factors, including laser irradiation parameters (e.g., wavelength, power density, and energy density) and the irradiated cell type. Even laser irradiation on its own, without using a photosensitizer, has a significant role in the stimulation of cell proliferation, differentiation, and apoptosis in various cell types [3].

When laser interact a biological tissue, application results one of mechanisms, which include photo-thermal, photo-chemical and photo-mechanical effect [4]. PDT has photo-chemical mechanisms without any temperature increase to cause damage tissue. Laser application with a photosensitizer results cytotoxic oxygen radicals that cause tumor destruction while normal cells are not affected [5,6]. Also, laser treatment is thermally monitored to determine the effect of photo-thermal interactions.

It appears that photosensitizer-free laser irradiation doses without any temperature rise are also important for maximum PDT effect. Appropriate light dosing is particularly critical to improve the therapeutic effect. Additionally, treatments by using different light sources with different tissue penetration characteristics will also increase PDT success rates.

For these reasons, we aimed to investigate whether different laser sources such as 635 nm

and 1071 nm have any effects on glioblastoma and normal fibroblast cell viability. We also observed whether laser energy doses cause any temperature increase throughout application.

2 Materials and Methods

2.1 Cell Lines

Rat glioblastoma cell lines (C6) and normal fibroblast cell lines (L929) were grown in the Dulbecco modified eagle medium (DMEM), supplemented with 10% foetal bovine serum (FBS), and 1% penicillin–streptomycin. Cells were kept at 37 °C in a humidified incubator with 5% CO₂.

Cell lines were seeded in a 96-well plate (TPP, Switzerland) and incubated for 24 h. Plates were divided into 3 major groups for each cell line (Control, Laser-635, and Laser-1071). The laser groups were divided further into 3 minor groups (15, 30, and 60 J/cm²) depending on the delivered laser dose.

2.2 Laser Irradiation System Set-up

Two laser sources with their dedicated drivers (Diode Laser, $\lambda = 635$ nm, VA-I-400-635, Optotronics – Ytterbium Fiber Laser, $\lambda = 1071$ nm, YLM-20-SC, IPG Photonics) were used for the irradiation of the cell lines. Both lasers were coupled to optical fibers, which were positioned above the well plate at a specific distance to ensure homogenous illumination of a single well. The output powers of the laser sources were adjusted using an optical power meter (1918-R, Newport) with a silicon detector (918D-SL-OD3, Newport) to realize a power density of 200 mW/cm² over the well plate. The exposure duration was regulated using an optical shutter with a dedicated controller (SH05 & SC10, Thorlabs) for the 635 nm laser (Figure 1), and an in-house developed controller for the 1071 nm laser (Figure 2). Cell lines were irradiated in continuous wave operation mode over 75, 150, and 300 seconds to deliver a laser dose of 15, 30, and 60 J/cm², respectively.

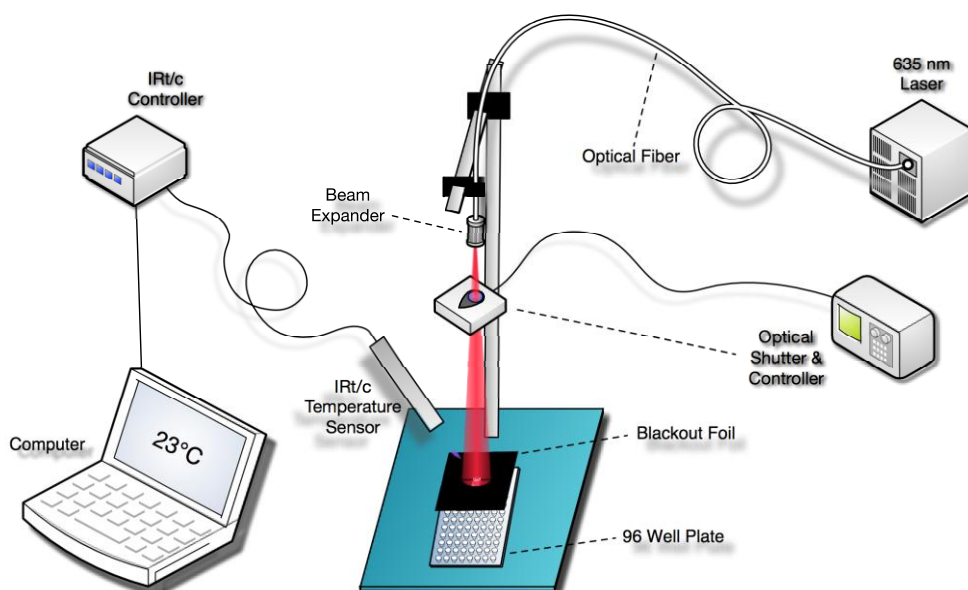


Figure 1 - Schematic diagram of 635 nm laser application.

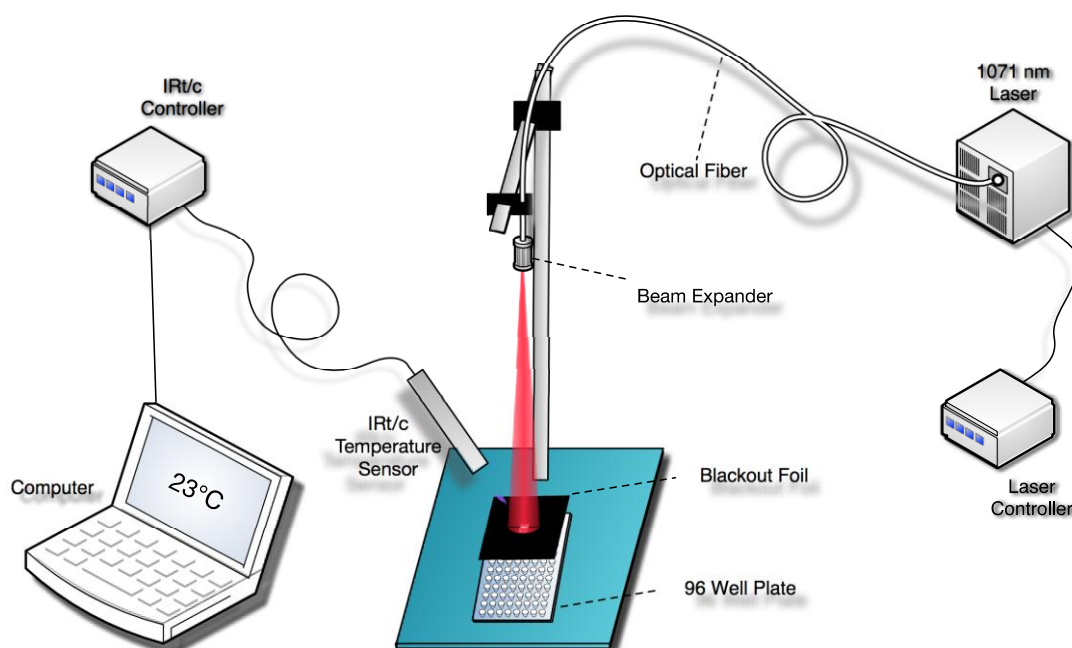


Figure 2- Schematic diagram of 1071 nm laser application.

2.3 Thermal Monitorization

An in-house developed non-contact IR thermometer system was used for temperature measurements (Fig. 1, Fig. 2). The system is composed of a narrow field of view IR thermocouple sensor (90614, Melexis) and a focusing IR lens to ensure a measurement spot diameter reasonably smaller than the well diameter, which is approximately 7 mm. The measurement system was calibrated using a blackbody calibration source (BB702, Omega) with an accuracy of 0.1 °C.

2.4 Cell Viability Assay

Cell proliferation was determined by MTT assay. The experiments were repeated three times. The cells were incubated for 3 h at 37 °C. The optical density was measured at 570 nm with a microplate reader (iMark Absorbance Reader, Bio-Rad). The cell counts were compared using one-way analysis of variance (ANOVA) and subsequently separated using Tukey's Honestly Significant Difference (HSD). Statistical analysis was performed using SPSS (v.18.0). Results were considered statistically significant ($p < 0.05$).

3 Results

In glioblastoma cell lines, all 635 and 1071 nm laser applications had inhibitory effects when

compared to control ($p < 0.05$). However, 15 J/cm² dose of 635 nm irradiation reduced cell viability more than the 30 J/cm² and 60 J/cm² of 635 nm laser application. An inverse relationship was observed between applied energy densities and cell viability for 1071 nm laser application (Fig. 3). It is expected that laser exposure on its own should not have any impact on normal cell proliferation in photodynamic therapy. In Figure 4, all laser treatments had no effect on cell viability of normal fibroblast cell lines, L929, as expected. Temperature monitorization did not reveal a significant temperature increase during laser exposure (Fig. 5).

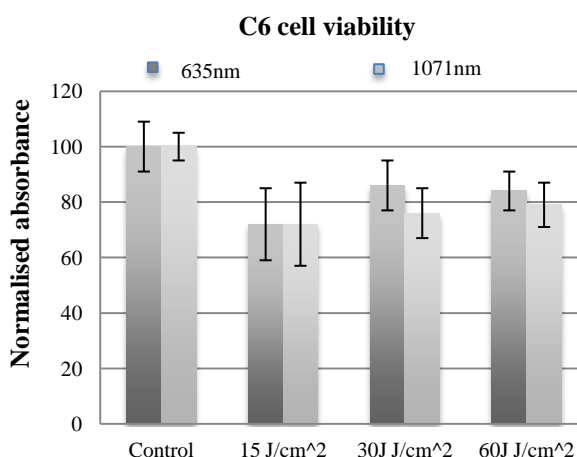


Figure 3- 635 nm and 1071 nm laser irradiations on C6 glioblastoma cell line viability.

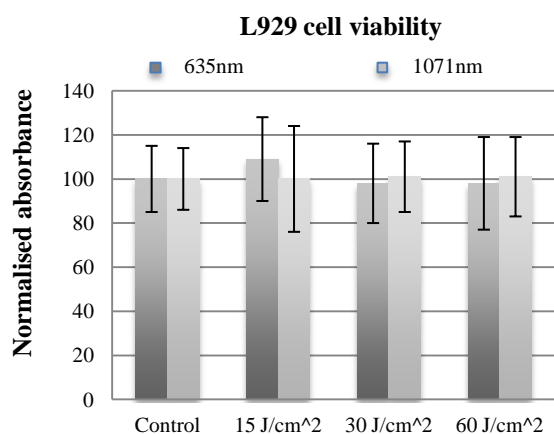


Figure 4- 635 nm and 1071 nm laser irradiations on L929 normal cell line viability.

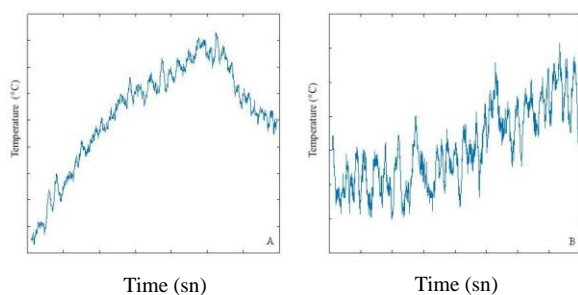


Figure 5- Temperature levels during 635 nm (A) and 1071 nm laser (B) applications.

4 Discussion

Laser irradiation with different wavelengths has different responses in cancer cell line and normal cell line. Laser irradiation at both wavelengths and doses showed remarkable results in terms of cell viability inhibition of C6 glioblastoma cell lines, while L929 cell viability was not affected in any way.

There are contradictory outcomes in the literature when using different wavelengths and energy densities. In several studies, it has been shown that different laser exposure doses and laser wavelengths have diverse responses (proliferative or ineffective) on normal or cancer cell lines [6,7,8,9,10].

In agreement with our findings, Frigo et al. showed that 9 J and 63 J laser irradiations have slightly inhibitory effects on melanoma cells for 24 h [11]. In another study, laser irradiation with

different parameters had positive results for treatment of facial skin cancer [12].

For photodynamic therapy applications, temperature rises are not desirable. In a study, in which five different cells were used, it was observed that infrared irradiation causes suppression of cell viability. The same study announced a small amount of temperature elevation, namely 3.8, and 6.9 °C in 20, and 40 J/cm² laser energy densities, respectively [13]. In our study, no significant temperature rise was observed in both major laser parameters. Moreover, only cancer cell viability was decreased. The lack of temperature increase is an indication that the inhibitory effect is only due to photochemical interactions. Depending on laser parameters, mitotic index of cells can also change which may lead to suppression of proliferation [14].

Cells that are irradiated have different cellular redox potentials. These differences may lead to different responses that are associated with stimulation or inhibition of cell proliferation [6].

5 Conclusion

In photodynamic therapy, if laser is used with a photosensitizer in cancer cells, various effects occur such as photochemical, photothermal or photomechanical. Our preliminary results show that when only laser is used at lower energy densities of 635 nm and 1071 nm, they have cytotoxic effects on cancer cells, not on normal cell. These data contain valuable outcomes especially for photodynamic therapy which is used to treat cancer patients. Effect of laser exposure has to be taken into consideration when conducting PDT, depending on cell type. Laser effect should be investigated more detailed in other cell lines and relevant tissues by using different laser parameters such as wavelength, energy densities, power densities and longer post treatment periods. Our further research will include determining whether free radical formation occurs by only laser exposure in cellular levels of glioblastoma cell lines.

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References:

- [1] Marmur ES, Schmults CD, Goldberg DJ., A Review of Laser and Photodynamic Therapy for the Treatment of Nonmelanoma Skin Cancer., *Dermatol Surg.*, Vol. 30, 2004, pp. 264–271.
- [2] Brown SB, Brown EA, Walker I., The present and future role of photodynamic therapy in cancer treatment, *The Lancet Oncology*, Vol. 5, 2004, pp. 497-508.
- [3] Montazerabadi AR, Sazgarnia A, Bahreyni-Toosi MH, Ahmadi A, Shakeri-Zadeha A, Aledavoode A., Mitoxantrone as a prospective photosensitizer for photodynamic therapy of breast cancer, *Photodiagnosis and Photodynamic Therapy*, Vol. 9, 2012, pp. 46-51.
- [4] Jaunich M, Raje S, Kim K, Mitra K, Guo Z., Bio-heat transfer analysis during short pulse laser irradiation of tissues, *International Journal of Heat and Mass Transfer*, Vol. 51 2008, pp. 5511–5521.
- [5] Lsson SP, Gustafsson L, Bendsoe N, Thompson MS, Andersson-Engels S, Svanberg K., Kinetics of the superficial perfusion and temperature in connection with photodynamic therapy of basal cell carcinomas using esterified and non-esterified 5-aminolaevulinic acid, *British Journal of Dermatology*, Vol. 148, 2003, pp. 1179–1188.
- [6] Mognato M, Squizzato F, Facchin F, Zaghetto L, Corti L., Cell Growth Modulation of Human Cells Irradiated in Vitro with Low-Level Laser Therapy, *Photomedicine and Laser Surgery*, Vol. 22, 2004, pp. 523-526.
- [7] Powell K, Low P, McDonnell PA, Laakso EL, Ralph SJ., The Effect of Laser Irradiation on Proliferation of Human Breast Carcinoma, Melanoma, and Immortalized Mammary Epithelial Cells, *Photomedicine and Laser Surgery*, Vol.28, 2010, pp. 115-123.
- [8] Renno ACM, McDonnell PA, Parizotto NA, Laakso EL., The Effects of Laser Irradiation on Osteoblast and Osteosarcoma Cell Proliferation and Differentiation in Vitro, *Photomedicine and Laser Surgery*, Vol. 25, 2007, pp. 275-280.
- [9] Liu CD, Kwan D, Saxton RE, McFadden DW., Hypericin and Photodynamic Therapy Decreases Human Pancreatic Cancer in Vitro and in Vivo, *Journal of Surgical Research*, Vol. 93, 2000, pp. 137–143.
- [10] Kujawa J, Zavodnik IB, Lapshina A, Labieniec M, Bryszewska M., Cell Survival, DNA, and Protein Damage in B14 Cells under Low-Intensity Near-Infrared (810 nm) Laser Irradiation, *Photomedicine and Laser Surgery*, Vol. 22, 2004, pp. 504-508.
- [11] Frigo L, Luppi JSS, Favero GM, Maria DA, Penna SC, Bjordal JM, Bensadoun RJ, Lopes-Martins RAB., The effect of low-level laser irradiation (In-Ga-Al-AsP - 660 nm) on melanoma in vitro and in vivo, *BMC Cancer*, Vol. 9, 2009, pp.404.
- [12] Moskalik K, Kozlov A, Demin E, Boiko E. The Efficacy of Facial Skin Cancer Treatment with High-Energy Pulsed Neodymium and Nd:YAG Lasers. *Photomedicine and Laser Surgery*, Vol.2, 2009, pp.345-349.
- [13] Tanaka Y, Matsuo K, Yuzuriha S, Yan H, Nakayama J., Non-thermal cytotoxic effect of infrared irradiation on cultured cancer cells using specialized device, *Cancer Science*, Vol. 101, 2010, pp. 1396-1402.
- [14] Sroka R, Schaffer M, Fuchs C, Pongratz T, Schrader-Reichard U, Busch M, Schaffer PM, Dühmke E, Baumgartner R., Effects on the mitosis of normal and tumor cells induced by light treatment of different wavelengths, *Lasers Surg Med*, Vol.25, 1999, pp. 263-271.