Summary

Pulsed laser therapy has grown widely in medical applications as a new modality used alone or assisted with different chemotherapeutic agents. Our study presents an ongoing method to test and compare the actions of both laser physical cytotherapeutic and chemical cytotoxic agents on the cytoskeleton of transformed and cancer cell lines.

The study investigated first the energy calibration of the Neodymium Yttrium Aluminum Garnet (Nd:YAG) pulsed laser source, and secondly the effects of different laser wavelengths (principal, second and third harmonics), pulse rates and fluencies on the cytoskeleton of Hep-2 cancer cell line and Ref-3 transformed cell line. Cell monolayers morphology and cytoskeletal architecture patterns were microscopically monitored by different visible and fluorescent vital staining techniques, while monolayers viability and cytotoxicity were assayed by crystal violet and Lactate Dehydrogenase Release (LDH) assays respectively. Acridine orange and fluorescein have been proved as suitable vital inclusion fluorescent dyes for mammalian cells in suspension and in monolayer cultures.

Also the study handled the morphological changes of cell line monolayers by the effect of different pulsed laser treatments on tissue culture concerning proliferation, cell migration, polarization and adhesion influence in Hep-2 cancer cell line and Ref-3 transformed cell lines *In vitro*. The results had shown that low laser treatments induced an increase in the cell proliferation rate without affecting cell viability, while leading to cytoskeletal re-arrangement. Hep-2 cancer cells grew rapidly, doubling themselves in 2-3 days and were shown to be resistant to UV laser rays. The UV-Visible spectral analysis revealed that photodynamic therapy effect of pulsed laser was modulated by many proteins known to be involved in cell cytoskeleton organization and differentiation.

The results of *in vitro* fluence under principal-near infrared laser (1064nm) on Hep-2 cell monolayers viability had shown that 12 pulse treatment of 100mJ laser energy produced the highest inhibition (52.8%), while 3-pulse treatment of 400mJ produced the lowest inhibition (5.6%).

Ongoing results of *In vitro* fluence under second harmonic laser (532nm) on Hep-2 cell monolayers viability had shown that 3 and 6 pulse treatments of all laser energies; 100, 200, 300, 400, 500 (mJ) had no inhibition (-1.85% to -4.4%), while 9 and 12 pulse treatment produced the highest inhibition (13.6%- 43%).

The results of *In vitro* fluence under third harmonic laser (355nm) on Hep-2 cell monolayers viability had shown that all pulses used (3, 6, 9, 12) for laser treatments of all energies; 100, 200, 300, 400, 500 (mJ) had no inhibition (-13.4% to -52.3%), except exclusively laser treatment of 6-pulses 500mJ energy which had 14.6% inhibition on viability.

The results of *In vitro* fluence under third harmonic pulsed laser (355nm) on Ref-3 cell monolayers viability had shown that the highest inhibition was under 500mJ energy level at 9,12 pulses (40.6%, 78.1) respectively, while lowest inhibition was under 100mJ energy level at 3, 9 pulses (-3.1%, -21.8%) respectively. The 200mJ, 300mJ energy levels had exerted inhibition effects ranging between 21%- 34% at pulses 12, 3 respectively.

photothermal effects produced by high laser pulse rates can induce modifications in the architecture of cytoskeleton and other macromolecular networks which, in turn, applies a mechanical stress to the cells through the integrin clusters at the anchoring points. Third harmonic wavelength pulses of Nd:YAG laser (355 nm) absorbed by different cell layer components had show damage of microtubules and ECM.

Concentrations of two-fold dilution of anti-tubulin anticancer drugs were prepared and tested on each cell line after 24hrs. The cytotoxic effects of eight concentrations of taxol on Hep-2 cell line were tested, from 300µg/ml reaching 4.68µg/ml with eight replicates for each concentration. The optical density of cell growth by crystal violet method was read by ELIZA reader at 492 nm, while cytotoxicity effects were assayed using lactate dehydrogenase enzyme (LDH) release coupled assay.

Taxol with the highest concentration of 300µg/ml had shown a dramatic effect on growth of Hep-2 cancer cell line as compared to the control group, while decreased concentrations of taxol reaching 150 µg/ml and 75 µg/ml still had the dissociation power

of cells and disturbing cells polarity due to cytoskeletal network change. Reaching 37.5µg/ml and 18.75µg/ml had evoked changes on cells periphery, while having focal adhesion contacts and regaining of cell polarity. At concentrations of 9.37µg/ml, 4.6µg/ml and 2.34µg/ml, lamellipodia and membrane blebbing had continued to appear throughout the monolayer along with cell adherence to substrate and establishment of cell-cell contact ruffles and cytoplasmic rarefaction had appeared.

Cell monolayers viability of Ref-3 responded to taxol concentrations of 0.293µg/ml, 0.586µg/ml and 1.172µg/ml in a steep highly significant independent effect ($p \leq 0.01$), while concentrations of 75µg/ml and 150µg/ml had exerted a significant effect ($p < 0.05$) in comparison to control, followed by a significant drop in the effect response under 300µg/ml concentration. Hep-2 cells had appeared to be more resistant to anticancer drug (Taxol) than Ref-3 cells which had appeared to be more sensitive showed by increased IC-50 (198.2 µg/ml > 15.8 µg/ml).

Vincristine concentrations of 0.0391µg/ml, 0.781µg/ml and 0.1565µg/ml had shown extremely significant cytotoxic effect on growth of Hep-2 tumor cell line. While higher concentrations from 1.25µg/ml up to 20µg/ml had shown fluctuating cytotoxic effects ending with an extremely significant ($p \leq 0.001$) cytotoxic effective drug concentration at 40 µg/ml.

Vincristine concentrations from 0.391µg/ml till 0.156µg/ml had shown a gradient decrease in Ref-3 cells viability with highly significance ($p \leq 0.01$). Gradual significant increase ($p \leq 0.05$) in monolayer viability upon increased drug concentration occurred at increased concentrations from 0.312µg/ml to 20µg/ml, followed by acute drop at 40µg/ml. Hep-2 cells had appeared to be more sensitive to anticancer drug (Vincristine) rather than Ref-3 cells, showed by decreased IC-50 (10.8µg/ml < 26.5 µg/ml). Cytotoxic effects of anticancer drug on cancer cell line was not time –dependent but dose-dependent.

Both, laser and cytotoxic drugs had induced shear stress on the adherent cells in its vicinity sufficiently strong to break adhesion forces between cells and substrate, making cytoskeleton collapse and cells destroyed.