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Near-Infrared Photobiomodulation of Living Cells, Tubulin, and Microtubules in Vitro

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We report the results of experimental investigations involving photobiomodulation (PBM) of living cells, tubulin, and microtubules in buffer solutions exposed to near-infrared (NIR) light emitted from an 810 nm LED with a power density of 25 mW/cm^2 pulsed at a frequency of 10 Hz. In the first group of experiments, we measured changes in the alternating current (AC) ionic conductivity in the 50 - 100 kHz range of HeLa and U251 cancer cell lines as living cells, exposed to PBM for 60 minutes, and observed increased resistance compared to the control experiments. In the second group of experiments we investigated the stability and polymerization of microtubules under exposure to PBM. The protein buffer solution used was a mixture of Britton-Robinson buffer (BRB80 aka PEM) and microtubule cushion buffer. Exposure of Taxol™-stabilized microtubules ($\sim$ 2 μM tubulin) to the LED for 120 minutes, resulted in gradual disassembly of microtubules observed in fluorescence microscopy images. These results were compared to controls where microtubules remained stable. In the third group of experiments we performed turbidity measurements (absorbance readings at 340 nm) throughout the tubulin polymerization process to quantify the rate and amount of polymerization for exposed versus unexposed tubulin samples, using tubulin re-suspended to final concentrations of $\sim$ 22.7 μM and $\sim$ 45.5 μM in the same buffer solution as before. Compared to the unexposed control samples, absorbance measurement results demonstrated a slower rate and reduced overall amount of polymerization in the less concentrated tubulin samples exposed to PBM for 30 minutes with the same parameters mentioned above. Paradoxically, the opposite effect was observed in the 45.5 μM tubulin samples, demonstrating a remarkable increase in the polymerization rates and total polymer mass achieved after exposure to PBM. These results on the effects of PBM on living cells, tubulin, and microtubules are novel, further validating the modulating effects of PBM and contributing to designing more effective PBM parameters. Finally, potential consequences for the use of PBM in the context of neurodegenerative diseases are discussed.

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