Photobiomodulation as an antioxidant substitute in post-thawing trauma of human stem cells from the apical papilla

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Introduction

Photobiomodulation (PBM) is an application of certain wavelengths of light to biological systems to change their cellular activity. Cytochrome c oxidase (CCO) acts as the primary cellular photoacceptor in PBM. According to a previous study, PBM often induces a dose-dependent increase in reactive oxygen species (ROS) production in biological systems but decreased ROS levels in cells exposed to oxidative stress at 750 and 950 nm[1]. Therefore, PBM can be used as a substitute for antioxidants in post-thawing trauma to overcome the limitations of chemical antioxidants. In this study, we used human stem cells from the apical papilla (SCAP). To identify the optimal pulsed wave, delayed luminescence (DL) is needed. DL is a measure of the intensity of light emitted from cells after the light source is turned off. The emitted photons are a response to modulation of ROS production by PBM. Thus, DL represents mitochondrial activity in the cells[2]. To determine the optimal effectiveness of PBM on modulation of ROS production, it is necessary to determine the decay time of DL to resolve post-thawing trauma. DL was used to determine the optimal PBM condition for all following measurements.

Keywords: Photobiomodulation, Post-thawing trauma, Stem cell, ROS

Materials and Methods

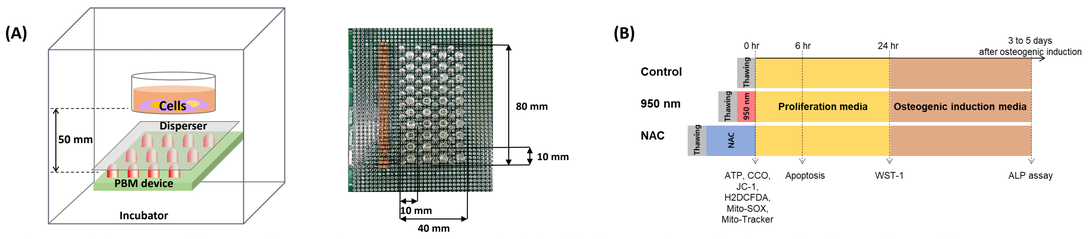

Figure 1. Schematics of the PBM device and experimental time schedule. (A) The PBM device for this study. (B) Timelines of the experiments.

Schematics of the PBM device and experimental time schedule is in figure 1. The light source is placed in a 37 °C incubator to block light other than the light source. The 950 nm LEDs are placed at 10 mm intervals, and a disperser is used to produce uniform irradiation. The transparent acrylic cell culture plate is placed 50 mm above the LEDs. All frozen cells were thawed in a 37 °C water bath for 2 min. All measurements, except those of apoptosis, were performed immediately after cells were irradiated with 950 nm NIR or treated with N-acetylcysteine (NAC). Apoptosis of cells was measured after 6 hours.
Delayed luminescence follows Eq. (1) which is the hyperbolic function[3]. The curves of NIR intensity were fitted in Matlab.

$$I(t) = I_0/(1 + t/\tau)^{\beta}. \quad (1)$$

Here, I_0 is the initial intensity, β is the delayed luminescence index factor, τ is the delayed luminescence characteristic, and T is the decay time. Decay time was calculated by Eq. (2).

$$T = \left(e^{1/\beta} - 1\right)\tau. \quad (2)$$

Results

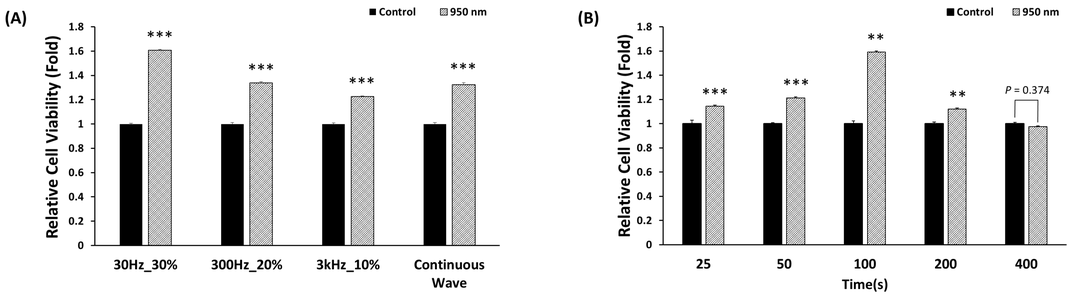

Figure 2. Optimization of 950 nm NIR irradiation conditions for cell viability. (A) Relative cell viability at energy density of 175.68 mJ/cm2. (B) Relative cell viability with different energy densities. Light with the same frequency and duty cycle, 30 Hz and 30%, was applied with different exposure times. Data are mean ± standard deviation (SD) (n = 10). **P < 0.01, ***P < 0.001 versus controls. P values were determined with one-way ANOVA.

Cell viabilities were observed for various duty cycles and frequencies of irradiation to determine the correlation of viability of irradiated cells after thawing with decay time. For viability measurements, the pairs of frequency and duty cycle with the shortest decay time, the longest time, and a moderate value were used with CW mode at the same energy density of 175.68 mJ/cm2 (Fig. 2A). A frequency of 30 Hz and a duty cycle of 30% were chosen for the subsequent experiments using 950 nm NIR irradiation.

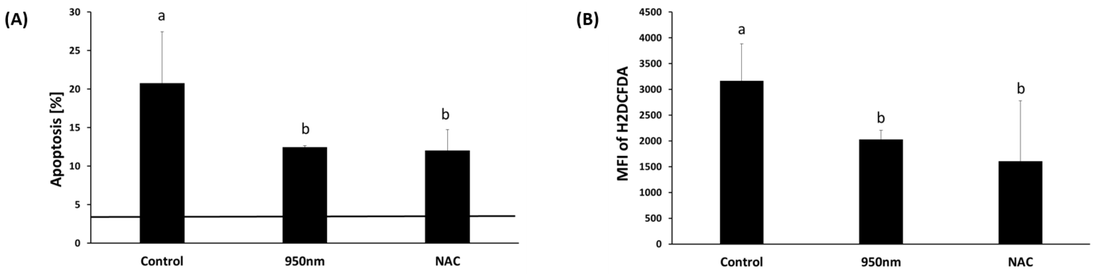

Figure 3. Irradiation with 950 nm NIR reduced apoptosis and ROS as NAC. (A) Apoptotic cell rate. The solid line on the graph is the results of the non-cryopreserved group. (B) MFI of H2DCFDA. Data are mean ± SD (n > 5). Different letters at the tops of columns are significantly different at P < 0.05 according to Duncan's test.

Cryopreservation induces programmed cell death and explosive oxidative stress[4,5,6]. The MFI of H2DCFDA and the percentage of apoptosis were proportional. Thus, increased ROS by cryopreservation was one of the main factors inducing apoptosis in post-thawing trauma. PBM with 950 nm NIR reduced both programmed cell death and ROS concentration.

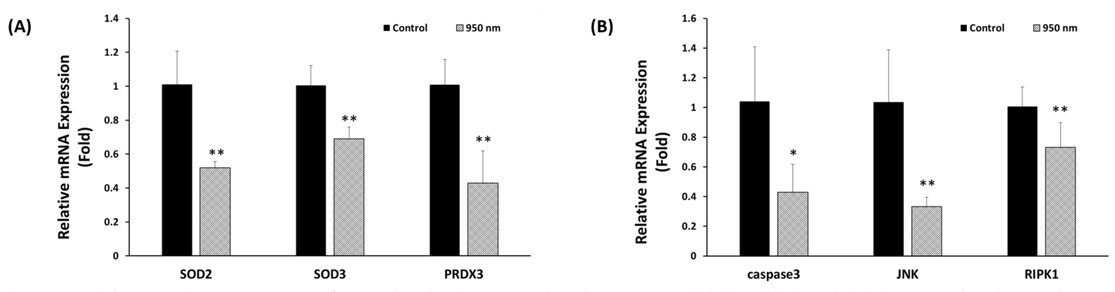

Figure 4. Relative mRNA expression of control and 950 nm -irradiated group. (A) SOD2, SOD3, and PRDX3 are related to oxidative stress. (B) Caspase 3, DUSP1, JNK, and RIPK1 are related to apoptosis. Data are mean ± SD (n > 5). *P < 0.05, **P < 0.01 versus controls. P values were determined by one-way ANOVA (A,B).

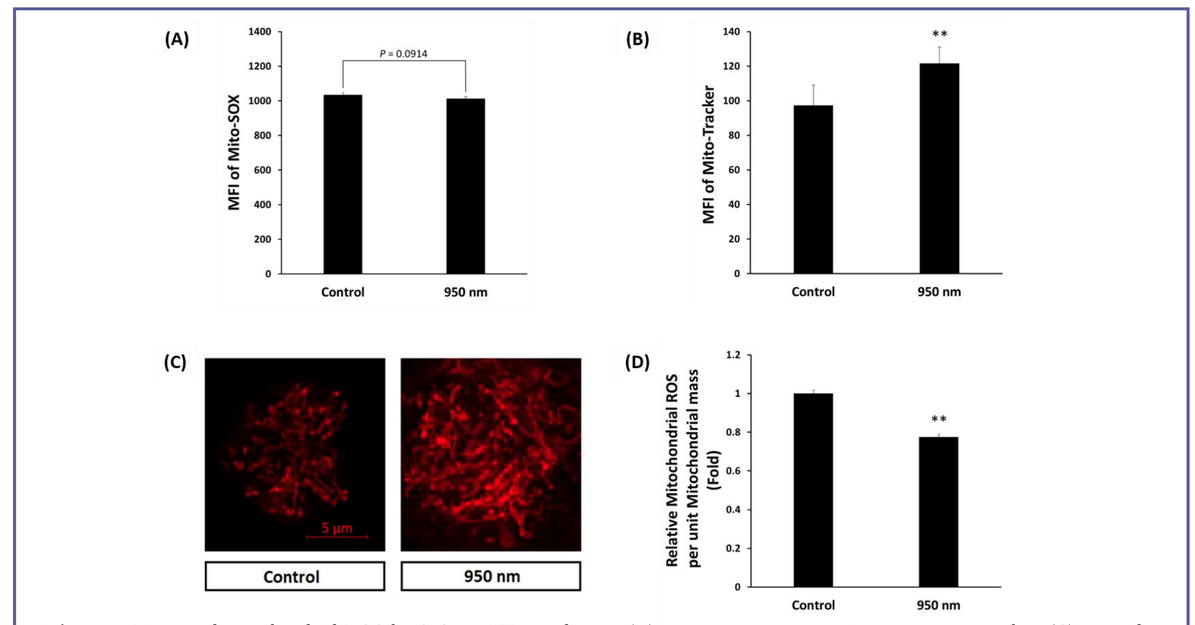

Figure 5. Decreased mitochondrial ROS by 950 nm NIR irradiation. (A) MFI of Mito-SOX. (B) MFI of Mito-Tracker. (C) Typical confocal microscopic images of Mito-Tracker-stained cells. (D) Relative amount of mitochondrial ROS per unit mitochondrial mass. Data are mean ± SD (n > 5). **P < 0.01 compared to controls. P values were determined by one-way ANOVA (A–D).

The 950 nm group did not show a statistically significant difference in MFI of Mito-SOX compared with controls. However, the two groups showed a statistically significant (P < 0.01) difference in mitochondrial mass. The MFI of Mito-SOX was divided by the MFI of the Mito-tracker, and the result indicates the amount of mitochondrial ROS per unit mitochondrial mass. It decreased significantly (P < 0.01) in the irradiation group compared to the control. PBM highly relates to mitochondrial activity because photons of NIR light activate cytochrome c oxidase (CCO) in mitochondria. Mitochondrial membrane potential (MMP), ATP generation, and CCO activity were measured to analyze mitochondrial activities.

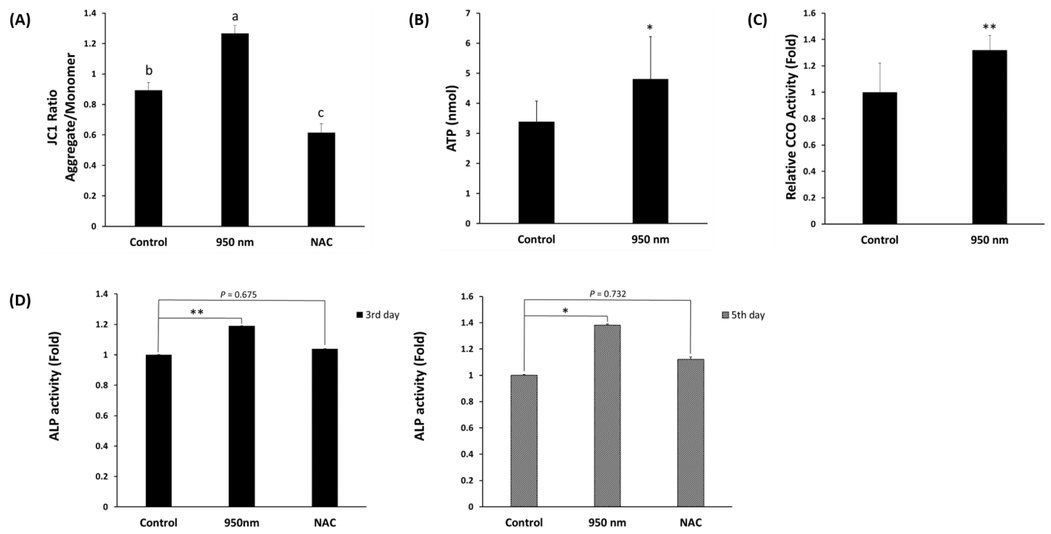

Figure 6. Relative mRNA expression of control and 950 nm -irradiated group. (A) SOD2, SOD3, and PRDX3 are related to oxidative stress. (B) Caspase 3, DUSP1, JNK, and RIPK1 are related to apoptosis. Data are mean ± SD (n > 5). *P < 0.05, **P < 0.01 versus controls. P values were determined by one-way ANOVA (A,B).

Conclusion

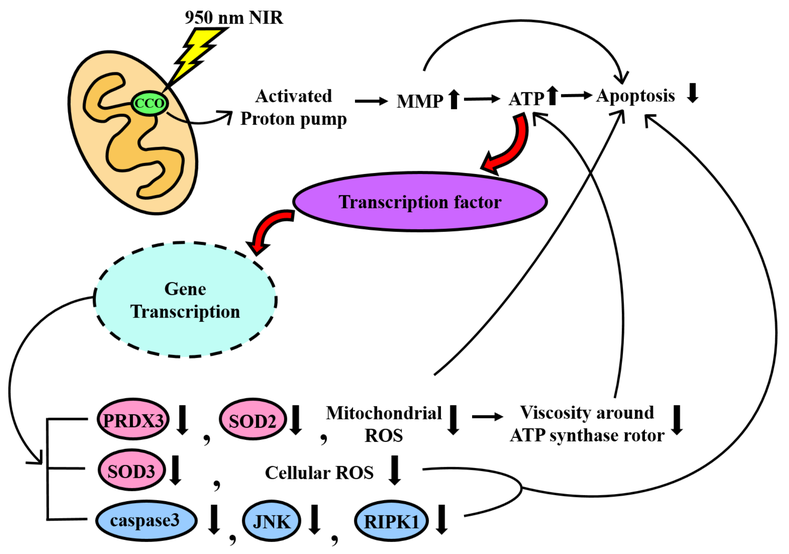

Figure 7. Relative mRNA expression of control and 950 nm -irradiated group. (A) SOD2, SOD3, and PRDX3 are related to oxidative stress. (B) Caspase 3, DUSP1, JNK, and RIPK1 are related to apoptosis. Data are mean ± SD (n > 5). *P < 0.05, **P < 0.01 versus controls. P values were determined by one-way ANOVA (A,B).

In conclusion, we discussed cryodamaged stem cells and the mechanism for recovering the damage by PBM. Various indicators have demonstrated that PBM induces normalization of cells damaged by cryopreservation, indicating that PBM can be used as an alternative to antioxidants in post-thawing trauma. PBM is being used as a treatment in various oxidative stress-causing diseases, but the mechanisms of PBM under various cell types and conditions remain unclear. Ultimately, we need an understanding of the more generalized PBM mechanisms associated with different cell types and PBM conditions. Currently, stem cell treatments are a popular research topic, so it is important to treat post-thawing trauma because most methods of preserving stem cells are types of cryopreservation.

References

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Acknowledgements

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