#64: Photobiomodulated Differentiation of Adipose-derived Stem Cells into Osteoblasts

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ABSTRACT

Osteoporosis is a progressive, metabolic bone disease affecting millions across the globe. Stem cell regenerative therapy has demonstrated potential in treating osteoporosis, particularly when using Adipose Derived Mesenchymal Stem Cells (ADMSCs). Photobiomodulation (PBM) has gained international momentum due to its ability to aid in the proliferation and differentiation of stem cells. Additionally, PBM when combined with differentiation growth factors has revealed enhanced proliferation and ADMSC differentiation into osteoblasts. This in vitro study combined the use of osteogenic differentiation inducers and PBM at a near-infrared (NIR) wavelength of 825 nm using a single fluence of 5 J/cm^2 to determine the proliferation and differentiation effectivity of ADMSCs into osteoblasts. The cells were characterised via the use of flow cytometry. Morphology was investigated and biochemical assays performed including proliferation, viability, and cytotoxicity. The successful outcome of this study provides relevant scientific knowledge and a standardization for osteogenic differentiation in vitro using PBM.

INTRODUCTION

Osteoporosis is known as the most progressive mitochondrial bone disease in humans. Osteoporosis is characterized by the increase in bone fragility leading to the increase in fracture occurrence. Regenerative Medicine (RM) is currently the most promising branch of medical science used to repair or heal tissues and organs damaged by severe injuries, chronic disease or age. At the frontline of RM stands SC therapy because of its' ability to indefinitely self-renew and transdifferentiate into various cell types. Adipose derived mesenchymal stem cell (ADMSC) transplantation can facilitate the development and strength of new bones, increase bone consistency and decrease the risk of fractures. ADMSCs are easily isolated and harvested from adipose tissues via minimally invasive surgery, providing low risk of morbidity, a high abundancy and a high yield in cell numbers. The successful differentiation of ADMSCs into osteogenic lineages in vitro, requires the addition of biological and chemical growth factors (GFs) within the cell culture medium. However, ADMSCs have a tendency of favouring adipogenic lineages despite GF presence. Therefore, lineage specific differentiation control is required via a combinational use of GF presence and a mechanical stimulant such as photobiomodulation (PBM). The use of visible and near-infrared (NIR) light by coherent or incoherent light sources on cells and tissues is known as PBM. Once endogenous chromophore absorption occurs, the light within various ranges brings about photophysical and photochemical responses. PBM is understood to aid in cell function, proliferation, migration and tissue regeneration due to the increased mitochondrial oxidative metabolism. Significantly, the potential stimulatory and inhibitory outcomes of PBM on ADMSCs are wavelength and fluence dependant. However, cell proliferation has been suggested when stimulated by PBM using a wavelength of/between 660-850 nm and a fluence of/between 5 – 10 J/cm^2.

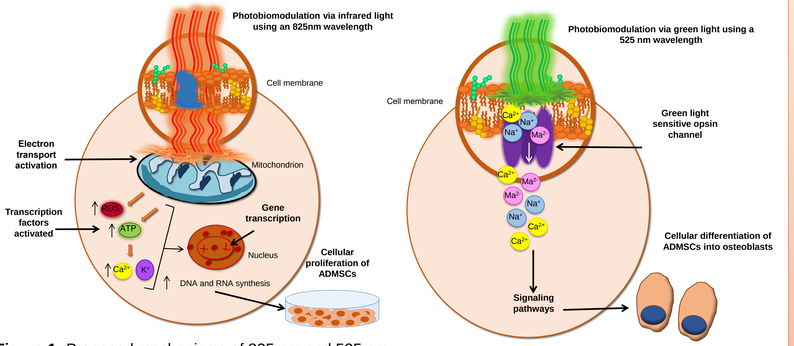

Figure 1: Proposed mechanisms of 825 nm and 525 nm.

METHODOLOGY

Cell Culture
Immortalized ADMSCs, hTERT ASC52telo (ATCC® SCRC-4000™)

Differentiation of iADMSCs into osteoblasts
[50 nM] Dexamethasone + [10 mM] Beta-glycerol Phosphate Disodium + [0.2 mM] Ascorbic Acid
+
PBM (825 nm, 525 nm and 825 nm + 525 nm + 0 and 5 J/cm²)

Characterisation of Protein Markers iADMSCs (CD44, CD166)

Antibody Identification
Flow Cytometry

Biochemical Analysis

- **Morphology** (ILM)
- **Viability** (Trypan Blue)
- **Proliferation** (ATP)
- **Cytotoxicity** (LDH)

Table 1: Laser Parameters.

Light source	Near Infra-red (NIR)	Green (G)
Wavelength (nm)	825	525
Type	Diode	Diode
Emission	Continuous wave	Continuous wave
Power output (mW)	100	574
Power density (mW/cm²)	10.394	59.66
Fluence (J/cm²)	5	5
Time of irradiation	8 min 1 sec	1 min 23 sec
Spot size (cm²)	9.52	9.52

RESULTS

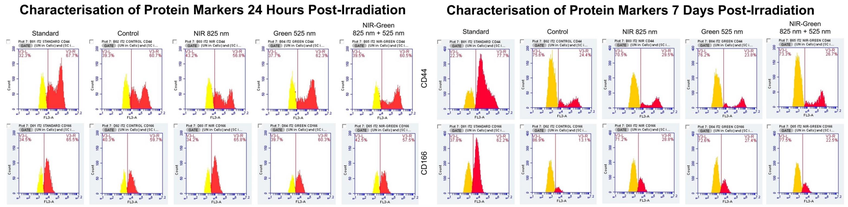

Figure 2: Flow cytometry characterization of immortalized ADMSC CD44 marker and CD166 marker at 24 hours and 7 days post-irradiation treatment. Flow cytometry analysis indicated a decline in CD44 and CD166 protein marker presence by all experimental groups at 7 days post-PBM treatment implying the effective use of PBM to reduce stem-ness and be an efficient tool for immortalized ADMSC differentiation. Noticeably, each experimental group reduced a particular protein marker more than another suggesting the individual influence a laser group may have on the function of a cell.

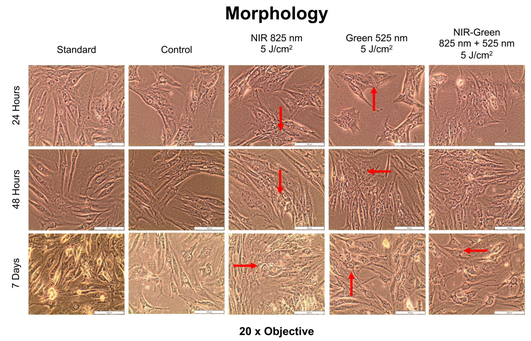

Figure 3: Morphology of immortalized ADMSC differentiation post-PBM treatment using Inverted Light Microscopy. Immortalized ADMSCs are characteristically thin and spindle in shape. A noticeable change in cell morphology occurred amongst the NIR and Green treated cell groups at 24 hours, 48 hours and 7 days and amongst the NIR-Green treated cell groups at 7 days post-PBM treatment. The cell shape had become rounded in appearance similarly to that of osteoblasts and a loss in the visibly thin and longitudinal initial cell shape.

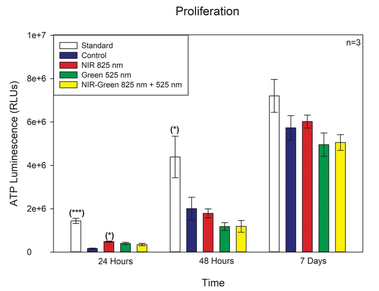

Figure 4 ATP Luminescence assay.
A significant increase in ATP was identified in the standard for 24 hours and 48 hours. PBM did not significantly increase cell proliferation, however, this may suggest that ATP is being redirected for cell differentiation instead of for cell proliferation.

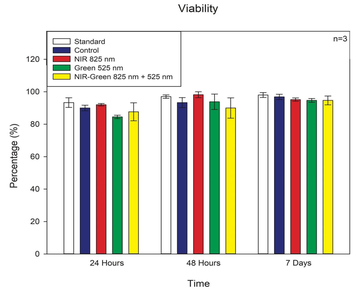

Figure 5 Trypan blue exclusion (%) assay.
The viability assay suggested a consistent cell percentage viability of cells at 24 hours, 48 hours and 7 days post-PBM treatment implying that PBM does not negatively impact cell health instead maintains the overall health of cells.

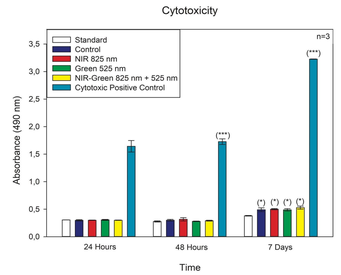

Figure 6 Lactate dehydrogenase (LDH) assay.
LDH results showed no significant increase in LDH production at 24 hours and 48 hours post-PBM treatment, however, a significant increase in LDH production did occur amongst the control and all the experimental groups at 7 days post-PBM treatment. Significantly, these increases are not of toxic concentrations in comparison to the cytotoxic positive control which represents a 100% cell toxicity and cell death.

CONCLUSION

- The results of this study have shown promising early osteogenic differentiation of immortalized ADMSCs using a combination of growth trans-differentiation inducers and PBM treatment. PBM aided in the differentiation of immortalized ADMSCs into osteoblasts according to characterisation and morphology, however, the biochemical assays indicated no significant effect within 24 hours, 48 hours and 7 days post-PBM treatment.
- From the experimental groups, the green laser experimental PBM group proved to be the most promising in inducing ADMSC differentiation as indicated by the reduction in all ADMSC protein markers, rounded cell morphology and slightly lower ATP proliferation, LDH cytotoxicity and ROS presence.
- Further studies will include early and late osteoblast protein marker characterisation and additional biochemical analysis for the establishment of efficient immortalized ADMSC differentiation into osteoblasts via the aid of PBM treatment. The genetic expression of these treated cells using both Real Time-PCR and ELISA will too be used to determine whether successful immortalized ADMSC differentiation into osteogenic cell lines occurs.

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