EFFECTS OF NEAR-INFRARED PHOTOBIOMODULATION AT 830 NM IN A DIABETIC WOUNDED CELL MODEL

LINDOKUHLE HADEBE [1] AND NICOLETTE HOURELD [1]

[1]Laser Research Centre, Faculty of Health Sciences, South Africa
University of Johannesburg

Hadebelindokuhle184@gmail.com

ABSTRACT

Diabetic wounds present a significant healthcare burden due to their failure to progress through the usual phases of healing within the expected time, leading to decreased cellular migration, skin ulceration, and impaired wound healing. Complications arising from these wounds, such as lower extremity amputation, can decrease patient quality of life and result in premature death. Photobiomodulation (PBM), previously known as low-level light therapy (LLLT) or cold laser therapy, is a non-invasive medical treatment that uses low-intensity light sources to stimulate cellular activity and aid healing in various tissues. This therapy has been researched for its ability to aid wound healing, decrease pain and inflammation, and increase tissue repair. However, the mechanisms that aid the induction of cell migration and survival using an 830 nm laser are poorly understood. Therefore, the present study was designed to evaluate the therapeutic efficacy of near-infrared (NIR) PBM at 830 nm in wounded and unwounded human skin fibroblast (WS1) cell models. Models were exposed to an 830 nm diode laser at a fluence of 5 J/cm^2, unirradiated models (0 J/cm^2) were used as controls. Post-PBM, the Trypan blue exclusion assay and CellTiter-Glo® 3D Cell Viability Assay (measures adenosine triphosphate (ATP) luminescence) were used to determine the population of viable cells at 24 and 48 h. Morphological changes and migration rate in wounded models was also evaluated at 0 h and 24 h. PBM significantly increased cellular migration and enhanced cell viability, thus improving wound healing in diabetic conditions *in vitro* at 830 nm.

BACKGROUND

Diabetes mellitus (DM) refers to a group of chronic metabolic disorders that are characterized by increased blood sugar levels over an extended period. It is worth mentioning that this medical disorder can occur when the normal physiology of the pancreas is dysfunctional (i.e., Type I DM) or cells are unable to use the insulin produced by the body (i.e., Type II DM). The role of insulin is to regulate the body's uptake and assimilation of blood glucose [1]. Patients with DM are likely to develop major complications, such as stroke, cardiovascular diseases, retinopathy, angiopathy, neuropathy, and chronic non-healing ulcers, often on the lower limbs. The projected prevalence of DM in individuals aged 20 to 79 is anticipated to rise to 643 million by the year 2030, with a further increase to 783 million by 2045 [2]. Current treatment options have shown to be largely ineffective, highlighting the need for developing innovative strategies to enhance diabetic wounds. PBM is the non-invasive application of predominantly red and NIR light to stimulate and accelerate cellular processes to promote tissue repair and regeneration, wound healing, and reduce inflammation [3]. PBM is useful in the treatment of various disorders and chronic non-healing wounds, including diabetic wounds [1]. Therefore, this study was designed to investigate the effects of NIR PBM at 830 nm in a wounded and diabetic wounded cell model.

METHODOLOGY

Cell culture and experimental models

This study used commercially available human skin fibroblasts, WS1 (ATCC®, CRL 1502™), to create four models, namely, normal (N), normal wounded (NW), diabetic (D), and diabetic wounded (DW). A wounded cell model was created by performing a central scratch on the monolayer of WS1 cells seeded in 3.5 cm diameter tissue culture plates using a 1 mL sterile pipette [4]. The cells were incubated for 30 min post-wounding and before irradiation to allow the cells to acclimatize. A diabetic model was made by continuously culturing the cells in Minimum Essential Medium (MEM) containing an additional 17 mM D-glucose. The diabetic cells were grown in a final glucose concentration of 22.6 mM since the media already contains 5.6 mM glucose [5]. Experimental cells were irradiated with a fluence of 5 J/cm^2 at a

wavelength of 830 nm and incubated for 24 and 48 h post-PBM. Non-irradiated cells (0 J/cm^2) served as controls. Schematic representation is shown in Figure 1.

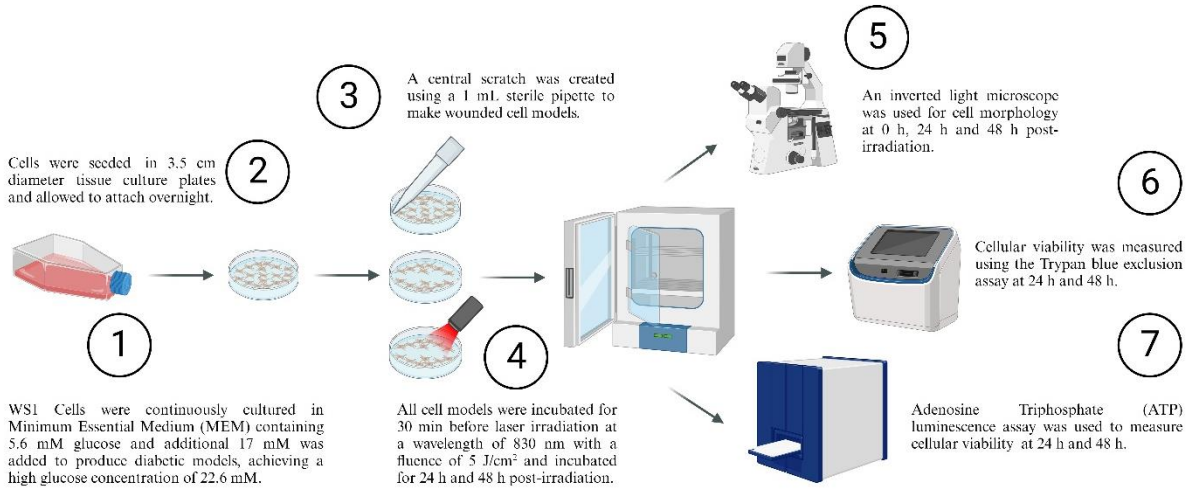

Figure 1: *General overview of the study methodology.*

Morphological analysis and cell migration

Cellular morphology and migration were determined by inverted light microscopy. Three images per cell culture plate were captured; images along the central scratch in wounded models (NW and DW) were taken in the same place. The wound distance (in µm) between the wound margins was measured using the microscope software (cellSens) at different time intervals over a period of 48 h (0, 24, and 48 h) to capture cell migration. The measured wound distances were used to calculate the migration rate using the following formula: At_{0h} - At_{time}/At_{0h} x 100, where At_{0h} is the scratch area at 0 h and At_{time} is the correspondent scratch area at different time points [6].

Cellular viability

The Trypan blue exclusion assay and CellTiter-Glo® 3D Cell Viability Assay (Promega, G9681) were used to determine cell viability. Trypan blue is a dye that is used to determine the number of viable cells, and its principle is based on dye exclusion; dead cells take up the dye due to their permeable cell membrane, while live (viable) cells do not take up the dye as their cell membranes are impermeable. Equal volumes (10 µL) of dye and cells were mixed and 10 µL loaded into a counting chamber. Viable (unstained) and non-viable (stained) cells was counted and reported in percentage using the automated cell counter (Invitrogen Countess II FL). The CellTiter-Glo® 3D Cell Viability Assay determines cell viability measuring the amount of ATP produced by metabolically acive cells. Briefly, equal amounts of Cell Tilter-Glo Reagent and cells (50 uL) were added and mixed on an orbital shaker for 2 min to induce cell lysis, followed by a 10 min incubation in the dark to stabilize the luminescent signal. Luminescence was read in Relative Light Unit (RLU) on a multilabel counter (Perkin Elmer, VICTOR3^{TM}, 1420).

Statistical analysis

All experiments were repeated three times (n=3). SigmaPlot version 14 (Systat Software, Inc.) was used for statistical analysis. Statistical differences between groups were determined by the Student t test. Results are shown as standard error of the mean (SEM), and statistical significance compared to the controls in the graphs are shown as *p<0.05, **p<0.01, and ***p<0.001.

RESULTS AND DISCUSSION

Morphological analysis and cell migration rate

Morphological changes were observed using an inverted light microscope across all cell models, namely, N, NW, D, and DW at 0 h, 24 h, and 48 h post-PBM. In both non-irradiated (0 J/cm^2) and irradiated (5 J/cm^2) cell models, high glucose concentrations had no effect on cellular shape. The cells kept their flat, elongated spindle form (Fig. 2). However, irradiated wounded cells demonstrated an accelerated cell migration from the wound margins into the wound site (central scratch) compared to the non-irradiated cells (Fig. 3), where the wounds were partially or entirely closed at 48 h. The results of the

present study are similar to those reported by Kasowanjete et al., [7] and Mokoena et al., [8]. This is in agreement with the results for migration rate, where a significant increase was observed in both NW (P=0.03) and DW (P=0.005) at 24 h (Fig. 4). This suggests that PBM at 830 nm with a fluence of 5 J/cm^2 stimulates cellular migration and promotes wound closure.

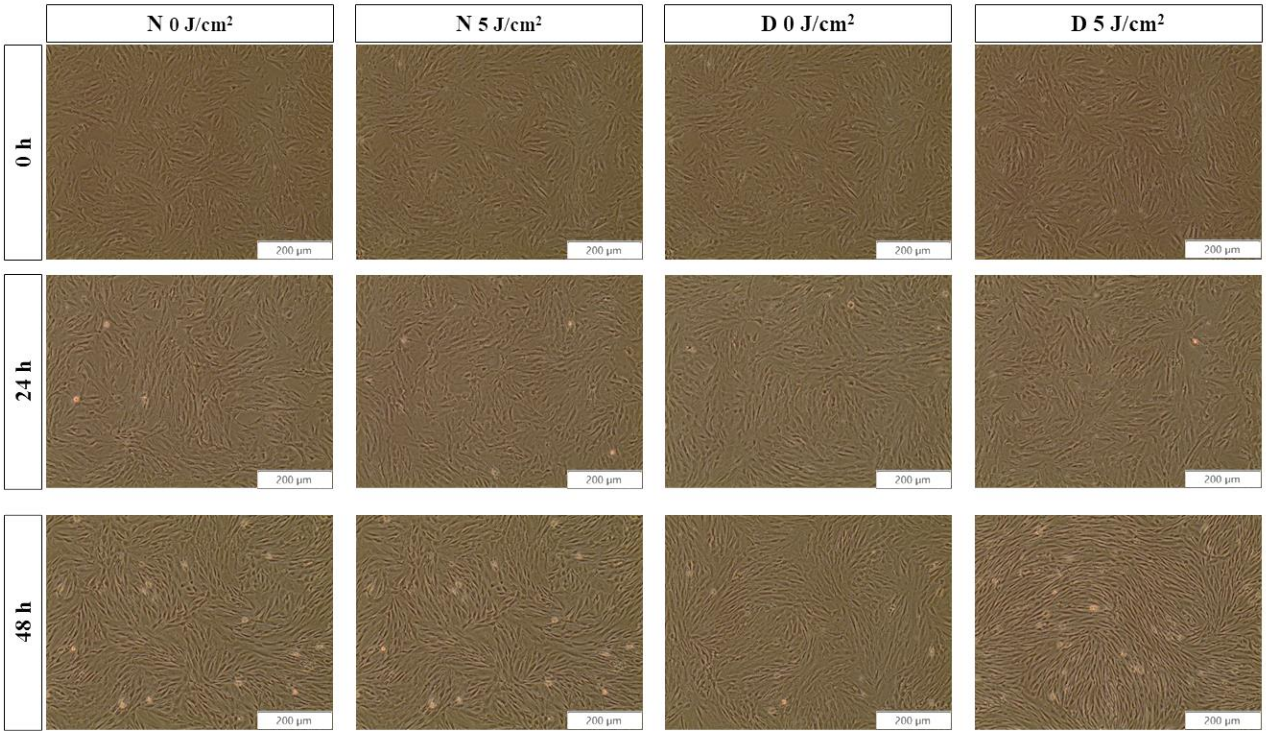

Figure 2: *Micrography showing the morphology of non-irradiated (0 J/cm^2) and irradiated (5 J/cm^2) normal (N) and diabetic (D) WS1 cell models at 0 h, 24 h and 48 h post-PBM using an 830 nm laser diode. No cellular morphological differences were observed across all models as they retained their basic morphology post-PBM.*

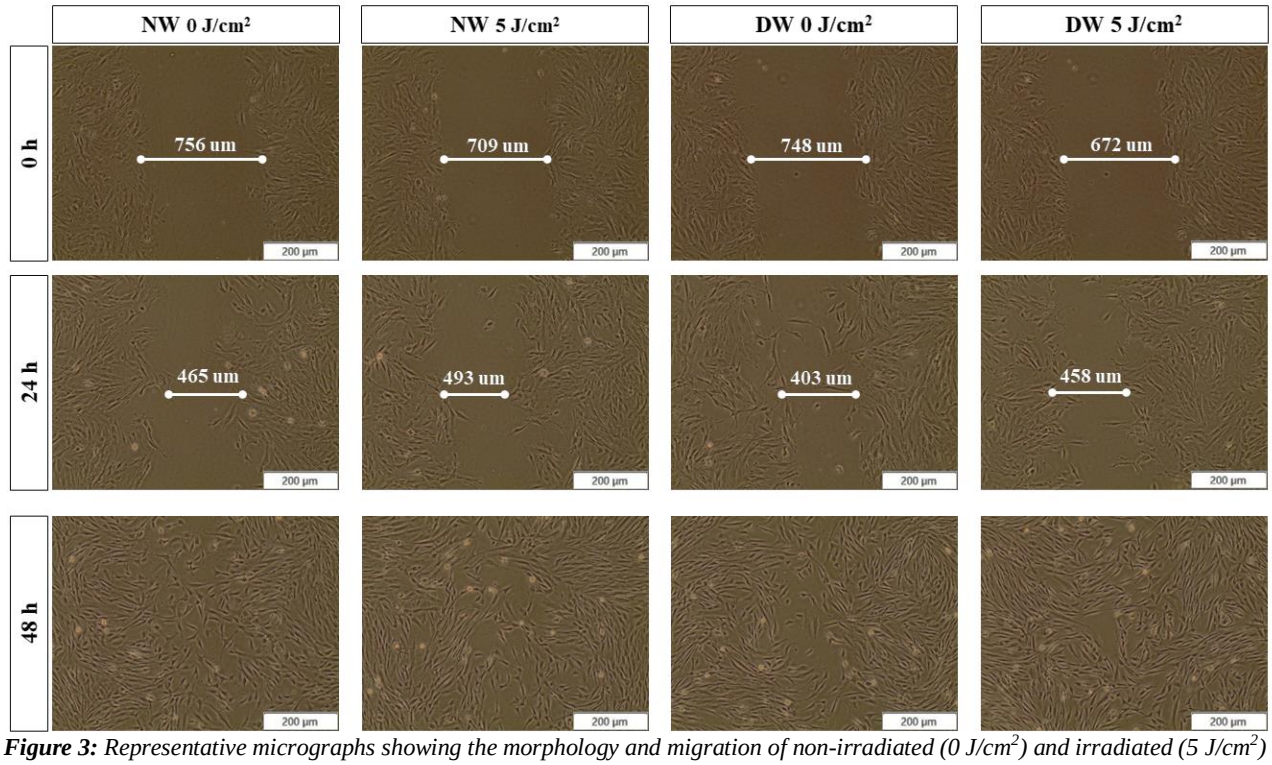

Figure 3: *Representative micrographs showing the morphology and migration of non-irradiated (0 J/cm^2) and irradiated (5 J/cm^2) normal wounded (NW) and diabetic wounded (DW) WS1 cells at 0 h, 24 h, and 48 h post-PBM using an 830 nm laser diode. Irradiated cells displayed accelerated cell migration into the central scratch to establish wound closure.*

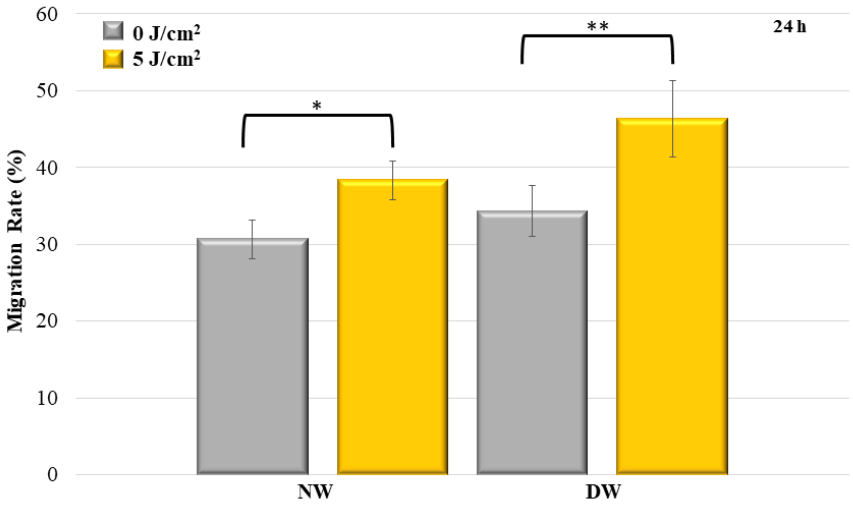

Figure 4: *Migration rate (%) in non-irradiated (0 J/cm^2) and irradiated (5 J/cm^2) normal wounded (NW) and diabetic wounded (DW) WS1 cells 24 h post-PBM using an 830 nm laser diode. Significant probability is shown as *p<0.05 and **p<0.01 (±SEM).*

Cellular viability

Cellular viability was assessed using the Trypan blue exclusion assay, which measures the number of viable and healthy cells in irradiated and non-irradiated cell models at 24 and 48 h (Fig. 5 and 6). At 24 h, the variation between the controls and PBM-treated N, NW, D, and DW cell models was not statistically significant (P=0.06, P=0.08, P=0.06, and P=0.07, respectively). However, all cell models (N, NW, D, and DW) showed a significant increase in cell viability (P=0.02, P=0.008, P=0.005, and P=0.03, respectively) at 48 h. This indicates the necessity of more extended incubation periods post-PBM to fully observe photobiomodulative effects *in vitro*. It also demonstrates that PBM at 830 nm is able to increase cellular viability under normoglycemic and hyperglycemic conditions. Irradiated cell models displayed higher (insignificant) ATP luminescence at 24 and 48 h (Fig. 7 and 8, respectively). Moreover, diabetic models have shown higher ATP production than the normal cell models at 24 and 48 h. However, there was no statistical significance observed.

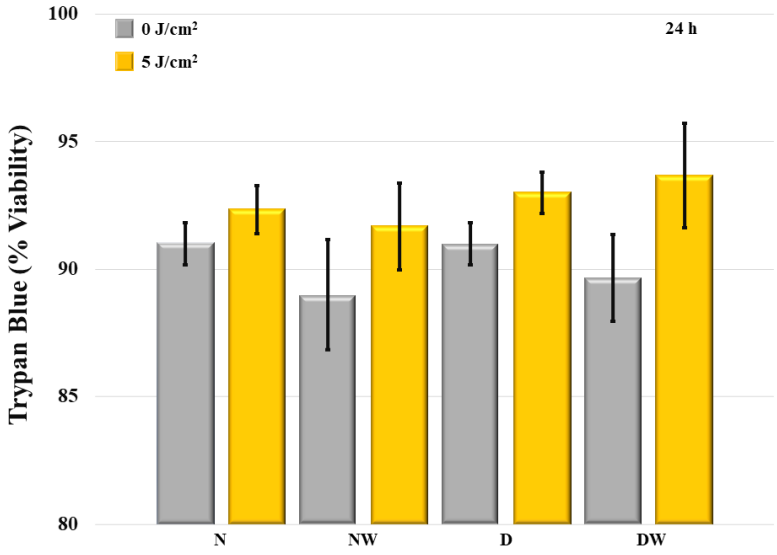

Figure 5: *Cellular viability (%) of normal (N), normal wounded (NW), diabetic (D), and diabetic wounded (DW) cell models was determined by the Trypan blue exclusion assay 24 h post-PBM at 830 nm (±SEM).*

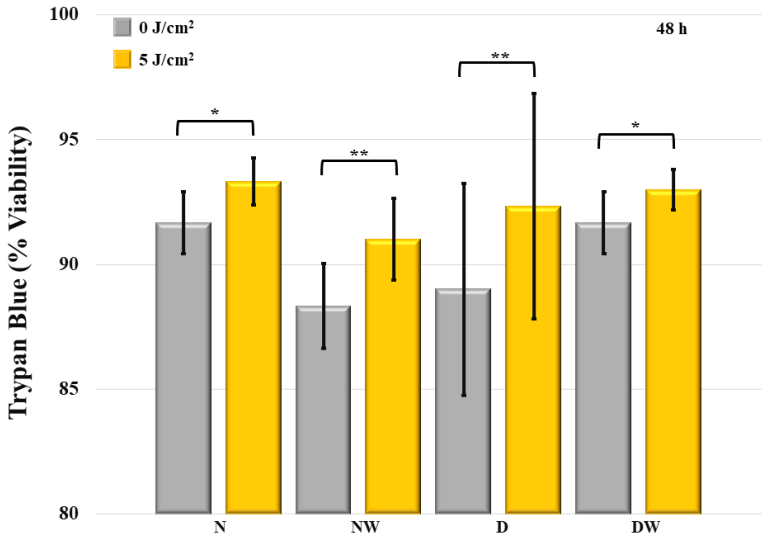

Figure 6: *Cellular viability (%) of normal (N), normal wounded (NW), diabetic (D), and diabetic wounded (DW) cell models was determined by the Trypan blue exclusion assay 48 h post-PBM at 830 nm. Significant probability is shown as *p<0.05 and **p<0.01 (±SEM).*

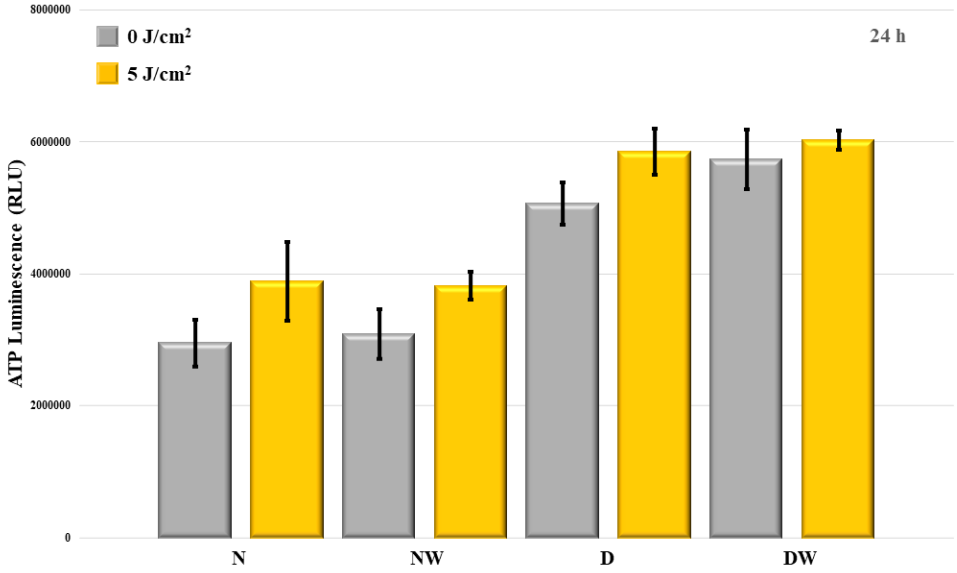

Figure 7*: Adenosine Triphosphate (ATP) luminescenc (relative light units, RLU) as a function of cellular viability was determined in normal (N), normal wounded (NW), diabetic (D), and diabetic wounded (DW) cell models 24 h post-PBM at 830 nm (±SEM).*

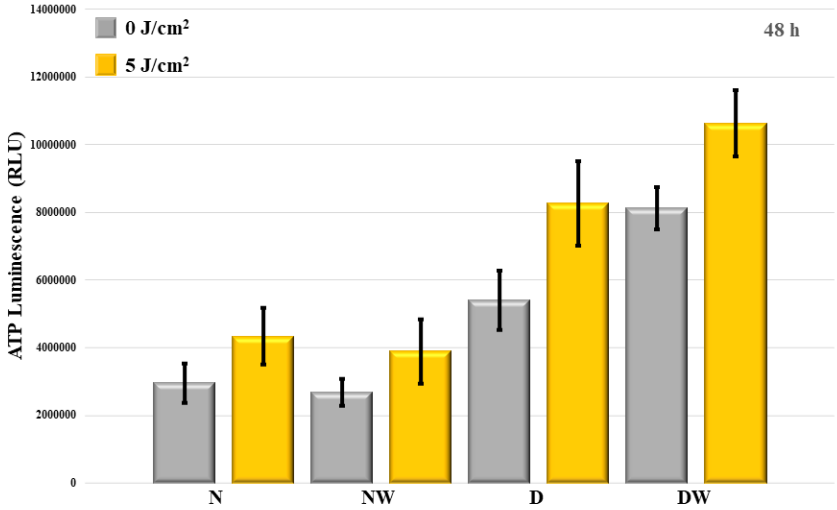

***Figure 8**: Adenosine Triphosphate (ATP) luminescenc (relative light units, RLU) as a function of cellular viability was determined in normal (N), normal wounded (NW), diabetic (D), and diabetic wounded (DW) cell models 48 h post-PBM at 830 nm (±SEM).*

CONCLUSION

In conclusion, this study revealed that high glucose concentrations did not affect cellular morphology in non-irradiated (0 J/cm^2) and irradiated (5 J/cm^2) cell models. The cells maintained their elongated flat spindle shape. The study also revealed that PBM at 830 nm with a fluence of 5 J/cm^2 enhances cellular migration rate, increases cellular viability, and promotes wound closure, even under hyperglycemic conditions. PBM at 830 nm could be used to stimulate the healing of slow, non-healing wounds.

ACKNOWLEDGMENTS AND FUNDING SOURCES

This work was funded by the South African Research Chairs Initiative of the Department of Science and Technology (DST) and National Research Foundation (NRF) of South Africa (Grant No 98337), the University of Johannesburg (URC), the NRF Competitive Programme for Rated Researchers (Grant No 1293270) and Council for Scientific and Industrial Research (CSIR) - National Laser Centre (NLC), Laser Rental Pool Programme. All lasers were supplied and set up by the NLC.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

[1] S. Ponnusamy, R. Mosca, K. Desai, P. Arany, Photobiomodulation therapy in diabetic wound healing. In: Wound Healing, Tissue Repair, and Regeneration in Diabetes, D. Bagchi, A. Das, S. Roy, Eds.; Academic Press: San Diego, CA, USA, p. 305–321, 2020.
[2] International Diabetes Federation, IDF Diabetes Atlas. 10th edition. Brussels, 2021.
[3] M. R. Hamblin, Photobiomodulation or low-level laser therapy. J Biophotonics. 1122–4, 2016.
[4] N. N. Houreld, H. Abrahamse, Low-Intensity laser irradiation stimulates wound healing in diabetic wounded fibroblast cells (WS1). Diabetes Technology & Therapeutics. 971-978, 2010.
[5] N. N. Houreld, H. Abrahamse, In vitro exposure of wounded diabetic fibroblast cells to a helium-neon laser at 5 and 16 J/cm^2. Photomedicine and Laser Surgery. 78-84, 2007.
[6] F. Felice, et al., Effect of different chitosan derivatives on in vitro scratch wound assay: a comparative study. Int J Biol Macromol. 236-241, 2015.
[7] P. Kasowanjete, H. Abrahamse, N. N. Houreld, Photobiomodulation at 660 nm stimulates in vitro diabetic wound healing via the Ras/MAPK pathway. Cells. 1080, 2023.
[8] D. R. Mokoena, N. N. Houreld, S. S. Dhilip Kumar, H. Abrahamse, Photobiomodulation at 660 nm stimulates fibroblast differentiation. Lasers Surg. Med. 671-681, 2020.