PHOTOBIOMODULATION PROMOTES WOUND HEALING IN A DIABETIC CELLULAR MODEL

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ABSTRACT

Wound healing is a critical process that activates tissue repair after injury. In diabetes mellitus however, this process is perturbed and leads to the development of non-healing diabetic ulcers. Current treatments which include glycemic control wound debridement are moderately effective as there is a challenge of recurrence. Therefore, improved therapeutics strategies are required for the treatment of diabetic ulcers. Photobiomodulation (PBM) at the wavelength range of 520 and 830 nm has been shown to improve the healing of cutaneous wounds by promoting cell proliferation and wound closure. The aim of this study was to determine if PBM at the wavelength of 660 nm and a fluence of 5 J/cm^2 can induce wound healing in a diabetic (D) cellular model. Human dermal fibroblasts were continuously cultured in high-glucose environment to induce the diabetic state. Prior to irradiation, a central scratch was created on the cell culture plates to create the "wound". Cell migration and cell viability analyses were performed 24 and 48 h post-irradiation. The results showed that cell migration was increased in irradiated diabetic wounded (DW) cells compared to the non-irradiated cells. The trypan blue and MTT data showed no statistical difference in cell viability between irradiated and non-irradiated cells at 24 and 48 h post-irradiation. The preliminary findings of this study indicate that PBM does not cause significant cell death and promotes "wound" closure in diabetic wounded cells.

INTRODUCTION

Wound healing is a tightly regulated process that promotes tissue repair following injury. However, it is deregulated in disease conditions such as diabetes mellitus, leading to poor wound healing and the development of chronic wounds. Diabetes mellitus is a metabolic disorder that is caused by high blood glucose due to insulin deficiency or insulin resistance. The hyperglycemic state leads to poor wound healing and development of non-healing diabetic ulcers, which increase the risk of lower limb amputations in diabetic patients [1]. Current wound treatments include glycemic control, wound dressing, debridement, and pain management. However, there is the reoccurrence of diabetic ulcers therefore, improved treatment strategies are required. Photobiomodulation (PBM) has been shown to improve the healing of diabetic wounds at wavelengths between 520 nm and 830 nm [2], but the mechanism(s) of action have not been fully described. Studies have indicated that PBM activates the TGF-β signaling pathways [3], but it has not been determined if it activates other pathways such as Wnt/β-catenin for wound healing. The aim of this study was to investigate if PBM induces healing in diabetic wounded cells at a wavelength of 660 nm and fluence of 5 J/cm^2.

METHOD

Human dermal fibroblasts (WS1) were continuously cultured in high glucose medium (22.6 mM D- glucose) to create an *in vitro* diabetic cellular model. Diabetic cells (D) were seeded at a density of 6 x 10^5 in 35 mm diameter culture plates 24 h prior to irradiation. To create a "wound', a central scratch was created in the middle of the cell culture plate using a 1 mL pipette tip. The cells were subjected to laser irradiation at a wavelength of 660 nm and a fluence of 5 J/cm^2 (100 mW/cm^2; 445.05 s) using the formula shown below.

$$mW/cm^2 = \frac{mW/cm^2}{\pi\,(r^2)}$$

$$W/cm^2 = \frac{mW}{1000}$$

$$Time\ (s) = \frac{5\ J.cm^2}{W/cm^2}$$

The sample groups were analyzed 24 and 48 h post-irradiation. Cell migration was analysed through inverted microscopy in diabetic wounded (DW) cells. Cell viability was measured using the trypan blue exclusion assay where the number of colorless (viable) and blue (non-viable) stained cells are counted, and the percentage viability calculated. Cell viability was also analyzed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay where live, metabolically active cells reduce the water-soluble yellow tetrazolium salt to water-insoluble purple formazan crystals of which the absorbance was detected spectrophotometrically at a wavelength of 570 nm. Differences between groups were

determined using the Student's t-test for each independent variable and were considered statistically significant when P< 0.05.

RESULTS

Cell migration was analyzed (Fig. 1) at 0 h, 24 h and 48 h post-irradiation (0 J/cm^2; 5 J/cm^2) and showed an increase in cell migration in irradiated cells compared to the non-irradiated cells. The cell viability data showed that PBM does not cause cytotoxicity in irradiated cells at 24 (Fig. 2a) and 48 h (Fig. 2b) post-irradiation, which showed similar viability of 70%±7 in irradiated sample groups when compared with the non-irradiated group. Similarly, the MTT data showed no statistical difference in cell viability between the irradiated and non-irradiated sample groups at 24 h (Fig. 3a) and 48 h (Fig. 3b) post-irradiation.

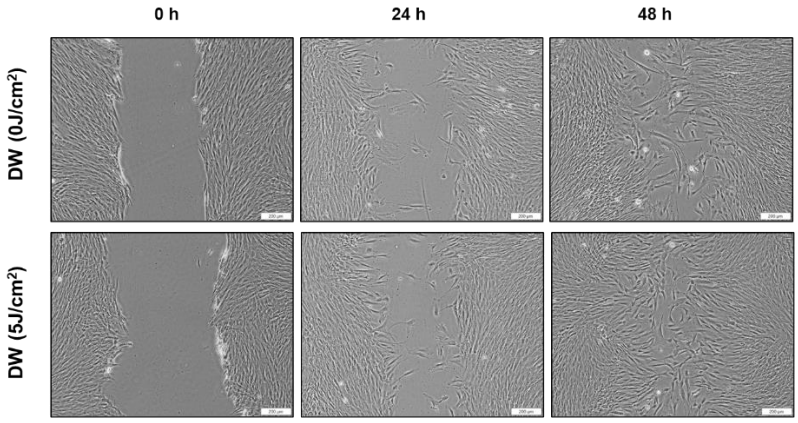
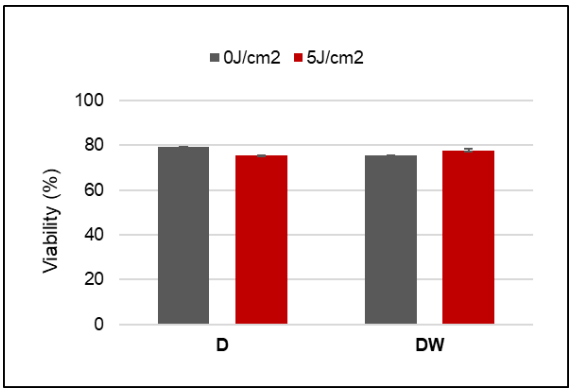
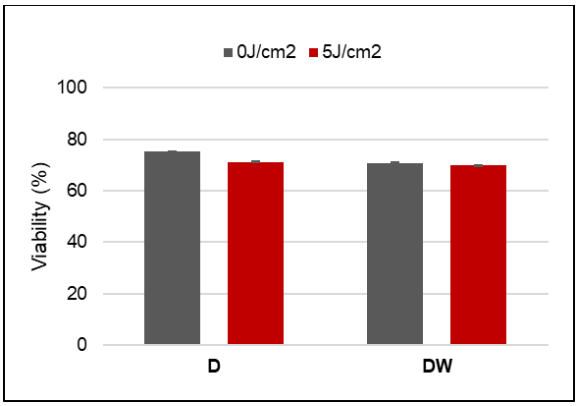


Figure 1: *Inverted light microscopy was used to assess cell migration in diabetic wounded cells (DW) at 0, 24 and 48 h after irradiation at a wavelength of 660 nm and a fluence of 0 J/cm2 or 5 J/cm2. Two biological repeats were performed.*

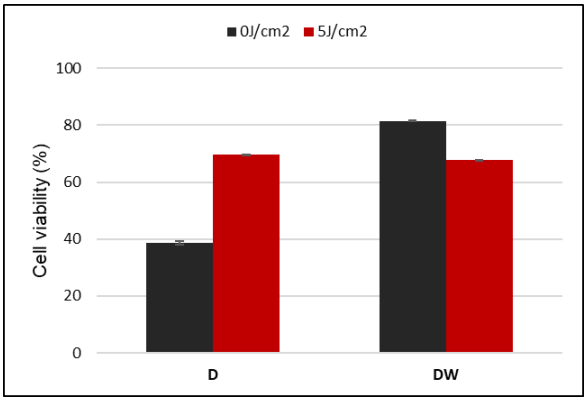


(a)

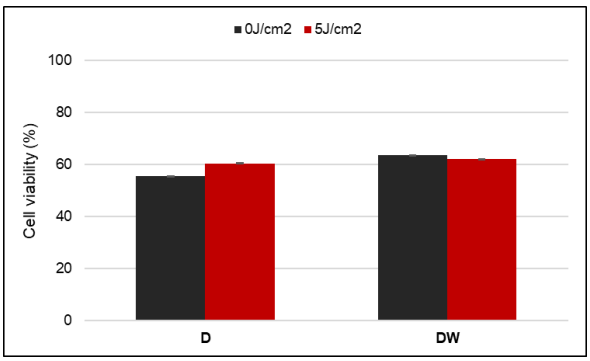


(b)

Figure 2: Trypan blue exclusion assay*. Cell viability (%) in irradiated diabetic (D) and wounded diabetic (DW) cells was determined by the trypan blue exclusion assay at 24 h (a) and 48 h (b) post-irradiation. Data is presented as means ± SD (n = 2). *P<0.05*



(a)



(b)

Figure 3: MTT assay. *Cell viability analysis of diabetic (D) and diabetic wounded cells (DW) by MTT assay at 24 (a) and 48 h (b) after irradiation at a wavelength of 660 nm and a fluence of 0 J/cm^2 or 5 J/cm^2. Data is presented as ±SD (n=2). *P<0.05*

CONCLUSION

The preliminary data show that PBM induces increased "wound" closure in irradiated DW cells compared to non-irradiated cells. The cell viability data further showed that irradiation at 660 nm and a fluence of 5 J/cm^2 does not induce significant cell death.

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