A comparison between photobiomodulation at 830 nm and 660 nm on differentiation in diabetic human skin fibroblast cells

Olajumoke Oyebode*and Nicolette Nadene Houreld
daramzjay09@gmail.com
Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, P.O. Box 17011, Doornfontein, South Africa, 2028

ABSTRACT

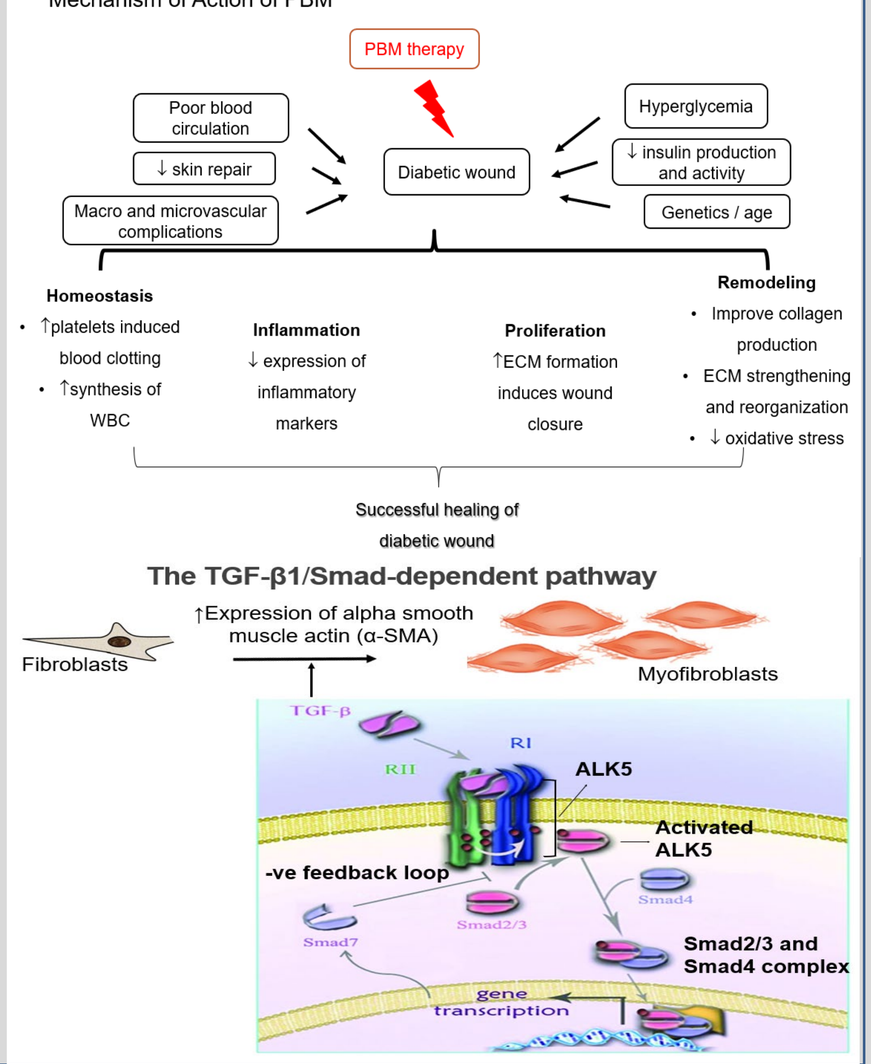
Different studies have proposed the efficacy of photobiomodulation (PBM) at different wavelengths (830 nm and 660 nm) to stimulate wound repair in diabetic cells. The TGF-β1/Smad cascade has proven to be an effective signalling pathway in differentiating fibroblasts into myofibroblasts. This study aims to compare the effects of both wavelengths on cellular viability and expression of fibroblast differentiation markers in WS1 fibroblast cells. The cells were modelled into groups; normal (N), normal wounded (NW) and diabetic wounded (DW). At 830 nm and 660 nm, cells were irradiated with 5 J/cm^2, while control cells were without irradiation (0 J/cm^2). At 24 and 48 h post-irradiation cell viability was investigated using the Trypan blue exclusion assay, while transforming growth factor-beta (TGF-β1) and p-Smad2/3 was ascertained using ELISA. Immunofluorescence was used to observe the presence of alpha smooth muscle actin (α-SMA). There was a significant increase in cell viability in the irradiated models using both wavelengths. A wavelength of 830 nm elicited a slight increase in the expression of TGF-β1 compared to 660 nm in diabetic wounded cells, while both wavelengths had no effect on the presence of p-Smad2/3. Both wavelengths were successful in initiating the differentiation of fibroblasts into myofibroblasts in diabetic wounded cells with no difference between wavelengths.

DIABETES MELLITUS

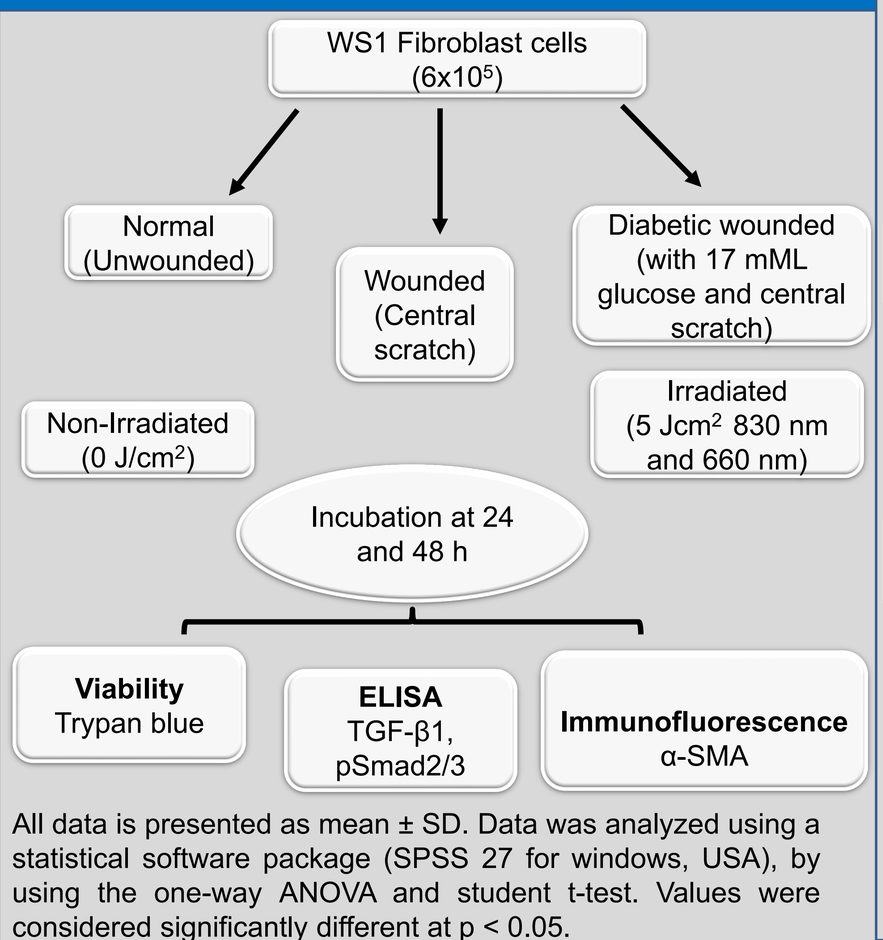
The Problem

- *Globally, it is estimated that by 2045, 700 million people will have diabetes*
- *Over 1 billion dollars spent on treatment annually*
- *Over 1.6 million new cases per year*
- *7th Leading cause of death*
- *Over 71,000 lower limb amputations so far*

Mechanism of Action of PBM



METHODS



All data is presented as mean ± SD. Data was analyzed using a statistical software package (SPSS 27 for windows, USA), by using the one-way ANOVA and student t-test. Values were considered significantly different at $p < 0.05$.

RESULTS

Table 1. Laser parameters

Variables		
Wavelength (nm)	830 nm	660 nm
Light source	Diode laser	Diode laser
Wave emission	Continuous wave	Continuous wave
Spot Size (cm^2)	9.1	9.1
Power Output (mW)	105	100
Power density (mW/cm^2)	11.56	11
Irradiation time	7 min 10 s	7 min 34 s
Energy density (J/cm^2)	5	5
Energy (J)	45.2	45.4

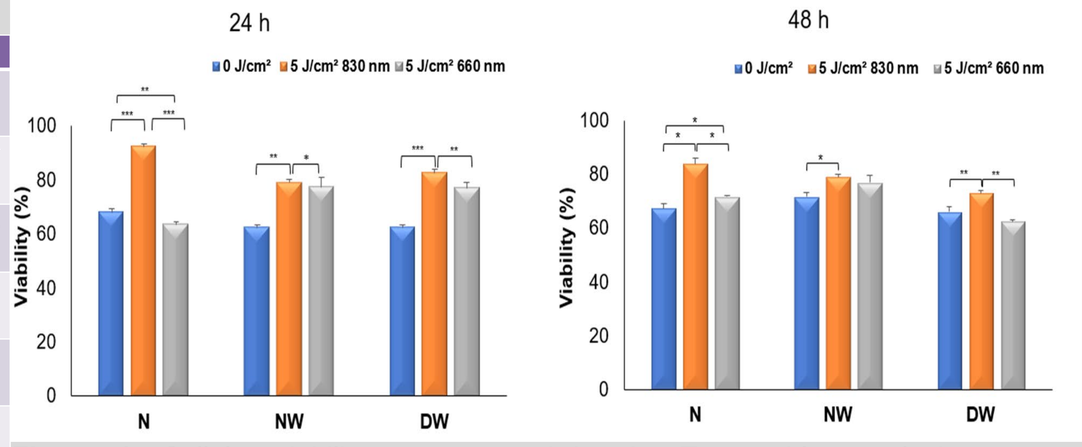


Figure 1: Cellular viability as assessed by the Trypan blue exclusion assay (measured in %). Viability was determined in non-irradiated (0 J/cm^2) and irradiated (5 J/cm^2 at 830 nm and 660 nm) normal (N), normal wounded (NW), and diabetic wounded (DW) cells, and analysed 24 and 48 h post-irradiation. Statistical significance is shown as *P<0.05, **P<0.01 and ***P<0.001 (±SEM). The bars show statistical significance between; control (0 J/cm^2) & irradiation at 5 J/cm^2 830 nm; control (0 J/cm^2) & irradiation at 5 J/cm^2 660 nm; and irradiation at 5 J/cm^2 830 nm & 5 J/cm^2 660 nm.

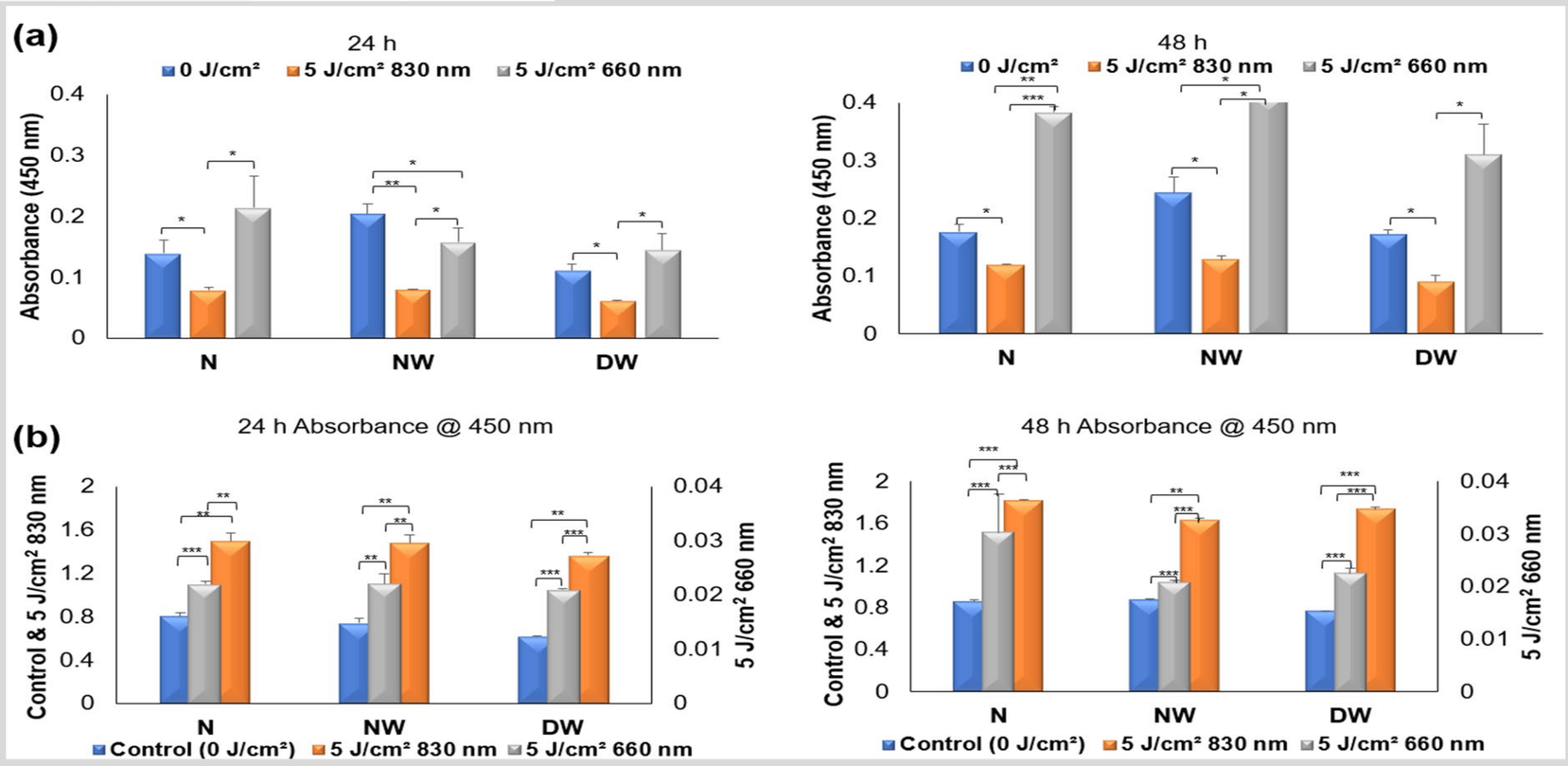


Figure 2: The presence of (a) p-Smad2/3 and (b) TGF-β1 was measured using ELISA at 24 and 48 h in Normal (N), Normal wounded (NW) and Diabetic wounded (DW) WS1 fibroblast cells irradiated with an 830 nm and 660 nm laser at 5 J/cm^2 while non-irradiated (0 J/cm^2) cells served as controls. Statistical significance is shown as *P<0.05, **P<0.01 and ***P<0.001 (±SEM). The bars show statistical significance between; control (0 J/cm^2) & irradiation at 5 J/cm^2 830 nm; control (0 J/cm^2) & irradiation at 5 J/cm^2 660 nm; and irradiation at 5 J/cm^2 830 nm & 5 J/cm^2 660 nm.

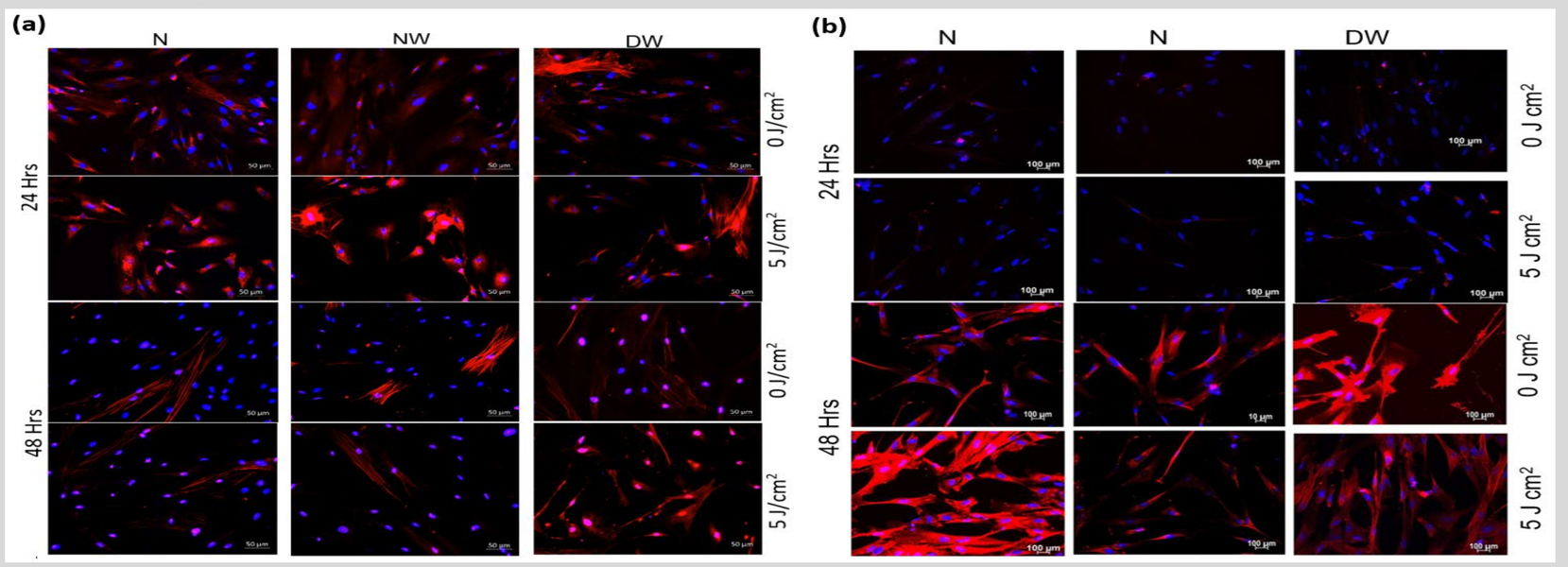


Figure 3: **(a)** Irradiation at 830 nm and **(b)** 660 nm wavelengths. Normal (N), Normal wounded (NW) and Diabetic wounded (DW) cells irradiated at a fluence of 5 J/cm^2 and control cells (0 J/cm^2). Cells were incubated for 24 and 48 h and stained for α-SMA with NL557-conjugated anti-Mouse antibody (red). Counterstaining of nuclei was done with DAPI (blue).

CONCLUSION

This study aimed to compare the effect of PBM at 830 nm and 660 nm on differentiation of normal, wounded and diabetic wounded fibroblasts into myofibroblasts 24 and 48 h after irradiation. The results discussed showed that PBM at both wavelengths influenced the irradiated cells in comparison with the non-irradiated cells. Although irradiation at 830 nm increased the expression of TGF-β1 in our study, we cannot conclude that it is the better wavelength for irradiation. Both wavelengths are equally successful in *in vitro* wound healing assays. More experiments (probably *in vivo* experiments) need to be done before we can justify the bases for a better wavelength.

ACKNOWLEDGEMENTS

Laser Research Centre
Laser Applications in Health
National Research Foundation
SARChI
science & technology
Department: Science and Technology
REPUBLIC OF SOUTH AFRICA
UNIVERSITY OF JOHANNESBURG
NRF National Research Foundation
NLC NATIONAL LASER CENTRE