U21 Health Sciences Group

Annual Meeting 2021

TecSalud Escuela de Medicina y Ciencias de la Salud

U21 Health Sciences

Laser irradiation at 660 nm affects cyclooxygenase 2, interleukin-6 and tumour necrosis factor-α levels in diabetic wounded fibroblast models

Asma Shaikh-Kader[1*], Nicolette N. Houreld,[1] and Heidi Abrahamse[1]

[1]Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, Johannesburg, 2028, South Africa.

Abstract

Impaired healing of diabetic wounds occurs due to numerous factors including sustained inflammation that occurs during the wound repair process. Increased levels of the inflammatory mediators' cyclooxygenase-2 (cox-2), interleukin–6 (IL-6) and tumour necrosis factor alpha (TNF-α) have been ascribed to diabetic non-healing wounds. Photobiomodulation (PBM) has many biological effects including the stimulation of processes important to wound repair. The effect of PBM at 660 nm on the levels of cox-2, IL-6 and TNF-α were determined in normal; normal wounded; diabetic and diabetic wounded fibroblast models. Cells in the experimental group were irradiated at 660 nm (5 J/cm^2) while control cells did not receive irradiation. Cell morphology (light microscopy), cell viability (Trypan Blue exclusion assay) and cox-2, IL-6 and TNF-α levels (ELISA) were determined at 0, 24 and 48 h after irradiation. Cell migration (over the 48 h period) and cell viability improved in the diabetic wounded groups at 0 and 24 h ($P \leq 0.05$ and $P \leq 0.01$, respectively); levels of cox-2 increased in the diabetic and diabetic wounded groups at 48 h ($P \leq 0.05$ and $P \leq 0.01$, respectively), and IL-6 levels decreased in the diabetic and diabetic wounded groups at 24 h ($P \leq 0.05$ and $P \leq 0.001$, respectively) and 48 h ($P \leq 0.05$) post-irradiation. The levels of TNF-*α* decreased at 48 h post-irradiation in both diabetic and diabetic wounded groups; however no statistically significant differences were found. PBM at 660 nm may therefore decrease inflammation by reducing the levels of IL-6 produced by diabetic cells.

Introduction

The inflammatory phase of wound repair is important to the wound healing process as it isolates the injured region from healthy tissue and thus prevents infection. The inflammatory phase must conclude before re-epithelialization, wound contraction and the deposition of extracellular matrix components can occur.[1,2] However, in patients with diabetes mellitus, an unresolved inflammatory phase is a contributing factor to wounds that do not heal effectively.[2] Macrophages enter the wounded area and release a variety of cytokines including interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α).[3] Cyclooxygenase-2 (cox-2) expression occurs and cox-2 is one of the enzymes responsible for metabolizing arachidonic acid to products that contribute to inflammation by increasing vascular permeability and by stimulating inflammatory cells.[4,5] In diabetes mellitus, the overexpression of cox-2 and high levels of IL-6 and TNF-α, lead to chronic inflammation, and ultimately wounds that display impaired wound healing.[6-8] Photobiomodulation (PBM) stimulates cellular signalling in diabetic wounds and affects inflammatory markers, cytokine production and oxidative stress.[9-12] In this study, the levels of cox-2, IL-6 and TNF-α were determined in diabetic wounded fibroblast cell culture models after irradiation at 660 nm and a fluence of 5 J/cm^2 as previous studies using the same laser parameters were successful at stimulating wounded diabetic cells.[13,14]

Methodology

The study was approved by the Research Ethics Committee, Faculty of Health Sciences, University of Johannesburg (REC-01-98-2017). Commercially obtained human skin fibroblasts (WS1; ATCC® CRL-1502™) were used in the study. Cells were divided into four models: normal (N), normal wounded (NW), diabetic (D) and diabetic wounded (DW). To achieve the diabetic model, 17 mmol/L D-glucose was added to the culture media. A diode laser emitting at a wavelength of 660 nm was used in the study. Control cells were not irradiated (0 J/cm^2); while experimental cells were irradiated (5 J/cm^2). Cell morphology was observed using the Olympus light microscope and cell viability was determined using the Trypan Blue exclusion assay. Cell culture media was collected at 0, 24 and 48 h post-irradiation and was used to determine the levels of cox-2, IL-6 and TNF-α using commercially available ELISA kits. Data were analysed using IBM SPSS Statistics v26 (n = 3; paired *t*-test; two-way ANOVA; $P \leq 0.05$ was considered significant).

Results

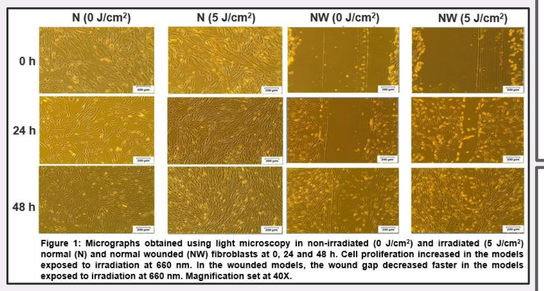

Figure 1: Micrographs obtained using light microscopy in non-irradiated (0 J/cm^2) and irradiated (5 J/cm^2) normal (N) and normal wounded (NW) fibroblasts at 0, 24 and 48 h. Cell proliferation increased in the models exposed to irradiation at 660 nm. In the wounded models, the wound gap decreased faster in the models exposed to irradiation at 660 nm. Magnification set at 40X.

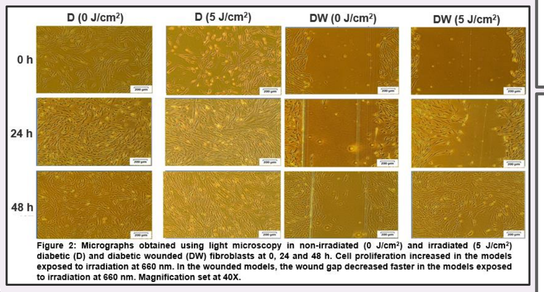

Figure 2: Micrographs obtained using light microscopy in non-irradiated (0 J/cm^2) and irradiated (5 J/cm^2) diabetic (D) and diabetic wounded (DW) fibroblasts at 0, 24 and 48 h. Cell proliferation increased in the models exposed to irradiation at 660 nm. In the wounded models, the wound gap decreased faster in the models exposed to irradiation at 660 nm. Magnification set at 40X.

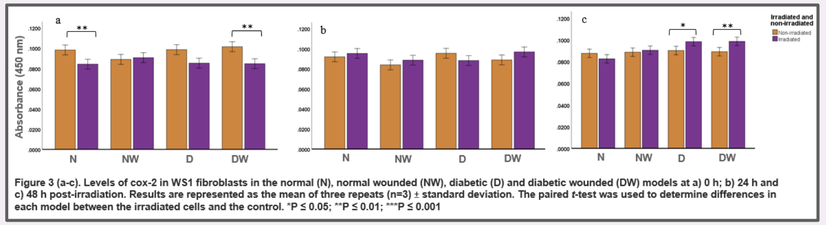

Figure 3 (a-c). Levels of cox-2 in WS1 fibroblasts in the normal (N), normal wounded (NW), diabetic (D) and diabetic wounded (DW) models at a) 0 h; b) 24 h and c) 48 h post-irradiation. Results are represented as the mean of three repeats (n=3) ± standard deviation. The paired *t*-test was used to determine differences in each model between the irradiated cells and the control. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

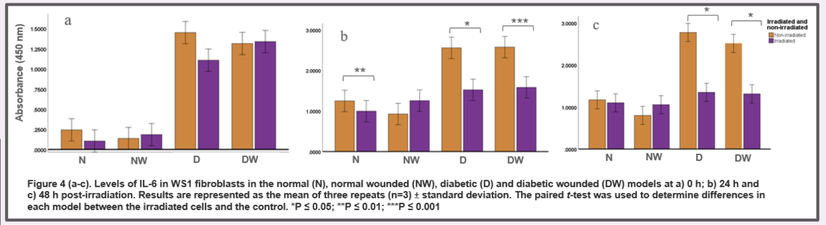

Figure 4 (a-c). Levels of IL-6 in WS1 fibroblasts in the normal (N), normal wounded (NW), diabetic (D) and diabetic wounded (DW) models at a) 0 h; b) 24 h and c) 48 h post-irradiation. Results are represented as the mean of three repeats (n=3) ± standard deviation. The paired *t*-test was used to determine differences in each model between the irradiated cells and the control. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

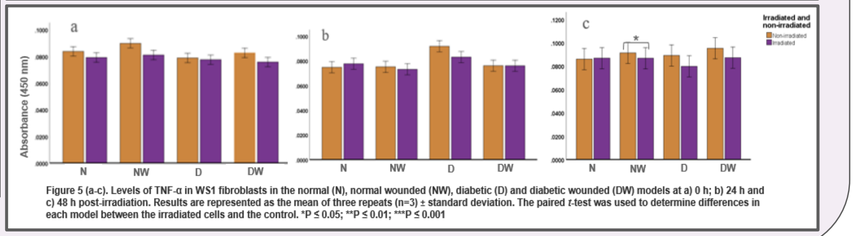

Figure 5 (a-c). Levels of TNF-α in WS1 fibroblasts in the normal (N), normal wounded (NW), diabetic (D) and diabetic wounded (DW) models at a) 0 h; b) 24 h and c) 48 h post-irradiation. Results are represented as the mean of three repeats (n=3) ± standard deviation. The paired *t*-test was used to determine differences in each model between the irradiated cells and the control. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

Conclusions

PBM at 660 nm and 5 J/cm^2 is successful at decreasing the gap of the wound and increasing cell viability in the diabetic wounded models. PBM at 660 nm and 5 J/cm^2 decreases the levels of the pro-inflammatory cytokine IL-6 in diabetic wounded WS1 fibroblasts, and this decrease is most apparent at 48 h post-irradiation. Immediately post-irradiation there was a decrease in cox-2 levels, however the levels of cox-2 increased at 48 h post-irradiation, but more research is required to determine if and why this increase may occur. TNF-α levels decreased at 48 h in the irradiated diabetic and diabetic wounded groups, but the decrease was not statistically significant.

References

Biography

Asma Shaikh-Kader graduated with a Bachelor of Science (BSc) at the University of the Witwatersrand and thereafter joined the School of Physiology in 2008 to complete a BSc with Honours. In 2011, she graduated with a Master of Science in Medicine at Wits University. Asma is currently a full-time lecturer in the department of Human Anatomy and Physiology at the University of Johannesburg, South Africa. She is also a part-time doctoral student in the Laser Research Centre at the University of Johannesburg and her research focuses on the effect of photobiomodulation on wound healing in diabetes mellitus.

Email: akader@uj.ac.za