Influence of 660 and 830 Nm Laser Irradiation on Genetic Profile of Extracellular Matrix Proteins in Diabetic Wounded Human Skin Fibroblast Cells

N.N. HOURELD*

Laser Research Centre, University of Johannesburg, Doornfontein, Gauteng 2028, South Africa

nhoureld@uj.ac.za

http://www.uj.ac.za/

*Corresponding author

Keywords: Diabetes, Extracellular Matrix, Lasers, Photobiomodulation.

Abstract. The extracellular matrix (ECM) provides tissue structural integrity and its synthesis plays a crucial role in wound healing. Impaired wound healing ensues following the destruction of the ECM, or the inhibition of its synthesis. An imbalance in ECM synthesis and degradation is seen in patients with diabetes. This has led to the development of novel therapies that aim to decrease ECM destruction and increase its synthesis. Photobiomodulation (PBM) has been shown to speed up the healing of these slow-to-heal wounds, and numerous studies are being conducted to determine the underlying molecular cause. This study aimed to ascertain the effect of laser irradiation at a wavelength of 660 or 830 nm at a dose of 5 J/cm^2 on the genetic expression profile of genes involved in ECM proteins. cDNA was reverse transcribed from total isolated RNA and used in real-time qualitative polymerase chain reaction (qPCR). Genes concerned with the basement membrane, collagen and ECM structural constituents, and ECM protesases and inhibitors were evaluated. Results showed that a similar genetic profile (although not identical) was seen post irradiation. PBM was able to reduce the expression of ECM proteases and increase mRNA levels of ECM proteins. This study fortifies the notion that PBM stimulates cellular activity and can influence ECM matrix synthesis and degradation during wound healing. This study has also shown that PBM is able to stimulate cells at a genetic level.

Introduction

The process of wound healing is a highly complex, well-orchestrated process which involves a variety of cells, cytokines and growth factors all aimed at reversing the loss of tissue structural integrity, usually by replacement with scar-forming connective tissue. Wound healing proceeds through 4 phases, namely homeostasis, inflammation, proliferation and remodeling. Extracellular matrix (ECM) synthesis plays a crucial role in all phases of wound healing, and provides structural integrity. Impaired wound healing ensues following the destruction of the ECM, or the inhibition of its synthesis. Diabetes Mellitus (DM) has been declared as a global burden, with 415 million cases (adults aged 20-79) worldwide in 2015, and a further estimated 193 million undiagnosed cases. The estimated number of people with DM on the African continent in 2015 was at 14.2 million, and is thought to increase to 140.2 million by 2040 [1]. It was also estimated that at the end of 2015 there would be 5 million deaths worldwide related to DM at a cost of between USD673 billion and USD1,197 billion in healthcare. To put this into perspective, there was only 1.5 million deaths related to

penetrating deeper into tissue, as well as the desired effects and target chromophores. The visible red (e.g., 660 nm) and infrared portions of the electromagnetic spectrum (e.g., 800 to 900 nm) have been shown to be highly absorbent in living tissues [21] and seem to provide the best results [22].

Diabetic wounded cells irradiated with a wavelength of 660 nm exhibited a total of 22 genes which were significantly down-regulated, while 10 were significantly up-regulated. The same cells irradiated with 830 nm exhibited a total of 22 significantly down-regulated genes, and only 4 significantly up-regulated genes. The functional groupings (basement membranes, collagens and ECM structural constituents, ECM proteases, ECM protease inhibitors and other ECM molecules) can be seen in Table 1. With the exception of a few genes, diabetic wounded WS1 cells irradiated at either 660 or 830 nm showed almost an identical gene profile, with a number of the same genes being significantly up- or down-regulated. The following genes were significantly down-regulated with both wavelengths: LAMA1, LAMB3, SPARC, COL5A1, COL6A1, COL7A1, COL12A1, COL16A1, FN1, KAL1, ADAMTS1, MMP1, MMP2, MMP8, MMP14, MMP16, SPG7, THBS1, and SPP1. The following genes were significantly up-regulated at both wavelengths: COL11A1, COL14A1 and ADAMTS8.

The ECM is a highly dynamic structure which provides extracellular structural support to cells, and is constantly being remodeled. The ECM is comprised of two classes of macromolecules, namely proteoglycans (PGs) which form a hydrated gel, and fibrous proteins which are collagen, elastin, fibronectin and laminin [23]. Collagen constitutes the most important fibrous protein in the ECM and are responsible for tensile strength, control cell adhesion, support chemotaxis and migration, and direct tissue development. Elastin interacts with collagen, and offers tissue elasticity. Fibronectin (FN) directs the organization of the ECM and plays a crucial role in mediating cell attachment and function [23]. Laminin forms part of the basement membrane and is involved in cellular attachment, shape and movement. The synthesis and degradation of the ECM are both important during wound healing. ECM degradation is necessary for cellular migration and is carried out by MMPs and other proteases. In recent years, new studies have also shown that MMPs play a role in regulating extracellular tissue signaling networks [24]. Due to their destructive nature, the activity of these ECM proteases have to be controlled. MMPs are controlled by TIMPs.

The failure of diabetic ulcers to heal has been linked to decreased ECM synthesis, and or increased ECM degradation [3]. In a study conducted by Liu and colleagues, it was found that high wound fluid concentrations of MMP-9 and high MMP-9–to–TIMP-1 ratios was a predictor of poor wound healing in diabetic foot ulcers [25]. In addition to increased MMP-9, other studies have also found increased levels of MMP-2 and -8 [4-6]. PBM has been shown to stimulate cells and wound healing, and has aided in the healing of chronic diabetic ulcers [8-13]. Aparecida Da Silva and co-workers irradiated diabetic induced male Wistar rats (660 nm, 4 J/cm2) and evaluated MMP-2 and -9, and Type I and III collagen 24 h post-irradiation [25]. The results showed that PBM significantly lowered MMP-2 and -9 expression as well as accelerate the production of collagen and increase the total percentage of collagen type III in diabetic animals [25]. Results from this study showed a significant reduction in mRNA in ECM proteases a disintegrin and metalloproteinase with thrombospondin motifs I (ADAMTS-1), MMP-1, -2, -8, -12, -14, and -16, and a significant increase in TIMP-1 in diabetic wounded cells irradiated with 660 nm. There was also a significant up-regulation in COL11A1, COL14A1 (coding for

collagen type XI and XIV respectively) and LAMA3 (Laminin, Alpha 3). Interestingly, there was also a significant up-regulation in ADAMTS8, MMP3, MMP7, MMP9, MMP11 and MMP13. However, despite this upregulation, it should be remembered that proteases are needed for ECM remodeling, and qPCR was done 48 h post-irradiation, also TIMP1 expression was up-regulated. Irradiation at 830 nm showed a significant reduction in ECM protease mRNA levels ADAMTS-1, MMP-1, -2, -2, -8, -14 and -16, while ADAMTS-8 was upregulated. Expression of type XI and XIV collagen was significantly upregulated.

Conclusion

Numerous biostimulatory effects of PBM has been demonstrated in several in vitro and in vivo studies, and the question is no longer whether it has an effect on the human cell, but rather what is the underlying mechanism of action? There is a rise in the number of studies being done on the influence of red and NIR laser light on biological systems, however there is not enough progress being made in the field of diabetes. If one looks at the literature, PBM is a non-invasive, non-thermal phototherapy with no reported side-effects when used at the optimal parameters, so why is this therapy not being used more frequently in the treatment of diabetic foot ulcers? As declared by the International Diabetes Federation, diabetes is one of the largest global health emergencies of the 21st century, and in light of the ever present threat of ulcers, infection, and amputation, and increasing incidence of diabetes, new improved therapies and the fortification of PBM in wound healing research deserves better attention.

Diabetic wounded WS1 cells irradiated at a fluence of 5 J/cm2 at a wavelength of either 660 (visible red) or 830 nm (NIR) showed a similar gene profile, with a number of genes significantly up- or –down regulated. It should be remembered that the gene profile of any cell type will differ depending on the stage of wound healing (and hence the time RNA is isolated post-irradiation), and even though there was an up-regulation in some proteases, these are essential for remodeling and breaking down old collagen and ECM proteins and making way for stronger, new collagen, as well as aiding in cellular migration. This study fortifies the notion that PBM stimulates cellular activity and can influence ECM matrix synthesis and degradation during wound healing. This study has also shown that PBM is able to stimulate cells at a genetic level.

Acknowledgments

This research was financially supported by the University of Johannesburg, the National Laser Centre /Council of Science and Industrial Research/National Research Foundation rental pool program as well as the African Laser Centre. Ms Sandra Ayuk conducted the lab experiments.

References

[1] International Diabetes Federation, IDF Diabetes atlas, 7th ed. 2015. Available at: www.idf.org/diabetesatlas (Last accessed March 8, 2016).

[2] K.N. Francis-Goforth, A.H. Harken and J.D. Saba, Normalization of diabetic wound healing, Surgery. 147 (2010).

[3] D. O'Reilly, R. Linden, L. Fedorko, J-E. Tarride, W.G. Jones, J.M Bowen and R. Goeree, A prospective, double-blind, randomized, controlled clinical trial comparing standard wound care with adjunctive hyperbaric oxygen therapy (HBOT) to standard wound care only for the treatment of chronic, non-healing ulcers of the lower limb in patients with diabetes mellitus: a study protocol, Trials. 12, (2011),http://www.trialsjournal.com/content/12/ 1/69

[4] S. Guo and L.A. DiPietro, Critical review in oral biology & medicine: factors affecting wound healing, J. Dent. Res. 89, (2010).

[5] N.J. Trengove, M.C. Stacey, S. Macauley N. Bennett, J. Gibson, F. Burslem, G. Murphy and G. Schultz, Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors, Wound Repair Regen. 7, (1999).

[6] J.W. Burrow, J.A. Koch, H. Chuang, W. Zhong, D.D. Dean and V.L. Sylvia, Nitric oxide donors selectively reduce the expression of matrix metalloproteinases-8 and -9 by human diabetic skin fibroblasts, J. Surg. Res. 140, (2007).

[7] N.N. Houreld, Mitochondrial light absorption and its effect on ATP production, in Handbook of Low-Level Laser Therapy, eds. M. Hamblin, M. Sousa and T. Agrawal, (Pan Stanford Publishing Pte Ltd., Florida, 2016) In Press.

[8] N.N. Houreld, Shedding light on a new treatment for diabetic wound healing: a review on phototherapy, The Scientific World Journal 2014, (2014),http://dx.doi.org/10.1155/2014/398412

[9] A. Schindl, M. Schindl, H. Pernerstorfer–Schon, K. Kerschan, R. Knobler and L. Schindl, Diabetic neuropatic foot ulcer: successful treatment by low-intensity laser therapy, Dermatology. 198, (1999).

[10] D.G. Minatel, A.C. Frade, S.C. Franca and C.S. Enwemeka, Phototherapy promotes healing of chronic diabetic leg ulcers that failed to respond to other therapies, Lasers. Surg. Med. 41, (2009).

[11] A. Kaviani, G.E. Djavid, L. Ataie–Fashtami, M. Fateh, M. Ghodsi, M. Salami, N. Zand, N. Kashef and B. Larijani, Randomized clinical trial on the effect of low-level laser therapy on chronic diabetic foot wound healing: a preliminary report, Photomed. Laser. Surg. 29, (2011).

[12] B. Nteleki, H. Abrahamse and N.N. Houreld, Conventional podiatric intervention and phototherapy in the treatment of diabetic ulcers, Semin. Vasc. Surg. 28, (2016).

[13] S.M. Ayuk, N.N. Houreld and H. Abrahamse, Laser irradiation alters the expression profile of genes involved in the extracellular matrix in vitro, International Journal of Photoenergy, 2014, (2014) http://dx.doi.org/ 10.1155/2014/604518

[14] M. Esmaeelinejad, M. Bayat, H. Darbandi, M. Bayat and N. Mosaffa, The effects of low-level laser irradiation on cellular viability and proliferation of human skin fibroblasts cultured in high glucose mediums, Lasers. Med. Sci. 29, (2014).

[15] P.A. Appleby, S. Shabir, J. Southgate and D. Walker, Sources of variability in cytosolic calcium transients triggered by stimulation of homogeneous uro-epithelial cell monolayers, J. R. Soc. Interface. 12, (2015) http://dx.doi.org/10.1098/rsif.2014.1403

[16] A. Teuschl, E.R. Balmayor, H. Redl, M. van Griensven and P. Dungel, Phototherapy with LED light modulates healing processes in an in vitro scratch-wound model using 3 different cell types, Dermatol Surg. 41, (2015).

[17] S.M. Ayuk, N.N. Houreld and H. Abrahamse, Collagen production in diabetic wounded fibroblasts in response to low-intensity laser irradiation at 660nm,Diabetes. Technol. Ther. 14, (2012).

[18] J.R. Basford, Low intensity laser therapy: still not an established clinical tool, Lasers Surg. Med. 16, (1995).

[19] N.N. Houreld and H. Abrahamse, Laser light influences cellular viability and proliferation in diabetic-wounded fibroblast cells in a dose- and wavelength-dependent manner, Lasers Med. Sci. 23, (2008).

[20] N.N. Houreld, P.R. Sekhejane and H. Abrahamse, Irradiation at 830nm stimulates nitric oxide production and inhibits pro-inflammatory cytokines in diabetic wounded fibroblast cells, Lasers Surg. Med. 42, (2010).

[21] M.V. Cabrero, J.M.G. Failde, O.M. Mayordomo, Laser therapy as a regenerator for healing wound tissues. In: International congress on lasers in medicine and surgery. June 27–28 pp 187–192 (1985).

[22] D.M. Harris, Laser biostimulation: review and hypothesis, Laser Topics 10, (1988).

[23] C. Frantz, K.M. Stewart and V.M. Weaver, The extracellular matrix at a glance, J. Cell Sci. 123, (2010).

[24] S. Löffek, O. Schilling and C-W. Franzke, Biological role of matrix metalloproteinases: a critical balance, Eur. Respir. J. 38, (2011).

[25] A. Aparecida Da Silva, E.C. Leal-Junior, A.C. Alves, C.S. Rambo, S.A. Dos Santos, R.P. Vieira and De T. Carvalho Pde, Wound-healing effects of low-level laser therapy in diabetic rats involve the modulation of MMP-2 and MMP-9 and the redistribution of collagen types I and III,J. Cosmet. Laser. Ther. 15, (2013).