Response of low intensity laser irradiation on collagen production in diabetic wounded fibroblast cells in vitro

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Abstract. Background: Collagen Type I (Col-I) is a major component of the extracellular matrix (ECM) and is important in wound healing processes. Several studies have shown that Low Intensity Laser Irradiation (LILI) biostimulates Col-I synthesis both in vitro and in vivo. Diabetic patients are known to suffer from slow-to-heal wounds and collagen production in these patients is impaired. This study aimed to determine if LILI affects collagen production and related cellular responses in an in vitro diabetic wounded fibroblast model. Method: This study was performed on isolated human skin fibroblasts. Different cell models namely; normal and diabetic wounded were used. Cells were irradiated with 5 J/cm$^2$ at a wavelength of 660 nm and incubated for 48 or 72 hours. Non-irradiated cells (0 J/cm$^2$) were used as controls. Biological responses were assessed. Cellular viability (Trypan blue), morphology (Bright Field Microscopy), proliferation (VisionBlue Quick Cell Proliferation Assay), and Col-I (Enzyme Linked Immunoabsorbent Assay, ELISA) were assessed. Results: Diabetic wounded cells irradiated with 5 J/cm$^2$ at 660 nm showed a significant increase in cell migration, viability, proliferation and Col-I. Conclusion: This study shows that LILI stimulates Col-I synthesis in diabetic wound healing in vitro at 660 nm.

1. Introduction

Impaired wound healing stands as a major diabetic complication and non-healing ulcers remain a serious problem in public health and clinical practice. It is common to note that out of about 15% of people with diabetic foot ulcers (DFUs), 3% suffer from lower limb amputation [1]. Wound healing comprises of a series of composite biological processes summarized in three phases namely inflammation, proliferation and tissue remodeling. These phases involves cytokines, chemokines, clotting factors, various cells and growth factors [2, 3] that must occur in the correct order, at a given time and at favorable intensities to ensure proper healing [4]. Fibroblasts secrete Col-I and other types of collagen which make up the major protein found in the ECM, provides structural scaffold of the cell that maintains the cell integrity and tensile strength in wound healing [5-7]. Studies have shown that diabetes affects collagen production as well as wound healing [8].

In the visible and Near Infra-Red (NIR) spectral range, Low Intensity Laser Irradiation (LILI) is known to accelerate wound healing processes in cell culture, in vivo and animal models [9, 10] at different wavelengths and doses [11]. This process is non-invasive and is achieved at wavelengths between 500-1,100 nm and power outputs of 10-200 mW during treatment [10]. Even though LILI is widely used in medical practice, however, there are disputable views on the use of treatment specifications since there is not enough scientific evidence and guidelines. More so, there is a need for these specifications to be stabilized under optimal conditions in both animal models and clinical practice; hence there is need for more research [12].

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The purpose of this study is to determine if LILI affects collagen production and related cellular responses in an in vitro diabetic wounded fibroblast model at 660 nm with a fluence of 5 J/cm².

2. Methods

2.1 Cell Culture and Laser Irradiation

In this study, normal (N) and diabetic wounded (DW) cells were utilized. Using standardized culture methods [13-16] isolated human skin fibroblast cells (Academic ethics Committee, Clearance Reference Number: 01/06, University of Johannesburg) were regularly cultured. Approximately 6 x 10⁵ cells were seeded into 3.3 cm diameter culture plates containing 3 ml complete media and incubated overnight for the cells to attach. Prior to irradiation, a central scratch was created from a confluent cellular monolayer by using a 1 ml sterile pipette to mimic a wounded model [17]. An in vitro diabetic wounded model was also established [18]. The normal cells were used as controls. Cells were irradiated using 660 nm diode laser (RGBlase, TECIRL-100G-650SMA, Fremont, California, USA) with a continuous wave emission, 92.8 mW power output, 9.1 cm² spot size, 10.22 mW/cm², and the cells were exposed to laser irradiation for 8 min 9 s in order to achieve a fluence of 5 J/cm². The effects of laser irradiation on cellular responses for cellular morphology, viability, proliferation and collagen production were observed after 48 h and 72 h (See Table 1). All lasers were supplied by the National Laser Center (NLC) of South Africa. Irradiations were performed four times (n=4) and each biochemical assay was done in duplicate and the mean used.

3. Results

Table 1. Summary of the methodology and results

<table>
<thead>
<tr>
<th>Method</th>
<th>Data</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N 0 J/cm²</td>
<td>DW 0 J/cm²</td>
<td>DW 5 J/cm²</td>
</tr>
<tr>
<td>Trypan blue exclusion test</td>
<td>Viability (%)</td>
<td>95</td>
<td>94</td>
</tr>
<tr>
<td>Proliferation test</td>
<td></td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>MTT (Cell Titre Non-Radioactive Proliferation Assay)</td>
<td>A_550 nm</td>
<td>0.163</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>VisionBlue (VisionBlue™ Quick Cell Proliferation Assay)</td>
<td>Ex/Em 560/595 nm</td>
<td>193</td>
<td>225</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±</td>
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<td></td>
<td></td>
<td>413</td>
<td>1513</td>
</tr>
</tbody>
</table>

*P<0.05, and ***P<0.001, compared to normal 0 J/cm²
†P<0.05 and †††P<0.001, compared to diabetic wounded 0 J/cm²
± SE
Figure 1. Cell Morphology in an *in vitro* normal, and diabetic wounded models. Fibroblasts appear as slender, spindle shaped cells. The wound, or central scratch (CS) and the direction of cell migration (CM) can clearly be seen.

Figure 2. Col I production in an *invitro* normal and diabetic wounded model. Col I was assessed using the Enzyme Linked Immunoabsorbent Assay. Significant probability is shown as *** $P<0.001$. 

*** $P<0.001$
4. Discussion and Conclusion

Cell migration, viability, and proliferation are various aspects that affect LILI in vivo and in vitro [19]. In this study, irradiated diabetic wounded cells showed an increase in cell migration, viability and proliferation compared to non-irradiated diabetic wounded cells, with no changes in morphology (Figure 1). Col-I increased in irradiated diabetic wounded cells compared to non-irradiated cells at 48 h and 72 h (Figure 2). Normal cells also showed a significant increase compared to diabetic wounded cells. According to Karu (2003) [20], LILI is thought to enhance these biological processes. Increase in cell proliferation could possibly have occurred through changes in the mitochondrial respiratory chain as a primary response to healing leading to increased ATP production. This in turn affects RNA synthesis and changes the expression of various proteins [21, 22]. However in a diabetic situation the process is impaired due to disturbed cell migration. As a result of this disorder, there is poor synthesis of growth factors and vasodilator agents [23].

These results showed that LILI increase Col-I production at 660 nm at a fluence of 5 J/cm². These findings are in agreement with Silveira and colleagues (2011) [11] who also observed that LILI produced a stimulatory effect in an animal model at 1 and 3 J/cm² [24, 25]. Their study further confirmed that shorter wavelengths and energy densities between 1-5 J/cm² increase the tensile force of tendons and speed up collagen production compared to higher energy densities and wavelengths [11].

In conclusion, this study adds to evidence that LILI stimulates Col-I synthesis in diabetic wound healing in vitro at 660 nm.

5. Acknowledgement

Many thanks to the National Research Foundation (NRF), South Africa, the University of Johannesburg (UJ), the National Laser Centre (NLC), South Africa, the African Laser Centre (ALC), and the Medical Research Council (MRC) who supported this project.

References