

Low Intensity Laser Irradiation (LILI) in Combination with Growth Factors in a Co-culture System Supports the Differentiation of Mesenchymal Stem Cells

B Mvula and H Abrahamse¹

Laser Research Centre, Faculty of Health Sciences, University of Johannesburg,
P.O. Box 17011, Doornfontein 2028, Johannesburg, South Africa

E-mail: habrahamse@uj.ac.za

Abstract. Mesenchymal stem cells have the capacity to differentiate into a variety of cell types that could potentially be used in tissue engineering and regenerative medicine. Low intensity laser irradiation (LILI) has been shown to have positive effects on different cell types, including a significant increase in cell viability and proliferation. Growth factors such as retinoic acid (RA) and transforming growth factor β 1 (TGF β 1) have been shown to play important roles in the differentiation of cells. The aim of this study was to investigate whether LILI in combination with growth factors could induce the differentiation of adipose derived stem cells (ADSCs) co-cultured with smooth muscle cells (SMCs). The study used primary and continuous ADSC cell lines and a SMC line (SKUT-1). Cells were co-cultured at a ratio of 1:1, with and without growth factors and then exposed to LILI at 5 J/cm² using a 636 nm diode laser. The cellular morphology, viability and proliferation of the co-cultures were assessed over a period of 24h. The expression of the cell specific markers was monitored over the same period of time. Cell viability and proliferation increased significantly in the co-cultured groups that were exposed to laser alone, as well as in combination with growth factors. Furthermore, there was a significant decrease in the expression of stem cell markers in the ADSCs over time. The results indicate that LILI in combination with growth factors not only increases the viability and proliferation of co-cultured cells but also decreases the expression of ADSC stem cell markers. This could indicate the possible differentiation of ADSCs into SMCs.

1. Introduction

Mesenchymal stem cells have the capability of self-renewal and differentiation. They have a high capacity for proliferation [1]. Adipose derived stem cells (ADSCs) are mesenchymal stem cells that are easily isolated in large numbers and have the ability to differentiate into several lineages [2]. Studies have shown that ADSCs could be differentiated into smooth muscle cells (SMCs) [3] and neuron cells [2]. So far studies have indicated that ADSCs co-cultured with adipocytes could not differentiate into adipocytes [4] while ADSCs co-cultured with melanocytes, increased melanocyte proliferation and migration [5].

SMCs play a vital role in angiogenesis and vasculogenesis during development of the embryos [6] and are active components of intestinal, urinary, cardiovascular and reproductive systems. These cells have been differentiated from mesenchymal stem cells in the presence of growth factors [7]. SMCs are known to play a role in diseases like asthma, hypertension, cancer and arteriosclerosis [8].

¹To whom any correspondence should be addressed

Low Intensity Laser Irradiation (LILI) has been shown to have positive effects such as increased viability and proliferation on different types of cells including ADSCs. It has had effects on these cells in combination with the growth factors as well [9, 10].

In rats, LILI promotes recovery from disuse muscle atrophy with satellite cell proliferation and angiogenesis [11]. Recent studies have also shown that LILI increased human periodontal ligament cell line at 670 nm [12]. It has also been recently found out that LILI up-regulates the genes involved in the electron transport chain in human fibroblast cells [13].

Growth factors have been shown to have an influence on cell differentiation, maturation, proliferation and apoptosis [14, 15]. Retinoic acid (RA) has been found to mediate neuronal differentiation from human embryonic stem cells [16]. Transforming Growth factor-Beta1 (TGF- β 1) promotes smooth muscle differentiation of genes in fibroblasts of rats [17]. It has been shown to act as a key physiological factor in maintaining the stem cell reserve by up-modulating CD34 [18]. Studies involving another growth factor, TGF- β 3, found that it could promote cartilage formation of ADSCs in a culture system [19].

This study aimed at investigating whether LILI in combination with growth factors could induce the differentiation of ADSCs co-cultured with SMCs. The success of the study could be beneficial in regenerative medicine and tissue engineering.

2. Materials and Methods

Primary and continuous ADSCs and a SMC cell line were used in this study. The cells were cultured individually to semi-confluency (ADSCs) and confluency (SMCs) and then co-cultured directly into 3.4 cm² diameter dishes in a ratio of 1:1. Growth factors (RA and TGF- β 1) were added at concentration of 0.1 μ M and 1 ng/ml respectively. The cells were then exposed to LILI at 5 J/cm² using a diode laser (National Laser Centre) with a wavelength of 636 nm. Six groups were established; co-cultured (CC - control group), co-cultured and LILI (CC+LILI), co-cultured and RA (CC+RA), co-cultured, RA and LILI (CC+RA+LILI), co-cultured and TGF- β 1 (CC+ TGF- β 1) and then co-cultured, TGF- β 1 and LILI (CC+ TGF- β 1+LILI).

Cellular morphology was assessed using light inverted and fluorescence microscopy. Trypan blue and adenosine triphosphate (ATP) assays were used to assess cellular viability. Cell proliferation was analysed using optical density (OD) assay and flow cytometry assessed the expression of the markers CD29 (β 1-Integrin) and CD90 (Thy-1) for ADSCs and heavy myosin chain for the SMCs. Statistically, the study used sigma plot 11.0. Significant differences between groups for viability, proliferation and expression of the markers were indicated by * $P \geq 0.05$, ** $P \geq 0.001$ and *** $P \geq 0.0005$.

3. Results

3.1 Cell morphology

Both types of cells were stained with CFDA-SE for morphological analysis. There was no difference in the morphology of ADSCs and SMCs (figure 1).

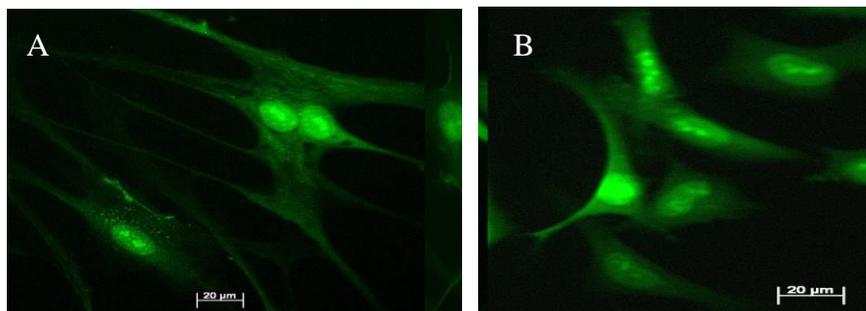


Figure 1: Showing the morphology of A - ADSCs and B - SKUT-1.Cells (SMCs) stained by carboxyfluorescein diacetate succinimidyl ester (CFDA-SE), a dye that stains the cytoplasm (green) and nucleus (bright green).

3.2 Cell viability and proliferation

ATP luminescence and trypan blue assays were used to analyse cell viability while OD assay was used for proliferation. There was an increase in cell viability and proliferation in cells that were co-cultured and irradiated as compared to cells that were co-cultured and with added growth factors. The co-cultured cells that were added with the growth factors and irradiated, increased in viability and proliferation as compared to those that were only co-cultured and with added growth factors (table 1, figure 2 and 3).

Table 1. Trypan blue assay showing percentage viability of the co-cultured cells. The black stars* indicate significant decrease in viability percentage of the co-cultures as compared to the control group (CC) while the black hash# indicate the significant increase.

Time Post Irradiation	CC	CC +LILI	CC + RA	CC+ RA + LILI	CC + TGF-β1	CC + TGF-β1 +LILI
24 h	74.5	72.8	66.3*	68.2	65.4*	71 [#]

The co-cultures that were added with the growth factors (CC + RA and CC + TGF-β1) showed significant decreases in percentage viability as compared to the control group (CC) while the group that was co-cultured with added growth factor, TGF-β1 and irradiated (CC + TGF-β1 +LILI) showed a significant increase in percentage viability as compared to its control (CC + TGF-β1).

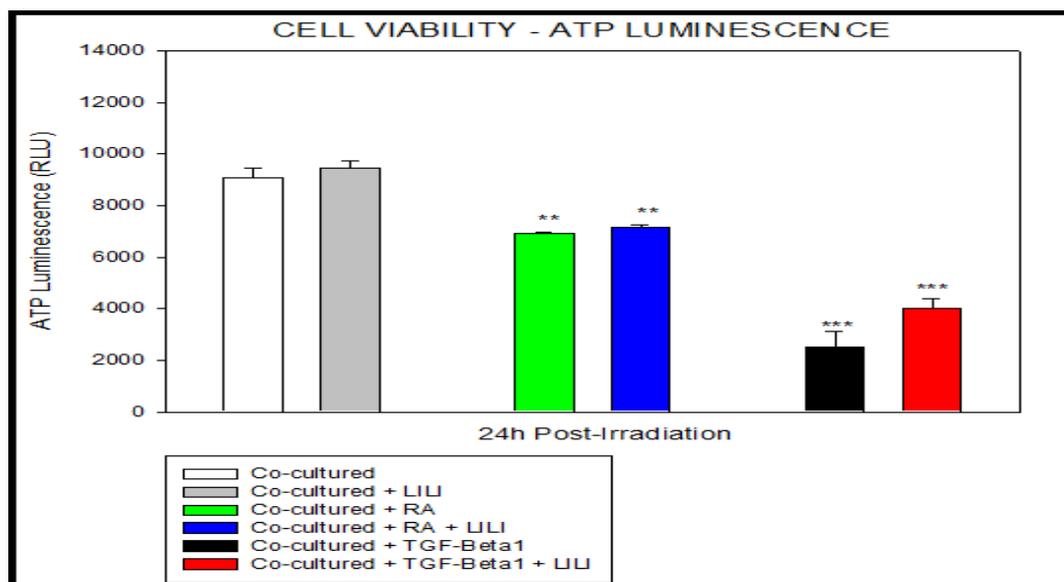


Figure 2: Cell viability as analysed by ATP luminescence at 24h after irradiation for the different six groups. There was an increase in viability in cells that were co-cultured and irradiated while a decrease was observed in groups that had growth factors. However there was an increase in viability in cells that had growth factors and irradiated as compared to those that had growth factors only.

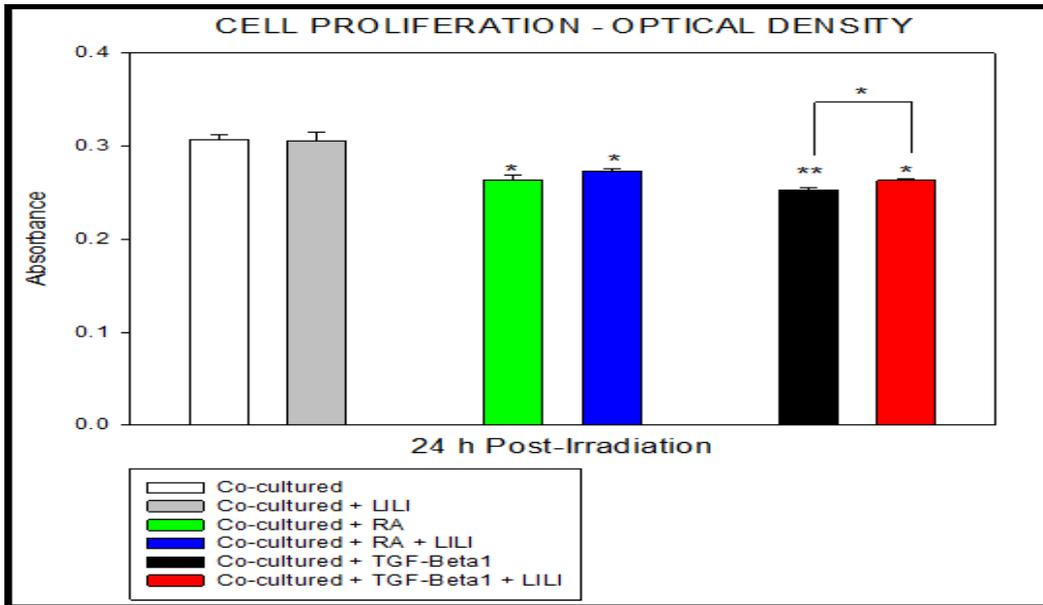


Figure 3: OD results showing cell proliferation at 24h after irradiation for the different six groups. There was a significant decrease in proliferation in groups with added growth factors as compared to the control groups at 24 h.

3.3 Expression of the markers

Expression of the ADSCs markers showed a decrease in cells that were co-cultured with added growth factors and irradiated (figure 4) while there was an increase in expression of the SMC marker (figure 5) and the increase was more in cells that were co-cultured, with added growth factors and irradiated cells.

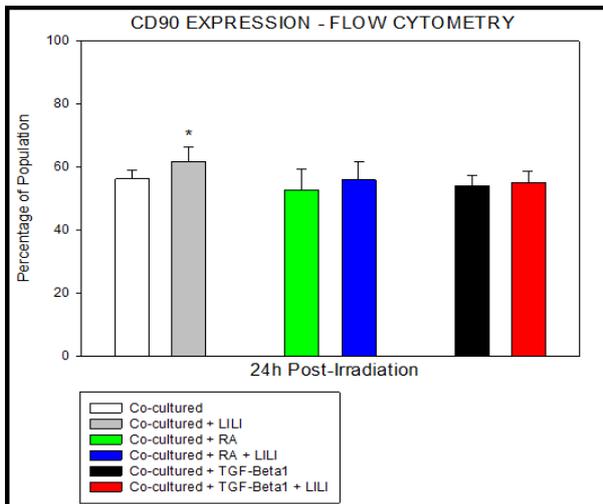


Figure 4: There was a decrease in the expression CD90, an ADSCs marker over the period of 24h in co-cultures with added growth factors though the decrease was not significant. But there was a significant increase in the expression of CD90 in

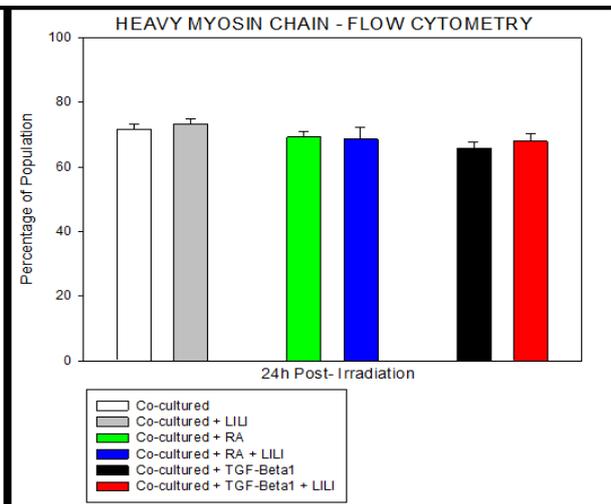


Figure 5: Heavy myosin chain, an SMC marker decreased in the co-cultures that were added with the growth factors but the decrease was not significant as shown in the figure above over the period of 24h.

4. Discussion and Conclusion

The findings in this study agree with those from other studies which found that LILI could increase viability and proliferation of cells [3, 9, 10]. Growth factors such as RA and TGF- β have been used to induce differentiation in previous studies [16]. TGF- β 1 has been used in the differentiation of human mesenchymal stem cells into osteoblasts and adipocytes [20]. Recent studies used TGF- β to induce differentiation of smooth muscle cells from human embryonic stem cells [21]. Chen and Lechleider [22] in their study, induced the differentiation of a neural crest stem cell line into smooth muscle cells with TGF- β . The current study found out that there was a decrease in the expression of the ADSC markers in the co-cultures with added growth factors. However, more investigations are required to prove that this could indicate a possible differentiation of ADSCs to SMCs. If this is achieved, the co-culture system with LILI and growth factors can be a promising method in research for treatment applications in tissue engineering and regenerative medicine.

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