Laser-Induced Differentiation of Human Adipose-Derived Stem Cells to Temporomandibular Joint Disc Cells

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Background and Objectives: Temporomandibular disorder (TMD) is an incapacitating disease with temporomandibular joint (TMJ) disc degenerative changes in patients. Despite several research attempts to find a definitive treatment, there is no evidence of a permanent solution. The objective of the current study was to observe the role of 660 nm diode laser in the differentiation of human adipose-derived stem cells (ADSCs) to fibroblasts and chondrocytes.

Study Design/Materials and Methods: After irradiation, the morphology, viability, and adenosine triphosphate (ATP) proliferation of the ADSCs were analyzed at different time intervals. The differentiation of ADSCs toward fibroblastic and chondrogenic phenotypes was supported using flow cytometry and immunofluorescence at 1- and 2-week post-irradiation.

Results: More than 90% of viable cells were observed in all experimental groups, with an increase in ATP proliferation. Flow cytometry analyses and immunofluorescence demonstrated the presence of chondrogenic and fibroblastic cell surface markers at 1- and 2-week post-irradiation.

Conclusion: This study has demonstrated methods to induce the differentiation of ADSCs toward fibroblastic and chondrogenic phenotypes with a 660 nm diode laser. The study also proposes a future alternative method of treatment for patients with degenerative TMJ disc disorders and presents a positive prospect in the application of photobiomodulation and ADSCs in the treatment of degenerative TMJ disc. Lasers Surg. Med. © 2020 Wiley Periodicals LLC

Key words: tissue engineering; temporomandibular joint disc disorder; photobiomodulation

INTRODUCTION

Tissue engineering and photobiomodulation are evolving as novel therapies for several diseases in medicine and dentistry [1,2]. The term low-level laser therapy has recently been superseded by the term photobiomodulation [3,4]. It has been confirmed that photobiomodulation turns cells into more alkaline and that cells are capable of achieving optimal function despite the low redox state and decreased oxidation [5]. The temporomandibular joint (TMJ) cells with decreased oxidation will generate reactive oxygen species (ROS) by direct mechanical injury, hypoxia-reperfusion, and arachidonic acid catabolism to the articular tissues. Further on, ROS affects the viscosity of synovial fluid and deterioration in the surface-active phospholipid layer leading to decreased lubrication of the articular surfaces of TMJ [6]. Photobiomodulation can be used as an effective tool to remove oxidative stresses within damaged TMJ disc cells.

The research studies have used stem cells with biomaterial scaffolds and/or biomolecules to form new cells to replace damaged TMJ disc cells [7,8]. The capacity to renew is a characteristic feature of stem cells that clearly distinguishes them from the other cells [9,10]. An in vitro study established biodegradable polylactide disks (PLA) with adipose-derived stem cells (ADSCs) that could be encouraging choice for tissue engineering of the fibrocartilaginous TMJ disc [11]. The ADSCs are part of mesenchymal cell lineages that have many advantages. One of ADSCs’ very important advantages is that they discharge cytokines and growth factors to an unhealthy tissue, which stimulates recovery in a paracrine manner [12]. The ADSCs at the ischemic site generate antioxidants or free radical scavengers and remove damaging elements encouraging the revival of the surviving cells [13]. Moreover, a research study reported that intra-articular injection of ADSCs and laser irradiation prevented knee joint degeneration with induced osteoarthritis [14]. Application of photobiomodulation on the bone marrow to stimulate patient’s stem cells and to induce regeneration of damaged tissue is one of the most recent approaches [15,16]. Another study used a 660 nm diode laser on damaged skeletal muscle in rats, reported positive effects and an increase in...
the muscle regeneration process itself [17]. Similarly, a study that used a 660 nm laser on an injured sternomastoïd muscle triggered by bupivacaine reported an improvement in the muscle regeneration process [18]. This indicates that photobiomodulation, alone or in combination with ADSCs, could offer a better treatment for patients with degenerative changes of the TMJ disc and temporomandibular disorder (TMD).

TMD is the most pervasive recurrent chronic, nondental-related persistent pain condition in dentistry [19]. To confirm the seriousness of this disorder, the worldwide Research Diagnosis Criteria for TMD (RDC/TMD) was developed [20–22]. Photobiomodulation has been introduced as a new method for the treatment of TMD patients [23]. Additionally, photobiomodulation increases stem cell migration and stimulate the proliferation and regeneration [24,25]. Studies have confirmed positive results for occlusal splint and relaxation and psychological treatment modalities [26,27]. However, none of them proved to have a long-term effect.

The current study observes the role of a 660 nm diode laser in the differentiation of ADSCs to fibroblasts and chondrocytes. The study also identifies a future alternative method of treatment for patients with degenerative TMJ disc disorder.

MATERIALS AND METHODS

Culture of ADSCs

The immortalized adipose stem cells procured from ATCC were used (ATCC Cat # SCRC4000™ [Lot # 70003596], Virginia, USA) for all the experiments. The Academic Ethics Committee of the Faculty of Health Sciences, University of Johannesburg, approved this research study (REC-241112-035). A monolayer of ADSCs was cultured in Dulbecco’s modified Eagle’s medium (D5796; Sigma Life Science, Johannesburg, South Africa) supplemented with 10% fetal bovine serum (Gibco™ 10270 106, Johannesburg, South Africa), 1% penicillin/streptomycin (P4333; Sigma Life Science), and 1% Amphotericin B (A2942; Sigma Life Science). The stem cell cultures were incubated at 37°C with 85% humidity and 5% carbon dioxide (CO2).

Laser Differentiation of ADSCs to Fibroblasts and Chondrocytes

The ADSCs were characterized and their identity was confirmed before seeding. The cells were cultured in a 175 cm2 flask and harvested at 80% confluence with TrypLE™ Select (12553-029; Gibco®). The cell viability percentage was determined with an automated cell counter (Countess® II FL; Invitrogen, LTC Tech South Africa Pty LTD, Fairland, Johannesburg, South Africa) using trypan blue assay and the viable cell number was used to optimize cell seeding density (Table 1). The experimental groups include cells grown at 24, 48, and 72 hours and at 1, 2, and 3 weeks.

### Table 1. Seeding Density Optimization of Adipose-Derived Stem Cells

| Time period                  | Number of cells |
|------------------------------|-----------------|
| 24 hours post-irradiation group | 5 × 10^5        |
| 48 hours post-irradiation group | 3 × 10^5        |
| 72 hours post-irradiation group | 3 × 10^5        |
| 1-week post-irradiation group | 1 × 10^4        |
| 2 weeks post-irradiation group | 5 × 10^3        |
| 3 weeks post-irradiation group | 5 × 10^3        |

To observe suitable results beyond 72 hours required different seeding densities. Cell growth was monitored in a 3.4 cm diameter culture dish (430588; Corning, The Scientific Group, Johannesburg, South Africa) with 2 ml of complete media. The images were captured using an inverted light microscope (Wirsam Scientific, Olympus CKX41, Johannesburg, South Africa).

The experiment was divided into four groups. Group C was a control group of ADSCs with no laser irradiation and basic fibroblast growth factor (bFGF) (Sigma-Aldrich, Merck Group, South Africa, GF003, Johannesburg, South Africa), Group LB with bFGF added at 10 ng/ml, before irradiation, Group B with bFGF (10 ng/ml) and no irradiation and ADSCs exposed to laser irradiation alone, Group L. The bFGF used in this study is a known strong mitogen for many cell types and it is found as a part of basement membranes, and subendothelial extracellular matrix of blood vessels in the normal tissue [28–30]. The irradiated and non-irradiated ADSCs were re-incubated at 37°C in a humidified atmosphere of 5% CO2.

The irradiation of ADSCs was done with 660 nm diode laser at a fluence of 5 J/cm2 with a continuous wave (FC-655-300-MM2-SMA-1-0; RGlase LLC, Fremont, CA), provided and set up by the National Laser Centre (NLC) of the Council for Scientific and Industrial Research (CSIR), South Africa. The power output was read by using the Field Mate Laser Power Meter (Field Mate, Power Sens detector, 0496005; Coherent, Western Cape, South Africa). The read values of power output were used to calculate the duration (time) of each exposure with the following formula:

\[
\text{mW} = \frac{\text{mW} \times 4}{\pi \times 3.4 \times 2}
\]

\[
\text{W} = \frac{\text{mW}}{1000}
\]

\[
\text{Time (s)} = \frac{((J \text{cm}^2) \text{W})}{(\text{cm}^2)}
\]

All the laser parameters were recorded (Table 2).
TABLE 2. Laser Parameters Used for Irradiation of Adipose-Derived Stem Cells With 660 nm Diode Laser

| Laser type          | Semiconductor diode |
|---------------------|---------------------|
| Wavelength          | 660 nm              |
| Wave emission       | Continuous          |
| Spot size           | 9.1 cm²             |
| Output power (mW)   | 24, 48, and 72 hours—97.1 |
|                     | 1, 2, and 3 weeks—98.25 |
| Power density (mW/cm²) | 24, 48, and 72 hours—10.6 |
|                     | 1, 2, and 3 weeks—10.8 |
| Fluencies           | 5 J/cm²             |
| Irradiation times calculated with reading of power output | 24, 48, and 72 hours—7.48 minutes |
|                     | 1, 2, and 3 weeks—7.42 minutes |

Morphology and Trypan Blue Exclusion Assay

Microscopic evaluation of cells was performed with an inverted light microscope at 24, 48, 72 hours and 1-, 2-, and 3-week post-irradiation. The trypan blue viability assay was performed (T8154; Sigma-Aldrich, Johannesburg, South Africa) to determine cellular viability. The trypan blue only enters impaired, leaky membranes of dead cells, resulting in unstained and colorless viable cells. An equal volume of cells and trypan blue (10 μl) were mixed by pipetting. The cell viability percentages were obtained from the automated cell counter.

Adenosine Triphosphate (ATP) Proliferation

The ADSCs were trypsinized and resuspended in complete media to perform ATP proliferation experiments. The luminescent cell viability assay (CellTiter-Glo® 3D; Promega, Anatech Instruments, Johannesburg, South Africa) was performed to establish the number of viable cells in the stem cell culture. The change of ATP to adenosine monophosphate (AMP) by the enzyme luciferase produces luminescence, which is measured with this assay. This assay enables the quantification of intracellular ATP and the assessment of mitochondrial activity, which is an indication of the existence of energy-storing active cells as a direct indicator of cell proliferation [31,32]. Equivalent volumes of cell suspension and reagents (50 μl) were added in a white-walled 96-well plate (353, 296; BD Biosciences, Woodmead, Johannesburg, South Africa) and mixed on a shaker for 5 minutes to provoke cell lysis; and incubated for 25 minutes at room temperature in the dark to stabilize the luminescent signal. Triplicate of each experimental group (C, LB, B, and L) was maintained during the study. The emission of the light signal was read in relative light units (RLU) with Victor 3 multiplate reader (Perkin-Elmer, Midrand, Johannesburg, South Africa).

Flow Cytometry Analysis

After repeated attempts, little to no differentiation was observed in groups, other than at 1 and 2 weeks. Hence, the percentage of ADSCs' differentiation toward fibroblastic and chondrogenic lineages at 1- and 2-week post-irradiation was determined with the expression of CD26 (clone M-A261, mouse anti-human; Bio Rad Laboratories (Pty) Ltd., Johannesburg, South Africa) used at 1:100 μl phosphate-buffered saline (PBS) dilution in all experimental groups and CD49C (clone P1B5, mouse anti-human; Bio Rad Laboratories (Pty) Ltd.) at 1: 200 μl of PBS dilution in L group. The ADSCs were cultured to 80% confluence and trypsinized. The ADSC suspension was centrifuged at 1 ml of room temperature PBS (A2153 and S8032; Sigma, Johannesburg, South Africa). A cell count and viability were performed using the automated cell counter. After cell count, 1 × 10⁶ of the cells were added into a focus tube in 100 μl cold (4°C) PBS. Ten microliters of the primary antibody were added into 100 μl of cells in PBS and vortexed. The cells were then incubated in the dark for 30 minutes and washed three times using PBS and centrifuged at 400g for 5 minutes at room temperature. In the next stage, 10 μl of the secondary antibody (FTTC Goat anti-Mouse; Santa Cruz Biotechnology, Anatech Instruments, Johannesburg, South Africa) was added into 100 μl cell suspension and vortexed. Thereafter, the cells were incubated in dark for 30 minutes and rinsed three times with PBS and centrifuged at 400g for 5 minutes at room temperature. The cells were then resuspended in 300 μl of PBS for the immediate flow cytometry reading. The detection of fluorescence (dye-stained cells) was performed using the Accuri C6 flow cytometer (BD Biosciences) and compared with the gated control unlabeled group of ADSCs. The fluorescence was detected using FL-1 with a 533/30 filter and a 488 nm laser.

Immunofluorescence

The surface markers of the cells, intracellular proteins, and other cellular antigens can be detected under a fluorescent microscope. The direct, indirect, and complement binding immunofluorescence methods are the best-recognized ones [33,34]. In this study, the indirect immunofluorescence protocol was used to confirm the differentiation of ADSCs to fibroblasts in all experimental groups (L, LB, and B) and to chondrocytes in L group at 1- and 2-week post-irradiation. The cells were cultured on a heat sterilized coverslip in a 3.4 cm diameter culture plate with 2 ml of complete media at a concentration of 1 × 10⁴ for 1-week and 5 × 10³ cells/plate for 2-week post-irradiation. The ADSCs were then irradiated at a fluence of 5 J/cm² with 660 nm diode laser and cultured for 1 and 2 weeks. Thereafter, the cells in separate experimental groups were washed three times with cold PBS (A2153; Sigma) and fixed in 4% paraformaldehyde (P6148; Sigma) for 15 minutes in the dark. Cells were then incubated with blocking buffer (10% [w/v]) bovine serum albumin for 30 minutes at room temperature as a blocking step and washed three times again using cold PBS. After blocking, the cells were incubated with 100 μl primary antibody CD26 (mouse anti-human; 1:100 μl of PBS dilution) for 1 hour in the dark and rinsed three times with PBS. The slides were then labeled and incubated with 100 μl of the
secondary fluorescent FITC Goat anti-mouse antibody (1:100 μl of PBS dilution) for 1 hour in the dark. In another set of experiments, the steps were repeated with 100 μl of CD49C (1:200 μl of PBS dilution). After the final rinse with PBS, the cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (D1306, 358Ex/461Em; Invitrogen™) and mounted on glass slides using Fluoromount™ the aqueous mounting medium (F4680; Sigma). The slides were viewed under a fluorescent microscope live-cell station from Carl Zeiss Axio Z1 Observer using AxioVision imaging software (Carl Zeiss, Randburg, Johannesburg, South Africa) at ×200 magnification.

Statistical Analyses

The Sigma Plot version 13.0 was used for statistical analysis. The statistical analysis of Student’s one tail $t$ test was applied. The one-tail $t$ test data was plotted on the graphs with * symbol indicating the statistical significance: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

RESULTS

Cell Morphology and Viability

At 24, 72 hours and 1-week post-irradiation, proliferation was evident in morphological images (Fig. 1). The morphology and confluence at 2 and 3 weeks were similar to that observed at 1 week. In control, a decline in proliferation was observed at 1, 2, and 3 weeks. However, proliferation was evident beyond 72 hours in all other groups with laser irradiation and bFGF.

More than 90% of viability was recorded in all experimental groups. A statistical significance of $P < 0.05$.
was recorded in group L at 72 hours compared with the control group (Fig. 2).

ATP proliferation

Since different seeding densities were used for the experimental groups, ATP proliferation of treatment sets was compared with their respective controls within the same time interval. A significant increase ($P < 0.01$) in ATP was recorded in groups L and B at 1 week, and in group LB at 72 hours post-irradiation. While at 48 hours, group L had the highest ATP proliferation (Fig. 3).

Flow Cytometry

The differentiation and expression of markers in the experimental groups up to 72 hours and at week 3 appeared minor. Hence, the results represented are of weeks 1 and 2. The expression of CD26 marker for fibroblasts was confirmed in LB, B, and L groups at 1- and 2-week post-irradiation.

Fig. 2. Trypan blue viability of ADSCs assessed at different time intervals post-irradiation with 660 nm diode laser. The statistical analysis was done with student’s one-tail $t$ test compared with their respective controls within the same time interval. All groups of cells indicated high viability. At 72 hours, depending on the cell seeding density, the laser-treated group was statistically significant. ADSC, adipose-derived stem cells.

Fig. 3. The ATP cellular proliferation was assessed at 24, 48, and 72 hours and 1-week post-irradiation with 660 nm diode laser. The statistical analysis of ATP proliferation in experimental groups was performed with student’s one-tail $t$ test compared with their related controls within the same time interval. The cellular proliferation of ADSCs as assessed by ATP. The ATP proliferation was evident in all irradiated groups of cells with and without bFGF compared with control cells. The result depicts the significant role of bFGF and laser in cell proliferation and an increase in ATP. As represented, at 72 hours, cells in the treatment group showed a significant increase in ATP compared with cells in other groups. ADSC, adipose-derived stem cells; ATP, adenosine triphosphate; bFGF, basic fibroblast growth factor.
Unlike for fibroblasts, ADSC differentiation to chondrocytes was observed in group L alone. The experimental groups were compared with a control group of ADSCs that had no markers. The highest percentage (28.27%) of CD26 expression was recorded at 1- and 2-week (25.37%) post-irradiation in group LB (Table 3). The expression of CD49C marker for chondrocytes was higher in group L, at 1-week (19.13%) than 2-week (1.23%) post-irradiation (Table 3). The Figures 4A and B and 5A and B represent the differentiation percentage of the best variable group and the table with average differentiation percentages of all experimental groups.

**Immunofluorescence**

A similar differentiation pattern was observed in groups for immunofluorescence study. The immunofluorescence results further supported the differentiation of ADSCs toward a fibroblastic phenotype based on the expression of CD26 marker in all the experimental groups (LB, B, L) at 1- and 2-week post-irradiation. Figures 6A and B represent the best-captured expression pattern of CD26 marker among the experimental groups and in group L. Further on, the expression of CD49C marker also supported the differentiation of ADSCs toward a

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**TABLE 3. Flow Cytometry Experimental Groups with Percentages of Differentiation**

| Flow cytometry experimental groups | B            | LB           | L            |
|-----------------------------------|--------------|--------------|--------------|
| CD26 at 1-week post-irradiation   | 23.46% ± 9.1%** | 28.27% ± 3.6%** | 21.63% ± 0.7%** |
| CD26 at 2-week post-irradiation   | 10.80% ± 0.2%** | 25.37% ± 3.0%*** | 18.40% ± 2.8%*** |
| CD49C at 1-week post-irradiation  | 19.13% ± 2.96% |              |              |
| CD49C at 2-week post-irradiation  | 1.23% ± 0.0% |              |              |

The experimental groups of cells are labeled as follows: C, control; LB, laser and bFGF; B, bFGF; and L, laser. The statistical significance is presented as **P < 0.01, and ***P < 0.001; SEM compared with control cells with no irradiation and basic fibroblast growth factor added within the same time interval.
chondrogenic phenotype at 1- and 2-week post-irradiation in group L (Figure 7A and B).

DISCUSSION

The purpose of this study was to observe the effect of 660 nm diode laser at a fluence of 5 J/cm² in the differentiation of ADSCs into fibroblasts and chondrocytes. Even though photobiomodulation and stem cell therapy research have recently emerged in greater numbers, only a few studies have reported interesting findings beyond 72 hours of post-irradiation [35–39]. The uniqueness of the current study is that the results have established the photobiomodulatory effects on ADSCs beyond 72 hours and at 1, 2, and 3 weeks. Additionally, at a fluence of 5 J/cm², the results of the current study confirmed an increase in ATP proliferation at 24 and 48 hours; and beyond 72 hours. This concurs with results of a similar study reported at 24 and 48 hours, diabetic-wounded fibroblasts showed a significant increase in proliferation when irradiated with 830 nm diode laser at a fluence of 5 J/cm² [40]. An increase in the mitochondrial oxidative mechanism caused by the excitation of components of the respiratory chain leads to an increase of ATP, which is an effect that photobiomodulation expresses on the cells [41,42]. Consequently, the current study results emphasize the importance of ATP luminescence and evidence on the therapeutic applications of 5 J/cm² against degenerative TMJ disc disorder.

The results also were detailed, with all experimental groups having a high percentage of cellular viability post-irradiation, confirmed by the trypan blue exclusion assay. A related result was reported in the studies that had an increase in cellular viability, proliferation, and differentiation after using photobiomodulation (980 and 660 nm) [43,44]. Furthermore, the flow cytometry and immunofluorescence results of the current study support differentiation of ADSCs toward fibroblastic and chondrogenic phenotypes at 1 and 2 weeks. Similarly, a laser-induced differentiation was tested in ADSCs, where a fluence of 5 J/cm² at 636 nm induced differentiation into smooth muscle cells [45]. Another study using 810 nm laser documented significant differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) into neurons [46].

The effect of 660 nm at 5 J/cm² was observed in a study that reported in diabetic-wounded cells differentiation of fibroblasts into myofibroblasts [47]. Likewise, the flow cytometry analyses to determine the differentiated
fibroblast-like cells showed statistically significant results in B group at 1- (mean value at 23.46%) and 2-week (mean value at 10.80%) post-irradiation. Additionally, the high percentage of differentiation was recorded in LB group at the same time intervals compared with non-irradiated-unlabeled cells. These results have given encouraging the possibility of combined application of laser and bFGF for the differentiation of ADSCs to functional fibroblasts.

In the differentiation of ADSCs to chondrocytes, the use of bFGF experimental groups was excluded to exclude the interference of fibroblast and to report on the effect of laser beam alone. Recently, a study used lasers alone (810, 660, 532, and 485 nm) on mesenchymal stem cells derived from rabbit iliac bone marrow for differentiation to bone or cartilage [48]. In the present study, the expression of the CD49C marker was confirmed using flow cytometry in group L only at 1- and 2-week post-irradiation compared with their respective control group. This was subsequently confirmed with immunofluorescence. A similar result of photobiomodulation was observed in the study that used 405 nm, blue laser to differentiate the pre-chondrogenic ATDC5 (Mouse 129 teratocarcinoma AT805 derived) cells into chondrocytes [49]. These results indicate that the duration of incubation after irradiation could be a crucial factor for the differentiation of ADSCs to fibroblasts and chondrocytes.

Hence, photobiomodulation on its own can be recommended as a possible novel approach for the treatment of degenerative TMJ disc disorder. Also, the combined application of laser irradiation with 660 nm at 5 J/cm², and nanomaterials without growth factors could improve the process of tissue regeneration [50,51]. Since the TMJ disc is made of two-thirds of fibroblasts and less than one-third of chondrocytes, the results of the current study are promising and could promote various future investigations in TMJ disc tissue regeneration. A study described a novel treatment modality with the regeneration of spinal cord disks after the transplantation of fibroblasts into degenerated spinal disks of the rabbit [52]. It could be postulated that one could use differentiated ADSCs to replace the apoptotic cells of TMJ disc by process of transplantation. However, the previous research attempts to use tissue regeneration for the TMJ disc have been mostly misconceived due to misunderstandings of the constitution and function of the TMJ disc [53]. Moreover, a better understanding of inflammatory mediators in TMJ and TMJ disks, which play a major role in the process of deformation of cartilage.

Fig. 6. Immunofluorescent microscopic images confirm the differentiation of ADSCs to fibroblasts. In the figure, green fluorescence (FITC) represents the expression of CD26 confirming the differentiation of ADSCs to fibroblasts in group L at 1- (A) and 2-week (B) post-irradiation. The nuclear counterstaining DAPI is represented in blue color. The fibroblast morphology can also be clearly seen in the figure. DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate.
and collagen, could lead to a permanent solution for the treatment of degenerative TMJ disc [54–58].

In the future, additional studies will examine the laser-induced differentiation of ADSCs to fibroblasts and chondrocytes using advanced techniques. These may include gene expression studies and western blot analysis to confirm the specific expression patterns of marker genes and proteins, such as collagen type I alpha 1 (COL1A1) and type II (COL2), SRY-Box transcription factor 9 (SOX9), and aggrecan (ACAN) genes, as well as collagen I and collagen II proteins.

CONCLUSION

In conclusion, this study has observed significant results in viability, ATP proliferation, flow cytometry analyses, and immunofluorescence after 72 hours of 660 nm diode laser irradiation and has provided evidence to support the differentiation of ADSCs to fibroblastic and chondrogenic phenotypes. This treatment approach can provide a novel aid to patients suffering from TMJ disc abnormalities. However, future research studies should examine sustainability, expression of specific genes to determine cell differentiation, confirm no presence of tumorigenic factors of differentiated fibroblasts and chondrocytes, and inspect a wider range of laser wavelengths with a profound observation of biochemical processes. The outcome of this study has demonstrated that the application of 660 nm diode laser at a fluence of 5 J/cm² and ADSCs could be a positive drive in the direction of better treatment of the degenerative TMJ disc.

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