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Differentiation Potential of Adipose-Derived Stem Cells When Cocultured with Smooth Muscle Cells, and the Role of Low-Intensity Laser Irradiation

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Abstract

Objective: The aim of the study was to investigate the differentiation potential of adipose-derived stem cells (ADSCs) when cocultured with smooth muscle cells (SMCs), and to determine the role of low-intensity laser irradiation (LILI). ***Background data:*** ADSCs isolated from adipose tissue are isolated with ease and in large amounts. SMCs constitute most parts of the intestinal, urinary, reproductive, and cardiovascular systems. LILI has been found to have positive effects on different cell types, including ADSCs. ***Methods:*** The study used ADSCs (Stempro Adipose Derived Stem Cells-R7788-115) and SMCs (SKU-T-1 American Type Culture Collection HTB-114) cell lines. These cell lines were cocultured in a 1:1 ratio with and without growth factors and then exposed to LILI using 636 nm at 5 J/cm^2. ***Results:*** Cell viability and proliferation increased significantly in the cocultured groups that were exposed to LILI alone, as well as in combination with growth factors. Further, there was a significant decrease in the expression of stem cell markers with a concomitant increase in SMC markers. ***Conclusions:*** These results suggest that ADSCs have the ability to differentiate into SMCs when cocultured with SMCs, whereas LILI potentially augments the differentiation potential and need. This further highlights the significant role that LILI has to offer ADSC therapy in regenerative medicine.

Introduction

ADIPOSE-DERIVED STEM CELLS (ADSCs) are mesenchymal stem cells that have been isolated from adipose tissue in large amounts, and are able to self-renew and differentiate into one or more specialized cells.[1,2] ADSCs have a doubling time of 2–4 days, depending upon the culture medium and passage number.[3] Stem cells reside in a niche, and there are several factors that regulate their characteristics. Some of these characteristics include cell interactions, interactions between themselves and other cells, and interactions between stem cells and the extracellular matrix.[4] Stem cells play important roles in regenerative medicine. Scientists have postulated that stem cells could play such roles through paracrine pathways by secreting cytokines and growth factors.[5,6] ADSCs are able to stimulate the stem cell niche to produce stem cells that could be differentiated into the required cells. The ADSCs could also produce antioxidants, free radical scavengers, and heat shock proteins to the ischemic area, thereby recovering the damaged cells. Mitochondria could also be delivered to the damaged cells,[7] and, finally, the ADSCs could differentiate into the required lineage.[5]

Studies have shown that mesenchymal stem cells can differentiate into smooth muscle cells (SMCs).[8] These cells are part of the cardiovascular, intestinal, urinary, and reproductive systems of the body, and, therefore, are important in controlling diseases affecting these organs.[9,10] These cells play important roles in angiogenesis and vasculogenesis during embryonic development.[9] ADSCs have been differentiated into cardiomyocytes in a cardiac microenvironment when cocultured directly or indirectly with cardiomyocytes.[11]

"Laser" is an acronym of light amplification by stimulated emission of radiation. It is monochromatic, coherent, and directional. Low-intensity laser irradiation (LILI) is a form of phototherapy that involves application of monochromatic light in the range of 630–905 nm wavelengths.[12] It has had stimulatory effects on many cells, including ADSCs. It has been shown to increase viability and proliferation of ADSCs.[13] Studies on LILI and stem cells have shown that LILI can change the metabolism of stem cells and increase adenosine triphosphate (ATP) production, and, therefore, increase migration of the cells.[14] It has also been found to accelerate wound healing as well as promoting angiogenesis.[15,16] Therefore, LILI may play an auxiliary

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TABLE 1. LASER PARAMETERS

Laser parameters	
Wavelength (nm)	636
Wave emission	Continuous wave
Power output (mW)	85
Spot size (cm^2)	9.08
Output density (mW/cm^2)	9.3
Irradiation duration	9 min 10 sec
Fluence (J/cm^2)	5

role in stem cell proliferation and differentiation and thus contribute to their role in regenerative medicine as described previously.

The aim of the study was to investigate the differentiation potential of ADSCs when cocultured with SMCs, and the role of LILI on these cocultured cells. Assessments were performed and monitored over a period of 1 week.

Materials and Methods

Cell culture

Primary ADSCs were isolated from human adipose tissue of the consenting donors undergoing abdominoplasty as described.[13] The Academic Ethics Committee of the Faculty of Health Sciences of the University of Johannesburg granted the ethical approval (Approval Number 01/06). After isolation, these cells were cultured in Dulbecco's Modified Eagle Medium (DMEM F12) (Sigma, D8062, SIGMA-Aldrich, Kempton Park, South Africa) with 10% fetal bovine serum (FBS) (Biochrom, S0615, Biocom biotech, Centurion, South Africa), 0.1% penicillin/streptomycin (Sigma, P4333, SIGMA-Aldrich, Kempton Park, South Africa), and 1 μg/mL Fungizone (Sigma, A2942, SIGMA-Aldrich, Kempton Park, South Africa) incubated at 37°C in an atmosphere of 5% carbon dioxide (CO_2) in a HERA CELL 150 incubator (Heraeus, 44857, Separation Scientific, Honeydew, South Africa). SMCs of a commercial cell line SKUT-1 (HTB-114, ATCC, USA) were also cultured in the same medium with similar conditions as ADSCs. After reaching semiconfluency for ADSCs and confuency for SKUT-1, both cell types were cocultured directly in 3.4 cm^2 diameter dishes in a 1:1 ratio with and without the growth factors in MCDB 131 medium (Gibco, 10372-019, Life technologies, Roosevelt, South Africa), with 2% FBS, 0.1% penicillin/streptomycin, and 1 μg/mL Fungizone incubated at 37°C in an atmosphere of 5% CO_2. The cells were cocultured at an individual concentration of 1×10^2/mL for each cell type. The cocultures were divided into six groups. Group 1 were cocultures without the growth factors and were not exposed to LILI (CC), group 2 were cocultures without growth factors but were exposed to LILI (CC+LILI), group 3 were cocultures with growth factor and retinoic acid (RA) (Sigma, R2605, Sigma-Aldrich, Kempton Park, South Africa) and not exposed to LILI (CC+RA). Group 4

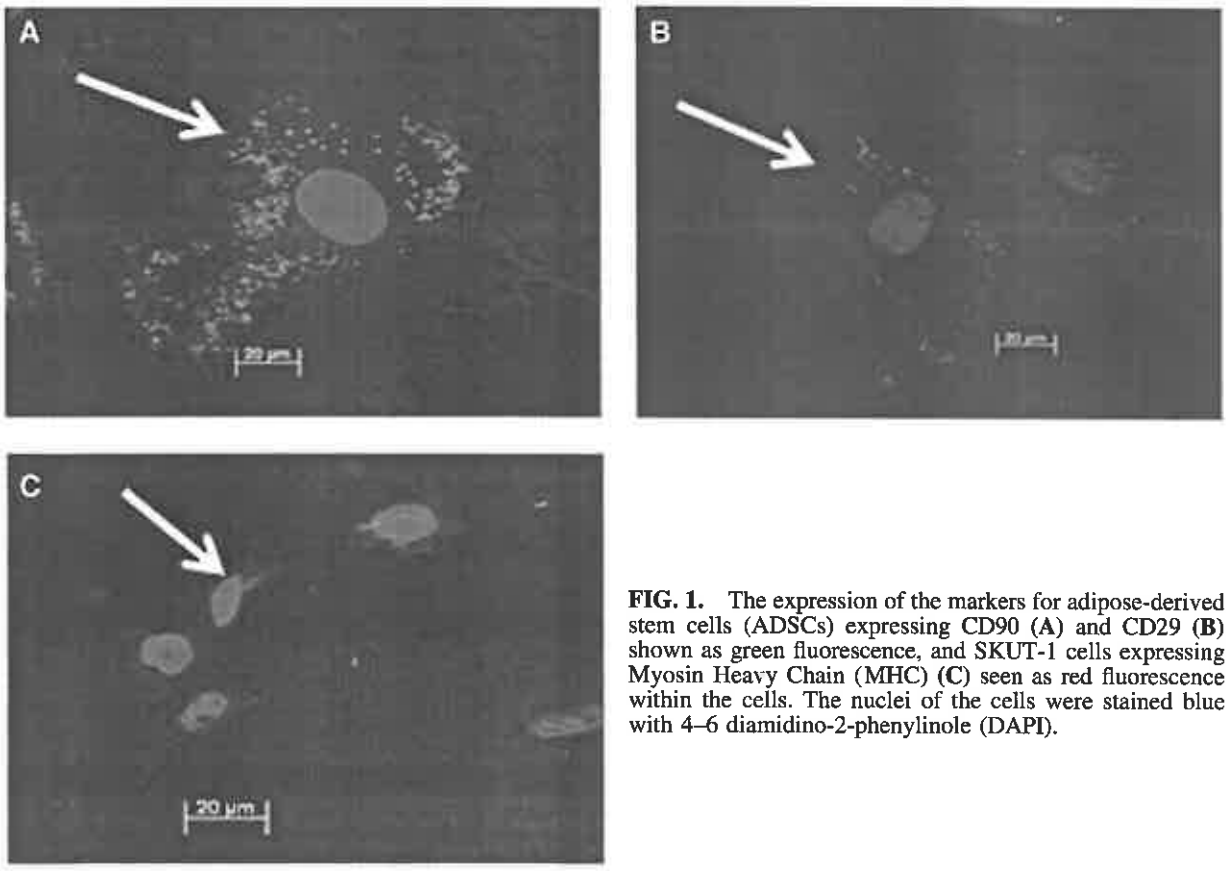

FIG. 1. The expression of the markers for adipose-derived stem cells (ADSCs) expressing CD90 (**A**) and CD29 (**B**) shown as green fluorescence, and SKUT-1 cells expressing Myosin Heavy Chain (MHC) (**C**) seen as red fluorescence within the cells. The nuclei of the cells were stained blue with 4–6 diamidino-2-phenylinole (DAPI).

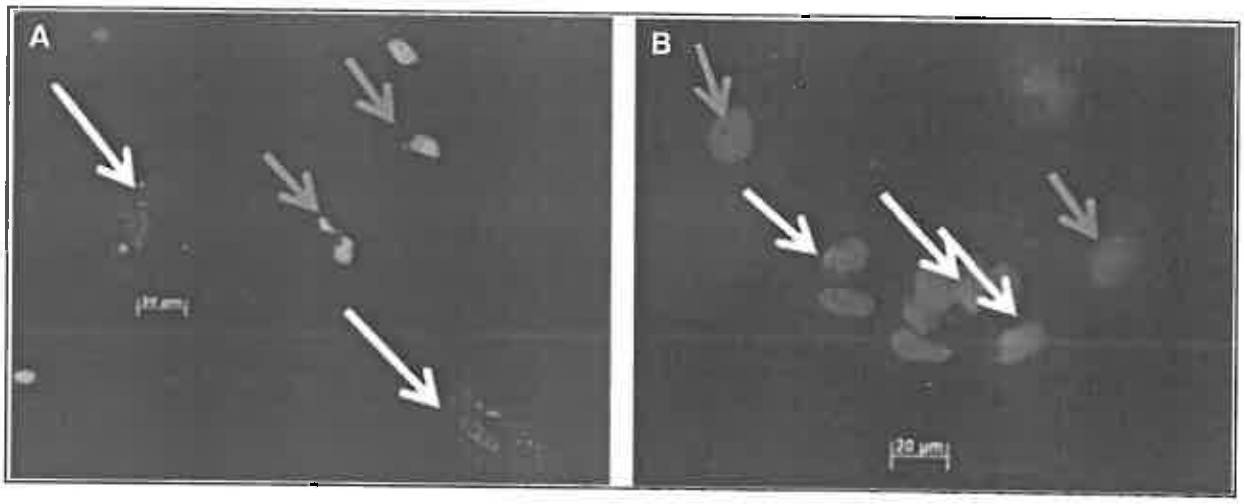

FIG. 2. Expression of the markers in a coculture. **(A)** Expression of the adipose-derived stem cell (ADSC) marker CD29 is shown by white arrows, and gray arrows are showing SKUT-1 cells, which did not express CDE29 after 24 h. **(B)** More cells expressed MHC after 1 week, as shown by white arrows. Gray arrows are showing cells that did not express MHC.

were the cocultures with RA exposed to LILI (CC+RA+LILI), group 5 were the cocultures with a growth factor, transforming growth factor beta 1 (TGF-β1) (Invitrogen, PHG 9204, Life technologies, Roosevelt Park, South Africa) but not exposed to LILI (CC+TGF-β1), and group 6 were the cocultures with TGF-β1 and were exposed to LILI (CC+TGF-β1+LILI). RA was added to the cocultures at a concentration of 0.1 μM and TGF-β1 was added at 1 ng/mL.

Laser irradiation

The cocultures of group 2, 4, and 6 were exposed to a diode laser (Oriel, LTIO00-PLT20, Oriel Corporation, USA) at 5 J/cm^2 with a wavelength of 636 nm in the dark. The medium was removed and cells were washed with Hanks Balanced Salt Solution (HBSS) and then complete medium was added to the plates. Low-laser irradiation was then delivered to the plate via the optical fibre as described previously.[13] Irradiation was performed once, and the cocultures were incubated for 1 week after which various assays were performed. The cocultures that were not irradiated were used as controls and kept under the same conditions as the irradiated ones. The laser parameters are shown in Table 1.

Cellular morphology

Cell morphology of individual cells was assessed by culturing the cells on heat sterilized cover slips and after confluency, the cells were stained with carboxyfluorescein diacetate succinimidyl ester (5[6]-CFDA,SE) (Invitrogen, C1157, Life technologies, Roosevelt, South Africa) and observed using the Carl Zeiss Axio Observer Z1 fluorescence microscope (195-041872, Carl Zeiss, Randburg, South Africa).

Cell viability

ATP luminescence was used to assess cellular viability. Cellular ATP was measured using the Cell Titer-Glo luminescence cell viability assay (Promega, G7573, Anatech, Randburg, South Africa) as described previously.[17] Trypan blue test was also used to assess cellular viability. Equal volume of the cell suspension and trypan blue reagent (Invitrogen, T10282, Life technologies, Roosevelt, South Africa) were mixed and analyzed as described in Mvula et al.[18]

Cell proliferation

Proliferation of the cocultures were analyzed by using optical density (OD) where the absorbance of one hundred microliter of the cell suspension was read at A_{540} nm in a Perkin Elmer, Victor^3 (Perkin Elmer, 1420, Separation Scientific, Honeydew, South Africa).[19]

Immunofluorescence

Cells cultured on heat sterilized cover slips were grown to semiconfluency, rinsed three times with phosphate-buffered

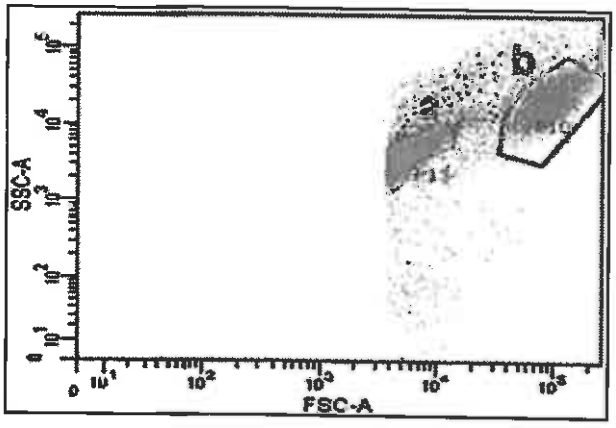

FIG. 3. Flow cytometry results showing the two distinct type of cells in a coculture 24 h after culturing in a ratio of 1:1 as seen in **(a)** SKUT-1 and **(b)** adipose-derived stem cells (ADSCs). At 1-week postirradiation and coculturing, flow cytometry results did not distinguish the two types of cell populations, but rather indicated one single population consisting of one cell type.

had growth factor TGF-β1 and were irradiated, as compared with the control group (CC) (Table 3). This marker showed no significant change in the group that had RA (CC+RA) but it increased in the groups that had RA and were irradiated (CC+RA+LILI) as well as in the group that had TGF-β1 (CC+TGF-β1), although the increases were not significant.

Discussion

The morphology of both types of cells did not show any difference postirradiation. These results have previously been published.[20] The characterization results confirmed that the cell lines used were ADSCs and SMCs, as both cell lines expressed the characteristic markers.[3,17,21] After co-culturing for 1 week, flow cytometry results could not show two distinct population of the two types of the cells co-cultured. This may be indicative of ADSCs differentiating into SMCs. Studies have shown that coculturing induces an increase in differentiation of stem cells. In the study done by Zuo et al.,[22] bone marrow mesenchymal stem cells differentiated into chondrocytes in a direct coculture system; however, a static coculture system has resulted in decreased viability and proliferation over a long period of time.[23] The viability and proliferation results in this study agree with what scientists have demonstrated in recent years. LILI increased cellular growth after culturing cells under conditions of fewer nutrients.[24] It increased cellular viability, proliferation, collagen production and the release of growth factors in ADSCs and other cells.[21,25] In this study, the cocultures that were irradiated increased in viability and proliferation compared with those that were not exposed to LILI.

Growth factors such as RA and TGF-β play important roles in many cells, including ADSCs and SMCs. There has been neural differentiation from embryonic stem cells promoted by RA. This growth factor was found to have inhibitory effects on fibroblast growth factor signalling, which prevented self-renewal of stem cells and increased their differentiation into neurons.[26] This could have been the reason why this study showed a decrease in proliferation in the cocultures that had growth factors. In another study done by Tong and Andrews, RA was found to be one of the mediators for neuronal differentiation from human pluripotent stem cell lines after a long exposure and intercellular communication.[27] Studies have shown that TGF-β can proliferate or inhibit the growth of cultured SMCs,[28] and TGF-β1 has been shown to promote differentiation of stem cells into SMCs.[29] TGF-β3 was found to promote differentiation of ADSCs into cartilage.[30] The results in this study found that the addition of growth factors decreased proliferation of the cocultures. This may be explained by the fact that after a period of 1 week in culture, the cells may have overgrown and, therefore, decreased in viability. The cocultures also showed a decrease in expression of the markers for ADSCs, whereas the SMC marker increased. This may be the result of differentiation of ADSCs into SMCs.

Conclusions

ADSCs play a major role in treating several regenerative diseases; however, they have to be produced in large numbers and differentiate into the required cell lineage like SMCs. SMCs have been differentiated from ADSCs after addition of growth factors. In this study, the expression of the ADSC markers decreased, and those of SMCs increased, at 1 week. This could indicate that the ADSCs were differentiating into SMCs. However, most studies performed on ADSCs revealed that differentiating into other cells could take a longer period of time. Coculturing and LILI could enhance differentiation of ADSCs into SMCs, but a longer period of culturing should be allowed in order to successfully achieve differentiation. If this is achieved, coculturing, addition of the growth factors, and exposing the cocultures to LILI would be a significant method in differentiating ADSCs into cells that would be required for therapeutic use in tissue engineering and regenerative medicine.

Author Disclosure Statement

No competing financial interests exist.

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