

The Use Of Laser Irradiation To Stimulate Adipose Derived Stem Cell Proliferation And Differentiation For Use In Autologous Grafts

Heidi Abrahamse

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

Abstract. Stem cells are characterized by the qualities of self-renewal, long term viability, and the ability to differentiate into various cell types. Historically, stem cells have been isolated from the inner cell mass of blastocysts and harvesting these cells resulted in the death of the embryo leading to religious, political and ethical issues. The identification and subsequent isolation of adult stem cells from bone marrow stroma have been welcomed as an alternate source for stem cells. The clinical use of Mesenchymal Stem Cells (MSCs) presented problems such as limited cell number, pain and morbidity upon isolation. Adipose tissue is derived from the mesenchyme, is easily isolated, a reliable source of stem cells and able to differentiate into different cell types including smooth muscle. Over the past few years, the identification and characterization of stem cells has led the potential use of these cells as a promising alternative to cell replacement therapy. Smooth muscle is a major component of human tissues and is essential for the normal functioning of many different organs. Low intensity laser irradiation has been shown to increase viability, protein expression and migration of stem cells *in vitro*, and to stimulate proliferation of various types of stem cells. In addition, the use of laser irradiation to stimulate differentiation in the absence of growth factors has also been demonstrated in normal human neural progenitor cells (NHNPCs) *in vitro* where NHNPCs are not only capable of being sustained by light in the absence of growth factors, but that they are also able to differentiate normally as assessed by neurite formation. Our work has focused on the ability of laser irradiation to proliferate adipose derived stem cells (ADSCs), maintain ADSC character and increase the rate and maintenance of differentiation of ADSCs into smooth muscle and skin fibroblast cells. Current studies are also investigating the effect of different irradiation wavelengths and fluences on ADSC viability and proliferation. This paper reviews the development of MSCs as potential therapeutic interventions such as autologous grafts as well as the contribution of low intensity laser irradiation on the maintenance of these cells.

Keywords: Adipose Derived Stem Cell; differentiation; Smooth Muscle Cell; Autologous grafts
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INTRODUCTION

Regenerative medicine refers to the medical intervention to regenerate cells, tissues, or organs lost after accidents or diseases and restore body functions. The idea of regeneration in medical care has a long history. It includes rehabilitation or training for recovery of physical functions; the use of artificial hands, legs, and joints made from synthetic materials; and living cell transplantation such as skin transplantation, bone marrow transplantation, and organ transplantation. Damaged organs or tissues used to be treated by using an intervention of drug therapy or surgery to slow the progression of disease. Surgical intervention by organ transplantation has further been

advanced where the challenging problems of lack of organ donors, rejection after organ transplantation, and ethical issues of transplantation of another person's organs have been overcome. There are different types of regenerative medicine, one of which is stem cell transplantation, eliminating many of these historical disadvantages of surgical interventions.

Stem cells have been extensively studied along with the recent developments in culture technology, molecular biology, tissue engineering, and genetic engineering, and stem cell-based regenerative medicine now draws attention as an international project. Stem cells have the ability to grow into specific cells and renew themselves in an undifferentiated state for a long time. In other words,

stem cells are master cells that grow into tissues or organs. The use of the patient's own cells to regenerate tissues or organs and restore function will overcome immunological rejection and ethical problems [1,2].

Regenerative medicine appears to be a medical treatment of unlimited potential, maximizing the innate regenerative ability of the body. Repair or replacement of defective tissue to restore its normal function involves three main components: inductive compounds (such as growth and angiogenic factors), tissue-relevant cells, and an extracellular matrix or other material to provide the proper structural information. The potential risk of using diseased tissue as a source of cells for tissue engineering necessitates the investigation of other possible cell sources. One promising source is human adult stem cells [3].

STEM CELL THERAPY

Stem cells are defined as cells that have the ability to perpetuate themselves through self-renewal and to generate mature cells of a particular tissue through differentiation. Due to their ability to differentiate into various tissue types by asymmetric replication, have presented significant new opportunities for therapy.

Sources of Stem cells

Embryonic Stem Cells

Early human embryonic tissue contains totipotent stem cells, which can give rise to all the tissues of the human body. Such cells can be isolated from normal blastocysts. Embryonic stem cells (ESCs) can be maintained in culture as undifferentiated cell lines or induced to differentiate into many different lineages. Between 4 and 6 week old embryonic tissue contain pluripotent stem cells while fetal tissue stem cells are pluripotent and multipotent. Potency of stem cells indicate the multilineage potential which is indicative of the different number of potential cell types that it can differentiate into.

Pluripotent stem cells are cells with the potential to become any differentiated cells in the body except those of the placenta. Multipotent stem cells can be differentiated into a limited number of cell types while unipotent stem cells can only be differentiated into one type of cell type of the body (Figure 1). In spite of their tremendous potential, however, ESCs are the subject of considerable controversy due to the nature of isolating and securing ESCs which result in embryonic death. Furthermore, ESCs may not be safe,

and neoplasia could be stimulated by the introduction of these totipotent cells as a form of stem cell therapy [4].

Adult Stem Cells

Ethical concerns surrounding the use of ESCs have stimulated investigators to explore the capabilities of adult stem cells (ASCs) to contribute to remodelling of diverse tissues and organs. Bone marrow stem cells have been a favoured target of investigation. The ability of bone marrow-derived stem cells to contribute to a number of different tissues has been demonstrated *in vitro* and *in vivo* in animal models and, more recently, in human transplantation chimeras [5].

Tissue damage caused by different mechanisms is believed to be responsible for the homing of stem cells and their differentiation into specific tissue-related cells. Many tissues in adult animals have been shown to contain reservoirs of stem cells with restricted differentiation capacity and are usually lineage-specific (Figure 1).









STEM CELL SOURCES AND POTENCY	EMBRYONIC		5-7 day Embryo		Totipotent
		4 week Embryo		Pluripotent	
		6 week Embryo		Pluripotent	
		Fetal			Pluripotent / Multipotent
	ADULT		Infant		Pluripotent / Multipotent
		Adult			Pluripotent / Multipotent

FIGURE 1. Embryonic and Adult Stem Cell Sources and multilineage potential.

ASCs are classified as pluripotent or multipotent and can proliferate in culture without senescence and can differentiate into mesodermal, endodermal, and neuroectodermal cell types. Interestingly, multipotent adult progenitor cells are not confined to the bone marrow and have been isolated from muscle, brain, and skin and can be made to differentiate into endothelium, neurons, hepatocytes, and other cell types. ASCs reside permanently in most organs.

These cells (known as tissue stem cells) can generate the mature cells of the organs in which they reside. Their differentiation commitment, however,

can change when they are transplanted into a different tissue. Tissue stem cells are located in sites called niches, which differ among various tissues. Niches have been identified in the bulge area of hair follicles and the limbus of the cornea and in the gastrointestinal tract at the isthmus of stomach glands. A change in stem cell differentiation from one cell type to another is also referred to as transdifferentiation, and the multiplicity of stem cell differentiation as plasticity [4]. Human umbilical cord blood has also been explored as an alternative source of stem cells. These cells are self-renewing and are capable of differentiating into multiple cells and tissues.

Human stem cells may offer considerable opportunities for providing differentiated cells for gene therapy, drug discovery, and regenerative medicine. Growth advantages of the corrected stem cells could offer new therapeutic approaches for genetic diseases [6].

Stem Cell Differentiation

Several soluble factors have been shown to direct differentiation of embryonic stem cells including IL-3 which directs cells to become macrophages, mast cells or neutrophils; IL-6 which directs cells to the erythroid lineage; retinoic acid which induces neuron formation; and transforming growth factor (TGF)- β 1 which induces myogenesis⁸. Bone marrow-derived stem cells are able to differentiate into epithelial cells of the liver, lung, gastrointestinal tract, and skin. Adipose tissue represents a potential alternative reservoir of cells with stem cell properties such as self-renewal and pluripotency. Cells isolated from the stromal vascular fraction of human adipose tissue termed processed lipoaspirate cells possess multilineage potential.

Additionally, Adipose Derived Stem Cells (ADSCs) isolated from lipo-aspirates have shown to improve postnatal neovascularization, muscle regeneration and have osteogenic, cardiomyogenic and stromal capacity [8]. The multilineage capacity and ease of isolating large quantities of ADSCs makes them promising candidates for stem cell and regenerative therapy.

Clinical Uses Of Stem Cell Therapy

Stem Cell Therapy has produced significant results in the treatment of many diseases previously believed to be incurable. It holds great promise against a wide variety of degenerative illnesses such as Alzheimer's Disease, Parkinson's Disease, diabetes and the consequences of diabetes, arthritis, Multiple Sclerosis, heart disease, blood cancers and other blood diseases,

skin disease, vascular disease and spinal cord injuries. There are 4 mechanisms whereby ASCs or MSCs can be applied in therapy. These include local implantation for localized diseases, systemic transplantation, combining stem cell therapy with gene therapy, and the use of ASCs or MSCs in tissue engineering protocols. Clinical trials have shown promising results with the administration of MSCs for osteogenesis imperfecta, Hurler's syndrome, and methachromatic leukodystrophy and to enhance engraftment of heterologous bone marrow transplantation [6].

A variety of sources, such as bone marrow, peripheral blood, umbilical cord blood, adipose tissue and skin/ hair follicles, have been utilized to isolate stem cells to modulate the healing response of acute and chronic wounds. The embryo with its developmental plasticity and high proliferative capacity has traditionally been the preferred source for the isolation of pluripotent stem cells. However, pluripotent somatic cells have been isolated that, by direct reprogramming to generate human dermal fibroblasts and other human somatic cells are comparable to the differentiation potential of human embryonic stem cells. In addition, differentiated adult tissues have been shown to harbor pluripotent stem cells with unexpected plasticity. Thus, stem cells derived from adult sources may have the same clinical and experimental potential as embryonic stem cells.

The development of cellular therapy using stem cells has been advanced by the study of basic mechanisms of cell proliferation and differentiation. It is evident that the plasticity of the different types of stem cells, both in vitro and in vivo, will have clinical applicability in the future. The intrinsic molecular mechanisms that keep stem cells pluripotent or direct them along particular differentiation pathways requires extensive further investigation [5].

ADIPOSE DERIVED STEM CELLS

Adipose-derived stem cells represent another available source of pluripotent cells with characteristics similar to bone marrow-derived mesenchymal stem cells. Human liposuction aspirates have been used to culture adipo-derived mesodermal stem cells and differentiate them into a number of different tissue cell types including cardiac, muscle, nerve, cartilage, adipose, liver and bone. In addition, ADSCs can also be used for gene therapy and promotes angiogenesis (Figure 2).

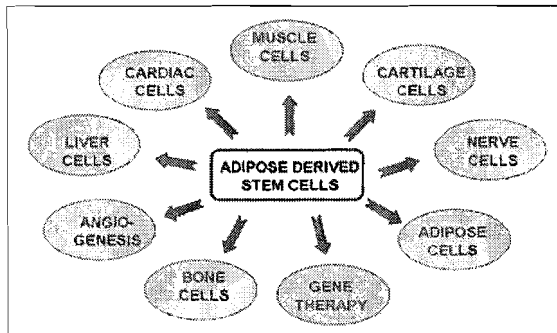


FIGURE 2. Multilineage potential of Adipose Derived Stem Cells in Human Stem Cell Therapy.

Autologous Adipose-Derived Stem Cells

Adipose is the richest and most accessible known source of stem cells. It contains a specialized class of stem cells comprised of multiple cell types that promote healing and repair. Adipose stem cells have been shown to differentiate into multiple cell types and beyond differentiation, may provide therapeutic benefit through the release of growth factors and other therapeutic healing mechanisms. The major advantages of adipose tissue as a source of regenerative cells are the yield which is much higher than other sources, the autologous capacity which by implication reduces immune rejection or transmission and the versatility of ADSCs in their mechanism of action¹⁰ (Figure 3).

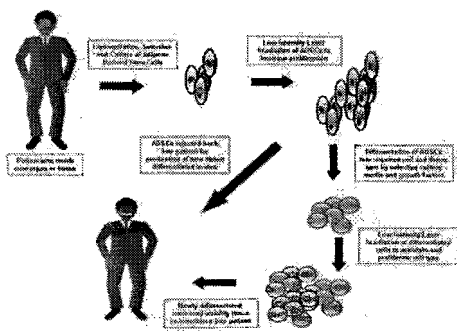


FIGURE 3. Autologous use of ADSCs and suggested intervention using LILI to proliferate and/or maintain differentiation prior to reintroduction into patient.

Harvesting adipose tissue by liposuction from which to obtain ADSCs is technically straightforward, safe, causes relatively little discomfort, and is free of ethical drawbacks [9]. Adipose-derived stem cells may represent another available source of pluripotent cells with characteristics similar to bone marrow-

derived mesenchymal stem cells. Recent studies indicate that adipose-derived stem cells promoted human dermal fibroblast proliferation by direct cell-to-cell contact and by secretory induced paracrine activation which significantly accelerated the re-epithelialization of cutaneous wounds [6].

The multilineage capacity of ADSCs makes them promising candidates for reconstruction of human smooth muscle related tissues and organs while adipose tissue can be harvested in large quantity with minimal morbidity [8]. The stromal compartment of mesenchymal tissues is thought to harbor stem cells that display extensive proliferative capacity and multilineage potential. Stromal stem cells offer a potentially large therapeutic potential in the field of regenerative medicine. Adipose tissue contains a large number of stromal stem cells, is relatively easy to obtain in large quantities and thus constitutes a very convenient source of stromal stem cells. Importantly, the number of stem cells obtained is compatible with extensive analyses of the cells in an uncultured, freshly isolated, form.

LASER IRRADIATION

Studies on Low Intensity Laser Irradiation (LILI) and stem cells have shown that laser irradiation increased migration of stem cells and suggests that it could affect the metabolism of stem cells, which in turn, could also be indicative of increased cell proliferation¹. LILI (frequently red or near-infrared) can have significant therapeutic effects on multiple classes of diseases, injuries and medical disorders. In particular it is effective for wound healing and pain control as well as reduction of inflammation and swelling. Laser irradiation at different intensities has been shown to inhibit and stimulate cellular processes. Recent findings suggest that at the cellular level, laser energy of a particular wavelength can initiate signalling cascades, such as those that promote cellular proliferation [10].

It is believed that the primary cellular chromophore that absorbs low levels of red and near-infrared light is cytochrome c oxidase, which is located in the inner mitochondrial membrane and an integral part of the electron transport chain associated with oxidative phosphorylation and ATP production. This absorption of energy may lead to increase in ATP synthesis and release of reactive oxygen species from the electron transport chain that can subsequently activate transcription factors and lead to cell proliferation and migration [10].

In our laboratories ADSCs were isolated from human adipose tissue, lipo-aspirates, and the effects of LILI alone, as well as in combination with growth factors inducing differentiation into smooth muscle cells or skin fibroblasts, were evaluated in vitro immediately after irradiation (0 h), 24 h and 48 h after irradiation. Western blot analysis confirmed that the isolated and cultured cells were, indeed, stem cells by the expression of $\beta 1$ -Integrin and Thy-1, both stem cell markers known to be expressed by ADSCs. In our study the increase in cell viability, expressed as a function of optical density, and proliferation expressed as a function of ATP concentration, was more pronounced in cells that had been exposed to 5 J/cm² LILI than in cells that had not received any irradiation [2,11] (Table 1). In addition, expression of stem cell and differentiation markers were more pronounced in cells exposed to irradiation in a time dependent fashion [2,4,11].

In most tissues, stem cells are rare. As a result, stem cells must be identified prospectively and

TABLE 1. Human ADSCs isolation, confirmation and response to growth factor treatment and LILI [2,4,11].

EXPERIMENT		TESTS	
Human ADSCs	Morphology	Viability and Proliferation	Expression of Markers
Isolation of ADSCs from lipo-aspirates	Normal Stem cell clusters that precipitate and become anchored cell lines	Slow but normal	$\beta 1$ -Integrin and Thy-1 expression
LILI (636 nm; 5 J/cm ²) 0 hours	Normal post-irradiation	Increased viability and proliferation	Optical density and ATP luminescence
LILI (636 nm; 5 J/cm ²) 24 h and 48 h post-irradiation	Normal increased growth towards fluency	Increased viability and proliferation at both 24 and 48 hours	Increased expression of $\beta 1$ -Integrin and Thy-1 at 24 hours – reduced expression at 48 hours
Differentiation into skin fibroblasts	Change of morphology from ADSC to typical Smooth muscle morphology	Irradiation maintains and increases viability and proliferation	bFGF expression
Differentiation into smooth muscle cells	Change of morphology from ADSC to typical Fibroblast morphology	Irradiation maintains and increases viability and proliferation	SMA-actin; Desmin; Smoothelin; SMMHC expression

purified carefully in order to study their properties. Although it seems reasonable to propose that each tissue arises from a tissue-specific stem cell, the rigorous identification and isolation of these somatic stem cells has been accomplished only in a few instances [12].

Despite many reports of positive findings from experiments conducted in vitro, in animal models and in randomized controlled clinical trials, LILI remains controversial. This likely is due to two main reasons; firstly the biochemical mechanisms underlying the positive effects are incompletely understood, and secondly the complexity of rationally choosing amongst a large number of illumination parameters such as wavelength, fluence, power density, pulse structure and treatment timing has led to the publication of a number of negative studies as well as many positive ones.

DISCUSSION

Compared with embryonic stem cells, adult stem cells have at least as great, if not greater, potential for biomedical application, but without the medical risks or the ethical controversy. The biomedical potential of

adult stem cells is enormous. Adult stem cells have already been used successfully in treatments for many diseases with encouraging results. Animal models

using ASC treatments indicate that therapeutic treatments for pernicious diseases such as diabetes, heart disease, Parkinson's, and stroke are well within the vast therapeutic capabilities of adult stem cells.

Additionally, science is continuing to discover human adult stem cells for an increasing number of cell and tissue types. Our work showed that LILI, a

treatment modality that involves the application of low-power monochromatic and coherent light in the treatment of numerous diseases, is indeed, a safe treatment and may actually stimulate adult stem cells in vivo to proliferate, which could aid in the healing process. However, the long-term effects of the exposure of stem cells to LILI require further investigation. ADSCs are considered to be a great source of stem cells for tissue engineering and regenerative medicine. Our work suggests that growth factors in combination with LILI can increase the numbers and viability of cultured ADSCs. This is an important step in the expansion of stem cell numbers in vitro, especially in light of the potential role that ADSCs could play in regenerative medicine and tissue engineering, particularly in the use of autologous tissue transplants [13].

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