Adipose Derived Stem Cells (ADSCs) and Low Intensity Laser Irradiation (LILI): Potential use in Regenerative Medicine

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Abstract. Adipose tissue is highly specialised and a reliable source of adult stem cells. ADSCs can be harvested easily and in large amounts from adipose tissue or lipo-aspirates. Collagenase digestion is one of the procedures used in generating these cells from adipose tissue. ADSCs have been shown to have the differentiation potential to differentiate into other cells including smooth muscle, bone, nerve, heart, cartilage, liver and fat. LILI, a form of phototherapy, involves the application of monochromatic light in the 630 to 905 nm (visible to near infrared) range to biological tissue. Application to a variety of different cell types has been found to enhance cell viability, proliferation and differentiation of ADSCs. The differentiated cells from ADSCs are a major component of cardiovascular, reproductive, urinary, neural and intestinal systems and play a key role in diseases like arterioscelosis, asthma, hypertension and cancer. These cells could be used in regenerative medicine and tissue engineering. This review discusses ADSCs and LILI and their potential use in regenerative medicine.

1. Adipose Derived Stem Cells

Adult stem cells can be isolated from adipose tissue and lipo-aspirates in significant numbers and exhibit stable growth and proliferative kinetics in culture. These cells are termed ADSCs [1]. ADSCs have been isolated from donors with reduced morbidity, they have been multiplied and handled easily compared to bone marrow cells [2]. In the past decade several studies have provided preclinical data on safety and efficacy of these cells, supporting the use of these cells in future clinical applications [3]. Adipose tissue as a source of these cells allows them to be obtained in large quantities without difficulties and at a minimal risk [4]. White adipose tissue can be obtained in large quantities from human tissue and the stem cells residing in it are easily harvested from the tissue with the ability to differentiate into several cell lineages [5,6].

Cells generated from adipogenic origin have also shown to differentiate not only into osteoblasts, chondrocytes, myocytes, cardiomyocytes, fibroblasts and adipocytes but also into vascular lineages such as endothelial, smooth muscle blood cells [7-11]. This is due to the fact that they can release potent angiogenic factors such as leptin and vascular endothelial growth factor [12,13]. In cell transplantation, ADSCs have been found to promote radiological ossification efficiently, 90% of fractures healed eight weeks after surgery and during this process, blood perfusion enhancement through neovasculatisation was observed [14]. These cells have been shown to restore dystrophin expression of Duchenne skeletal-muscle cells *in vitro* [15]. ADSCs may constitute a potential cell based therapeutic alternative for the treatment of pancreatic ductical adenocarcinoma (PDAC) after being found to strongly inhibit human PDAC cell proliferation both *in vivo* and *in vitro* [16].

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Due to their plasticity and easy harvesting, ADSCs are the preferred stem cells to be used in tissue engineering rather than bone marrow derived stem cells [17,18]. However, ADSCs have certain limiting characteristics due to several factors. First, their capacity is limited in terms of subculturing and secondly, adipose tissue varies in its metabolic activity and as well as its capacity for proliferation and differentiation depending on the location of the depot of tissue and the age and gender of the patient [19,20].

2. Low Intensity Laser Irradiation

LILI, a form of phototherapy, involves the application of monochromatic light to biological tissue to elicit a biomodulative effect within that tissue. LILI is now accepted in many countries and is used in medical and dental practices. It elicits both a photobiostimulative and a photobioinhibitive effect within the irradiated tissue, each of which can be used in a number of therapeutic applications. LILI is not thermic (it does not produce heat) [21], and uses monochromatic light in the 630 to 905 nm range of the electromagnetic spectrum [22]. It stimulates capillary growth, granulation tissue formation and alters cytokine production. Altered keratinocyte motility and fibroblast movement have also been shown following low intensity laser irradiation [23]. These effects aid in the treatment of disorders like acute or chronic tissue hypoxia, destruction of tissues, as well as altered cell metabolism. Studies on LILI and stem cells have shown that low intensity irradiation can alter the metabolism of stem cells, increase adenosine triphosphate (ATP) production and so increase migration [24].

It has been shown that 5 J/cm^2 of laser irradiation at a wavelength of 635 nm positively affects ADSCs by increasing cellular proliferation, viability, and expression of β1-Integrin and Thy-1 (established stem cell markers), [25] and LILI in combination with epidermal growth factor (EGF) enhances the proliferation of ADSCs [26]. Several studies have been conducted on ADSCs identifying the effects of LILI at a cellular and molecular level (Table1).

Table 1. Studies conducted on ADSCs and/or differentiation inducers and/or LILI.

Study	Laser Parameters/ Differentiation Inducer (DI)		Results	References
ADSCs & LILI	5 J/cm^2 635 nm	No DI	Increased viability & proliferation	[25]
ADSCs & LILI	5 J/cm^2 636 nm	EGF	Increased viability & proliferation	[26]
ADSCs & LILI	10 & 15 J/cm^2 830 nm	No DI	Decreased viability & proliferation	[37]
ADSCs	No LILI	Retinoic Acid	Increased viability, proliferation & differentiation	[38]
ADSCs & LILI	5 J/cm^2 636 nm	Retinoic Acid	ADSC differentiation into smooth muscle cells	[38]
ADSCs	No LILI	Angiotensin II, Sphingosylphospho-Rylcholine, TGF-β1	ADSC differentiation into smooth muscle cells	[39]
ADSCs trans-planted in ische-mic mouse limbs	No LILI	No DI	Enhancement of angiogenesis & osteogenesis	[40],[14]
ADSCs	No LILI	Heparin	Differentiation into smooth muscle cells	[41]

Studies have also shown that LILI can increase proliferation of cells, cellular attachment, differentiation and production of transformation growth factor beta1 (TGF-β1) in human osteoblasts cells indicating that, *in vitro*, LILI can modulate the activity of cells and tissues [27]. Fiszerman and Markmann, (2000), discovered that LILI enhances wound healing in chronic diabetic foot ulcers [28]. LILI has been successfully used for pain attenuation and to induce wound healing in non-healing defects [29]. In addition, Abrahamse and co-workers, (2010), demonstrated that increasing the fluence and wavelength caused a decrease in ADSC viability and proliferation in a reciprocal manner.

3. Regenerative Medicine

Tissue engineering and regenerative medicine is a multi-disciplinary science that has evolved in parallel with recent biotechnological advances. It combines biomaterials, growth factors and stem cells to repair organs [30]. Adult stem cells hold great promise for use in tissue repair and regeneration as a novel therapeutic option [31]. This can be done by culturing the cells and differentiating them into the required lineage *in vitro* and then introducing the differentiated cells into the failing organs. Plastic and regenerative surgeons are constantly burdened with the challenge of replacing lost soft tissue. More than 6.2 million individuals received reconstructive plastic surgery procedures in 2002, approximately 70% of them as a result of tumour removal [1]. Elective cosmetic procedures also require the placement of soft tissue implants to restore or improve tissue contour for the purpose of enhancing aesthetic appearance. Conventional soft tissue-grafting procedures have had some clinical success for soft tissue augmentation and reconstruction. However, the need for secondary surgical procedures to harvest autologous tissues and an average of 40-60% reduction in graft volume over time are considered drawbacks of current autologous fat transplantation procedures. It should be possible to overcome these problems with tissue-engineered soft tissue grafts generated from the patient's own adult stem cells [1].

Parkinson's disease, stroke and multiple sclerosis are thought to be caused by a loss of neurons and glial cells. These cells can now be generated from stem cells in culture and can be used to treat the above diseases in human patients through transplantation [32]. Clinical trials for the regeneration of soft tissue, craniofacial tissue and cardiovascular tissue have enrolled a number of patients. Breast reconstruction with ADSCs trials have been reported by Yoshimura and colleages [33]. These cells have also been used to stimulate bone repair in calvarial defects [34]. ADSCs have been used to heal chronic fistulas in Crohns disease [35] and hold great promise for the treatment of cardiovascular diseases [36].

4. Conclusion

More cells with increased differentiation potential are required for the treatment of various regenerative diseases. ADSCs have that differentiation potential and exposing them to LILI increases the proliferation rate. Therefore the novelity of using LILI in conjuction with ADSCs could improve tissue regenerative disease treatment by increasing the number of differentiated specialised cells. Much more research has to be conducted to develop standardise procedures for treatments.

5. References

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