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Review Article

Neuronal Differentiation of Adipose Derived Stem Cells: Progress So Far

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The nervous system is essential for normal physiological function of all systems within the human body. Unfortunately the nervous system has a limited capacity for self-repair and there are a plethora of disorders, diseases, and types of trauma that affect the central and peripheral nervous systems; however, current treatment modalities are unable to remedy them. Stem cell therapy using easily accessible mesenchymal stem cells, such as those found in the adipose stroma, has come to the fore in a number of biomedical disciplines as a potential therapeutic regime. In addition to substantial research already having been conducted on the *in vitro* differentiation of stem cells for the treatment of neurological repair, numerous strategies for the induction and culture of stem cells into terminal neural lineages have also been developed. However, none of these strategies have yet been able to produce a fully functional descendent suitable for use in stem cell therapy. Due to the positive effects that low level laser irradiation has shown in stem cell studies to date, we propose that it could enhance the processes involved in the differentiation of adipose derived stem cells into neuronal lineages.

1. Introduction

Stem cells are defined as cells that have the ability to perpetuate themselves through self-renewal and to generate mature, differentiated cells of a particular tissue. Different types of stem cells exist, as well as the degree of their differentiation potential or "potency." Both the type and potency of the stem cells are dependent on their provenance. Table 1 summarises the different types of stem cells, their origin, and their potency or differentiation potential. Embryonic stem cells are pluripotent, derived from the inner cell mass of a blastocyst, and capable of differentiating into cells of the three germ layers (endoderm, ectoderm, and mesoderm) [1, 2].

Primordial germ cells (the precursors of gametes) have been shown to be pluripotent and are able to be reprogrammed into embryonic germ cells, which are also pluripotent [3, 4]. Stem cells from more differentiated tissues such as those found in and around the developing foetus, extraembryonic tissues (placenta, chorion, and umbilicus), and the newborn infant and in older "adult" tissues are all thought to be multipotent. Multipotent stem cells are able to differentiate into different cell types but usually only to cell types that are derived from the same embryonic germ layer [5]. However, due to the ability of some multipotent "mesenchymal" stem cells (MSCs) derived from one germ layer to differentiate into cells from another germ layer, it is thought that some multipotent stem cells may in fact also be pluripotent. The defining characteristics of MSCs are inconsistent among investigators due, in part, to the lack of a universally accepted surface marker phenotype. Three characteristics of MSCs are defined and are universally accepted by most: (1) MSC populations are plastic adherent *in vitro*; (2) MSCs express antigens CD105, CD90, and CD73 and lack the expression of hematopoietic antigens CD45, CD34, CD14, CD19, or CD3 while expressing MHC Class I molecules *in vitro* but not Class II molecules unless stimulated; (3) MSCs have the capacity to differentiate to osteoblasts, adipocytes, and chondroblasts *in vitro* [6].

Table 1: Stem cell types, their origin, and their differentiation potential.

Origin	Blastocyst	Early embryo	Foetus/infant	Infant/adult
Stem cell type	Embryonic stem cells	Primordial germ cells Embryonic germ cells	Foetal stem cells Foetal/maternal stem cells Umbilical cord and placental stem cells	Adult stem cells Induced pluripotent stem (iPS) cells
Potency	Pluripotent	Pluripotent	Multipotent Pluripotent	Multipotent Pluripotent
References	[7, 8]	[3, 4]	[5, 9–12]	[13–17]

It is also possible to reprogramme normal "unipotent" somatic cells into pluripotent stem cells, through the introduction of four genes into the somatic cells. These cells have been termed induced pluripotent stem cells and have many of the same characteristics of embryonic stem cells [13, 14].

During wound healing, damaged cells are replaced with new cells, ascendant from a stem cell niche within the particular tissue. Most mammalian tissues have the potential and capacity, in varying degrees, for self-repair. The nervous system, however, has a much more limited, or indeed lacks, capacity for this. Although neural stem cells have been identified in the adult brain [18], their capacity and ability to functionally replace and repair damaged or diseased tissue in all parts of the brain and nervous system are limited [19]. Furthermore, although neural stem cells have been isolated and cultured from foetal and adult brain and other central nervous tissues [20–22], the harvesting and isolation of neural stem cells from these sources are not a practical solution for treatment. Stem cell therapy, with the use of mesenchymal stem cells, offers a more feasible option in seeking a treatment modality for neuronal replacement and repair.

2. Nervous System

The nervous system is the master control and communication core of the human body. Using chemical and electrical signals, the nervous system allows the body to react to sensory stimuli, usually in the form of motor responses. The nervous system is broadly divided into two principal parts—the central nervous system and the peripheral nervous system. The central nervous system consists of the brain and the spinal cord, and these decipher and translate sensory input and in turn direct an appropriate motor response. The peripheral nervous system is that part of the nervous system that falls outside the central nervous system and consists primarily of bundles of axons, that is, the nerves that extend from the spinal cord and the brain. The peripheral nervous system is further subdivided into the sensory and motor divisions. Sensory nerves act as the afferent division, transmitting impulses from the peripheral receptors to the central nervous system. Conversely, the motor nerves act as the efferent division conducting impulses from the central nervous system to the effector organs, primarily the muscles and glands. The peripheral nerves link and connect all parts of the body to the central nervous system and act as the communication system between the body and the brain.

3. Diseases and Trauma

Communication between the central nervous system and the rest of the body is essential for normal physiological functioning of the body. Neural damage, whether due to disease, physical trauma, or degeneration, can all result in the impairment or total loss of function of the affected organs or systems. Neurodegeneration is most commonly due to the aging and affects both nondividing neurons and neural stem cells, in both the central and peripheral nervous systems. Clinical issues resulting from neurodegeneration include losses of memory and cognition and sensory and motor deficits that in turn cause impairments to the functioning of many individual physiological systems such as the gastrointestinal tract (e.g., constipation), impaired vision and hearing, and normal muscular motor function [23].

Although neurodegeneration due to age could itself be considered a disease, there are specific diseases that affect the nervous system to devastating consequence. These include stroke, multiple sclerosis, spinal cord injury, and motor neuron diseases such as amyotrophic lateral sclerosis and spinal muscular atrophy. Spinal cord injury can be caused by both neurodegeneration and disease, but the most common cause is due to severe trauma to the spinal column and spinal cord by physical means, including falls in the elderly, motor vehicle and sporting accidents, and those brought about by physically violent incidents [24].

4. Therapies

Treatment of neural damage is, in general, primarily palliative. This includes the prevention of the progression of the disease/injury, management of sensory loss and spasticity, and teaching patients how to adapt to their disabilities. Many treatment regimens rely on drugs to attempt to improve neurological function and/or support, but few have the potential to repair and cure. These include steroids, hormones, and opiate antagonists; however, none of these agents provide major beneficial improvement of neurological function [25].

Due to the lack of any one or combinatory physical or chemical therapeutic remedy available, the investigation of cellular and molecular therapies is now at the fore of this research. Cell therapy, in particular stem cells, is showing promise as a potential therapy, especially in combination with tissue engineering applications [26, 27].

5. Multipotent Mesenchymal Stromal Cells

Adult stem cells are cells that possess the capacity of self-renewal, a proliferative potential, and the ability to differentiate into one or more terminal cell types [28]. Adult stem cells, unlike embryonic stem cells, circumvent the ethical and biological safety issues of embryonic stem cells, such as tumorigenicity [29] and histocompatibility [30]. It has been recommended by the International Society for Cellular Therapy that stem cells isolated from adult tissues are designated "multipotent mesenchymal stromal cells" (MSCs) [31]. This is due to the fact that not all adult stem cells that are being isolated and investigated are of true embryonic mesodermal origin. MSCs isolated from different adult tissues are proving to be a valuable research tool in cell therapy. Tissues from which MSCs have successfully been isolated include adipose [32, 33], bone marrow [34], muscle [35], skin [36], synovial membranes [37], and nervous tissue [38]. Most MSCs should by definition be able to be differentiated into osteogenic, adipogenic, and chondrogenic lineages using standard *in vitro* culture conditions [39]. However, dependent on the source tissue and the culture and differentiation conditions, MSCs have also successfully been differentiated into myogenic and neurogenic lineages [34, 40–42]. As our understanding of MSCs expands, so do their differentiation abilities and potential uses in tissue engineering for cell therapy.

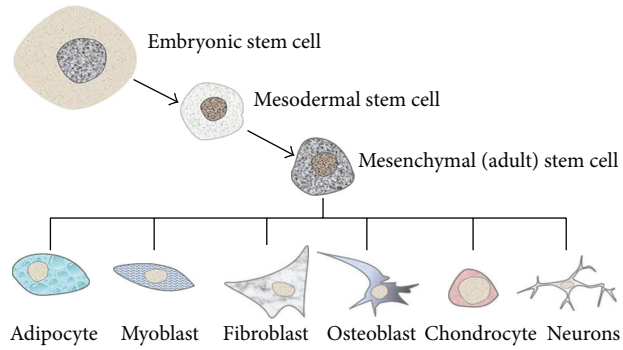

Figure 1: The hierarchical development of mesenchymal stem cells and their differentiation potential into various tissue lineages.

6. Adipose Derived Stem Cells

Autologous stem cell therapy, using adipose derived stem cells (ADSCs), could indeed be a much more practical, ethical, and readily accessible source of stem cells for the use in reparative and regenerative medicine. ADSCs, like haematopoietic stem cells (HSCs), are of mesenchymal origin; however, ADSCs are more abundant as well as being relatively easier and more cost effective to isolate. Moreover, the stem cell yield from adipose tissue is exponentially higher than from bone marrow [43]. ADSCs have been shown to be able to differentiate into adipogenic, osteogenic, chondrogenic, myogenic, and neurogenic lineages *in vitro* (Figure 1) by using cell specific culture and induction media [32, 43, 44].

In characterising ADSCs, they phenotypically identify as mesenchymal stem cells by expressing well documented cluster of differentiation (CD) markers, including CD13, CD29, CD31, CD34, CD44, CD63, CD73, CD90, and CD144 [45–49]. The expression of some of these markers has been shown to increase and/or decrease over time, *in vitro*, indicating an adaptive response to the culture environment [50]. Furthermore, the ability of the ADSCs to differentiate *in vitro* into the lineages mentioned above is another characteristic identification assay for their potency.

A great deal of work has been performed investigating the differentiation of various types of stem cells, including embryonic, induced pluripotent, and neural stem cells into neuronal lineages for the potential use in regenerative medicine. However, although these studies provide vital information and a greater understanding of stem cell biology, signalling, and differentiation processes, it is questionable as to the long term safety and efficacy of these cells for use in regenerative treatments. Further studies into the safety of using both embryonic and induced pluripotent stem cells are required, as they share the major disadvantage of being prone to forming teratomas after transplantation *in vivo* [14, 51]. The use of autologous multipotent mesenchymal stromal cells, however, potentially offers an easier, faster, and hopefully safer option.

7. Low Intensity Laser Irradiation

Laser radiation at different intensities has been shown to inhibit as well as stimulate cellular processes. Findings suggest that, at the cellular level, laser energy of a particular wavelength can initiate a signalling cascade that promotes cellular proliferation [52]. Low intensity laser irradiation (LILI), a form of phototherapy used in different clinical conditions to promote wound healing, appears to accelerate wound closure (i.e., the proliferation of new cells) [53]. LILI has been shown to be of neurological benefit in studies investigating stroke, degenerative brain disease, spinal cord and traumatic brain injury, and peripheral nerve regeneration as reviewed by Hashmi et al. [54]. Table 2 summarises selected studies conducted on ADSCs and neuronal stem cells with subsequent outcomes using LILI at different parameters.

8. Neuronal Induction and Characterisation

Several protocols have been employed by researchers in this field to induce the *in vitro* differentiation of ADSCs into neural lineages. Typically, isolated ADSCs are used and are either induced directly using chemicals and growth factors to stimulate differentiation or coaxed into generating neurospheres, which are then further induced using similar chemical and growth factor containing media.

Neurosphere generation is typically induced by the addition of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) to the ADSC culture media [69]. However, the spontaneous formation of spheres without

Table 2: Laser parameters used in stem cell studies.

Study	Laser parameters	Results	References
Human dental pulp stem cell (hDPSC) and LILI	20 mW/6 s, 40 mW/3 s, 660 nm	Increased viability and proliferation	[55]
ADSCS and LILI	0.153 W/cm^2 for 30, 45, 60, 180, and 300 s 532 nm	Increased proliferation at 30–60 s, longer exposure decreased proliferation, and autofluorescence	[56]
Neuronal embryonic rat brain cells and LILI	10, 30, 50, 110, 160, 200, and 250 mW for 1, 4, or 7 min, 780 nm	Increased migration and fiber sprouting, large size neurons with dense branched interconnected network of neuronal fibers	[57]
Primary neuronal culture and LILI	50 mW, 0.2, 4, 10, and 30 J/cm^2, 808 nm	Induced neuronal growth and neurite extension alter microglial phenotype	[58]
Human neural progenitor (NHNP) and LILI	50 mW/cm^2, 0.05 J/cm^2 808 nm	Increased ATP production	[59]
ADSCs and LILI	5 J/cm^2, 635 nm	Increased viability and proliferation	[60]
ADSCs and LILI	5 J/cm^2, 636 nm	Increased viability and proliferation	[61]
ADSCs and LILI	10 and 15 J/cm^2 830 nm	Decreased viability and proliferation	[62]
ADSCs	No LILI	Increased viability, proliferation, and differentiation	[63]
Human fibroblasts and primary rat cortical neurons and LILI	1 W, 10 mW/cm^2, 0.01, 0.1, 0.5, 2, 10, 50, 200, 1,000, 5,000 mJ/cm^2 810 and 980 nm	Improved neurite elongation, accelerated nerve regeneration, and improved functional recovery	[64]
ADSCs and LILI	5 J/cm^2, 636 nm	ADSC differentiation into smooth muscle cells	[63]
ADSCs	No LILI	ADSC differentiation into smooth muscle cells	[65]
ADSCs transplanted in ischemic mouse limbs	No LILI	Enhancement of angiogenesis and osteogenesis	[66, 67]
ADSCs	No	Differentiation into smooth muscle cells	[68]

the addition of growth factors has also been reported [70]. Dissociated neurospheres are then cultured in media containing a variety of differentiation factors.

Reports of experimental protocols successfully inducing neural differentiation by the addition of factors (alone or in combination with others) to culture media for ADSCs or ADSC derived neurospheres include all-*trans*-retinoic acid, forskolin, platelet derived growth factor (PDGF-BB), heregulin, B27, brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF), neurotrophin-3 (NT-3), β-mercaptoethanol (BME), dimethyl sulfoxide (DMSO), butylated hydroxyanisole (BHA), insulin, indomethacin, isobutylmethylxanthine (IBMX), valproic acid, hydrocortisone, and azacytidine [32, 69, 71–79].

However, the morphological and neuron-like appearance and changes noted in chemical induction protocols, particularly those using BME, DMSO, or BHA, have been reported to be due to cell shrinkage and not neural differentiation [80, 81]. Furthermore, the short time span in which these changes occur in these chemical induction protocols does not appear to be compatible with actual cellular differentiation. It would therefore appear that neural induction via growth factors alone or in combination with specific chemicals is able to truly induce differentiation that can be determined by both morphology and expression of neural specific markers.

Morphology alone can therefore not be an indicator of differentiation; hence, detection of the expression of neuronal markers is necessary. There are a plethora of neuronal markers dependent on the neuronal lineage being investigated. These include vimentin, nestin, glial fibrillary acidic protein (GFAP), β-tubulin III (Tuj-1), and microtubule associated protein 2 (MAP2). Undifferentiated ADSCs have been shown to express native immature neural proteins, such as vimentin, GFAP, and nestin [71, 82, 83], with the expression of these characteristic neural cell markers decreasing after neuronal differentiation [83]. Once ADSCs are more committed along their neural differentiation path, they express Tuj-1 and MAP2, indicative of immature neurons [83, 84]. However, GFAP expression has also been shown to increase after induction of neural differentiation [82, 84]. This illustrates the importance of understanding the expression profiles of the different markers and their significance during the differentiation process.

Dependent on the induction protocol and the degree of differentiation achieved (neuronal progenitor, immature neuron, or mature neuron), the next step in confirming functional differentiation would be to assess the expression of neurotransmitter receptors and electrophysiological studies [82, 83, 85].

9. Influence of Laser Phototherapy on Neuronal Differentiation of ADSCs

To date there is no data available on whether LILI can influence the neuronal differentiation of ADSCs. However, LILI has been shown to increase the expression of stem cell markers in ADSCs [60] as well as the proliferation and viability of stem cells *in vitro* [55, 56, 60]. Furthermore, LILI has been successfully used to demonstrate an increase in neuronal sprouting and migration in embryonic rat brain cultures [57], as well as inducing neuronal growth and neurite extension in primary neuronal and microglia cocultures [58]. Laser irradiation has also been shown to enhance the adenosine triphosphate (ATP) production of cultured normal human neural progenitor cells [59]. These studies all point to a positive effect of LILI on stem cells and neural progenitors, unequivocally indicating that laser irradiation can indeed play an important role in the differentiation of ADSCs into neuronal lineages, potentially at specific or, perhaps, all stages of the differentiation process.

10. Conclusions

It is now known that ADSCs exhibit the properties of multipotent stem cells, and hence their role in tissue engineering is invaluable. In the past decade, this relatively new and specialised research focus of differentiating ADSCs into neuronal lineages has progressed in leaps and bounds. However, attaining lineage specific, terminally differentiated, functional neurons has yet to be achieved. Results from *in vivo* studies have shown that ADSCs (isolated or differentiated) can promote nerve healing, but whether this is ostensibly by direct differentiation of the transplanted cells or via the paracrine effects of growth factors secreted by ADSCs on the endogenous neural progenitors has yet to be elucidated.

By understanding how neural progenitors function *in situ*, we are gaining a better understanding of what it is that we are aiming for in a terminally differentiated and functional neuron. Not only is it a prerequisite to understand the functional properties of the differentiated cells, but also deciphering the regulatory pathways and mechanisms of the ADSCs themselves will expedite the development and usage of ADSCs for autologous stem cell therapy in treating neurological diseases and disorders.

Disclosure

The material in this paper has neither been published nor been considered elsewhere for publication.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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