dard conditions for 48 hours. The differentiated SH-SY5Y and HOG cells exposed to H_2O_2 and cultured with MSCs-CM showed: a significant increase in ROS levels (3.4- and 2-fold of non-treated oxidized cells, respectively); an increase in GSH content (1.14- and 2-fold of oxidized cells, respectively); and, also, an increase in proliferative activity (1.8- and 1.5-fold of the oxidized cells, respectively, at 72 hours), recovering the proliferative rate of non-oxidized controls.
Discussion and conclusions: The properties of MSCs-CM, including a relevant antioxidant capacity and the possible presence of several growth factors, allow CNS cells counteract the harmful effect of oxidative stress. In fact, differentiated HOG and SH-SY5Y cells recover proliferation activity up to that of non-oxidized cells. Curiously, this recovery is mediated, at least in part, by an increase in ROS levels, which are implicated in signaling for proliferation and differentiation.
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The effects of human keratinocyte coculture on human adipose derived stem cells

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Introduction: Human adipose derived stem cells (hADSCs), mesenchymal stem cells within the subcutaneous adipose tissue, possess multipotent differentiation capabilities, and are influenced by the surrounding microenvironment. The wound healing effects of hADSCs are mainly mediated by interaction between dermal fibroblasts and keratinocytes through paracrine mechanisms. However, while the effect of keratinocytes on other mesenchymal stem cells has recently started to receive attention, there has been a paucity of studies on the effect of keratinocytes on hADSCs. While keratinocyes are known to induce neuroectodermal differentiation of delivered bone marrow derived stem cells, their effect on hADSCs is yet to be determined. This study aims to begin elucidating the mechanism by which human epidermal keratinocytes effect hADSCs *in vitro*.
Materials and methods: Human epidermal keratinocytes and human adipose derived stem cells (hADSCs) were cultured in keratinogenic growth media (CnT-57) divided into the following groups; hADSC monoculture (group 1), noncontact transwell coculture of hADSCs and human keratinocytes (group 2), and keratinocyte monoculture (group 3). Cell morphology was photographed and proliferation was assessed using the CCK-8 kit on days 1, 3, and 7 after culture initiation. Keratogenicity was analyzed through immunocytochemistry and polymerase chain reaction (PCR) of early, intermediate, and late keratogenic markers.
Results: Human ADSCs cocultured with keratinocytes showed a significant difference in proliferation rate compared to monocultured hADSCs, which seemed to decrease in number. Morphological character did not seem to change in the first few days of coculture. After a 7-day coculture period, immunohistochemistry findings revealed presence of specific keratinocyte markers including keratin-10, filaggrin, and involucrin in the ADSCs cocultured with keratinocytes. Reverse transcriptase PCR showed significant difference in expression of involucrin and filaggrin between the ADSC monoculture group and ADSC, keratinocyte coculture group.
Discussion and conclusions: This study demonstrates that ADSCs have the capacity to transdifferentiate into keratinocyte lineage cells, and suggests that adipose tissue may be a source of keratinocytes that may further be utilized in structuring bioengineered skin.
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Secretome of mesenchymal stem cells as a new tool for central nervous system regenerative medicine

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Introduction: The low regeneration potential of the central nervous system (CNS) represents a challenge for the development of new therapeutic strategies. Mesenchymal stem cells (MSCs) have been proposed as a possible therapeutic tool for CNS disorders. Their secretome possesses a broad range of neuroregulatory factors that could promote an increase in neurogenesis, inhibition of apoptosis/glial scar, immunomodulation, angiogenesis, neuronal and glial cell survival, as well as relevant neuroprotective actions into different pathophysiological contexts. In the present work the role of the secretome of MSCs, from different sources, *in vitro* and *in vivo* neuronal/glial survival was addressed. Additionally the possible applications of secretome based therapies (with no cell transplantation) for Parkinson's Disease (PD) regenerative medicine was also screened. Finally new trends on how to modulate the secretome MSCs were also explored
Material and Methods: MSCs were expanded in tissue culture flasks, and their secretome collected as previously described (1). Subsequently primary cultures of hippocampal neurons and cortical glial cells were incubated with MSCs for different periods of time. Addiotnally it was also injected intracaranially in the hippocampus and in a rat model of PD (2). In order to further modulate and enrich their secretome MSCs were also cultured in suspension biorectors, after which the same *in vitro* and *in vivo* experiments were performed.
Results: MSCs secretome demonstrated to be able to increase cell viability in primary cultures of glial cells and hippocampal neurons. When injected in the DG, the secretome of MSCs increased the levels of proliferation (ki-67+ cells) as well as the number of newborn neurons (DXC+ cells) and astrocytes (GFAP+ cells). When injected into a PD rat model it was also observed that the secretome of MSCs was able to increase the recovery of dopaminergic neurons (Figure 1). Finally, the use of dynamic culturing conditions seems to modulate and enrich the secretome of MSCs, leading to high levels of survival/differentiation of neuronal lineages *in vitro* and *in vivo* when compared with the secretome from static conditions.

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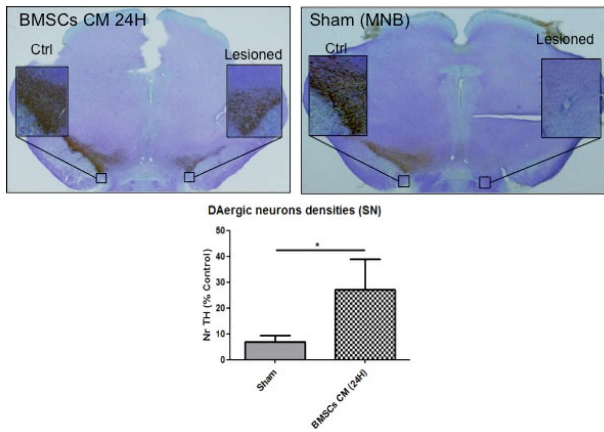

Figure 1 – BMSCs secretome promote the recovery of dopamigernic neuronal cell populations in a rat PD model

Discussion and Conclusions: The secretome of MSCs is a modulator of neuronal/glial survival and differentiation, potentiating neuronal and glial cell densities both *in vitro* and *in vivo*. When applied into PD model, the secretome of MSCs reveal to be neuroprotective in the dopaminergic system. The use of a bioreactor system seems to modulate the secretome of MSCs, enhancing their therapeutical potential in the survival and differentiation of neuronal lineages both *in vitro* and *in vivo* when compared with to the static conditions. These results indicate that the secretome of MSCs could represent an alternative for the development of novel CNS therapeutic strategies.

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