Insights in Stem Cells

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Stem Cell Research 2018-Cryopreservation Impacts Cell Functionality of Long Term Expanded Adipose-Derived Stem Cells- Ali El Othmani-, Pasteur Institute of Morocco

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Introduction

Adipose Tissue (AT) has gained more interests since reported to be enriched in multipotent stem cells .These cells having Mesenchymal Stem Cell (MSC) characteristics were called ADSCs and were identified within the freshly isolated Stromal Vascular Fraction (SVF) which contains also smooth muscle, circulating cells as leukocytes, endothelial and hematopoietic progenitors, pericytes, fibroblasts and pre-adipocytes [3,4]. Their ability to differentiate into different cells belonging to mesodermic, endodermic and ectodermic origins has been largely documented in the literature increasing their attractiveness in tissue repair and regenerative medicine. ADSC are known to secrete a large panel of cytokines and chemokines and their ability to produce inflammatory factors innately or after stimulation has been investigated. Native or auto-induced ADSC secreted IL-8, TNF-α, IL-10, TLR2 and higher levels of IL-6, being involved for distinct inflammatory signaling pathways. Also, in response to inflammatory stimuli, ADSC inhibited pro-inflammatory factors while increased that of the anti-inflammatory cytokines IL-10 and the TLR2 in vitro as well as in vivo. Nevertheless, it has been reported that stimulation of distinct TLR elicited different inflammatory signaling pathways leading to differentially expressed inflammatory factors and influencing the ability of MSC to suppress immune cell proliferation. Controlling these inflammatory properties might be very helpful in the treatment of perinatal morbidities-associated inflammation of colitis, respiratory distress, Crohn's disease, and lupus erythematosus. However, Graft Versus Host Disease (GVHD) has been the most studied so far. The biological mechanisms underlying ADSC immunological benefit observed have been variable over the clinical studies and might interfere with the cryopreservation protocols used to pool these cells. Being uncultured or expanded at a different time, ADSC presented different cell profiles having thus multiple therapeutic outcomes. It's widely accepted that ADSC proliferation changed significantly within successive culture passages regarding stem cell-associated profile and multilineage differentiation ability [22-24], reflecting functional cell changes relating themselves to different regulation pathways and effectiveness. This fact was reported for fresh ADSC and might not be similar when using cryopreserved/thawed cells [23], suggesting an additional effect of Cryoprotective Agents (CPA) and the time storage to culture passage on the paracrine activity and response efficiency of fresh ADSC. Because of cryopreservation protocols are mostly reported for freshly isolated ADSC or those derived from primary culture, ADSC efficiency and inflammatory cytokines profile might be modulated by the freezing at the different time point of expansion culture and are yet to be determined. Most of them were reported to impact negatively ADSC cellular viability, Colony Forming Unit-Fibroblast (CFU-F) ability, differentiation potency, phenotypic and gene expression and secretion profile [25-29]. When separated from their microenvironment, ADSC cryopreservation outcomes were also differentially appreciated [22,30- 32]. ADSC might be exposed to exogenous factors such as draining reagents since collection suggesting that the time delay between their collection and separation might be associated with their efficiency. All these parameters should be addressed to master ADSC use for therapeutic applications. In our study, we attempt to define the effects of a widely used freezing protocol at a different culture time point on ADSC yield,

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clonogenicity, protein, and total RNA (tRNA) secretion, and on IL-6 and TLR2 secretion.

Materials and Methods

Collection of AT

AT collection process has been endorsed by the institutional requirements of the Institut Pasteur Ethical Committee. Lipoaspirates were performed on consent young women aged from 18-48 years old undergoing esthetic treatments as previously described [33]. Samples were collected from abdominal subcutaneous tissue and stored at +4°C before and during transfer to the laboratory. More than 30 samples were collected and each of them was manipulated and tested separately. Separation of the SVF AT from each donor was separated on four identical samples. All the samples were placed at +4°C during either one day (24 h), two days (48 h), or five days (72 h), the last one was manipulated within 12h after collection. Cells were washed and digested with collagenase II (Gibco, Invitrogen) at 2.5% and incubated at 37°C for 30 min. At the end of incubation, cells were washed, and the final pellet was suspended in DMEM medium (Gibco, Invitrogen). Cells were tested for viability using the trypan blue (Gibco, Invitrogen) dye exclusion method. Only SVF processed less than 12 h were seeded in culture for ADSC expansion and cryopreservation. In-vitro ADSC expansion SVF from lipoaspirates were cultured in 75 cm2 flasks at 2 × 104 / cm2 density. Culture medium containing DMEM supplemented with 10% FBS (Gibco, Invitrogen), 2% Penicillin-streptomycin (Gibco, Invitrogen), and 1% fungizone (Gibco, Invitrogen) was added. Flasks were incubated for confluence in a humidified atmosphere at 37°C and 5% CO2 . For successive passages, adherent layers were treated with trypsin-EDTA (Gibco, Invitrogen), and cells were tested for viability before seeding in culture. Cell expansion was maintained up to 4th passages, and ADSC aliquots were cryopreserved after each passage. Cryopreservation protocol Freshly isolated SVF and expanded ADSC (P0, P1, P2, P3) were used for cryopreservation. The freezing medium was prepared as 90% FBS and 10% DMSO. Cell suspensions were washed at +4°C and kept on ice and were assimilated at 5 × 106 cell/ml for SVF and 1 × 106 cell/ ml for expanded ADSC. Cryopreservation medium was added gently to the vials transferred rapidly latter at -20°C for 24 h and in a freezing system ensuring a steady freezing rate of -1°C/min down to -80°C. Cells were frozen at -80°C for 6 months to a year.

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