Influence of adipose tissue harvest condition on the *in vitro* adipogenic differentiation of human stem cells in three-dimensional spheroid culture

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Statement of Purpose: Better understanding of adipose tissue structure and function is critical to develop novel strategies at the cellular level to reduce the deleterious effects of obesity. Mesenchymal stem cells are commonly used for adipose related *in vitro* studies. Within this cell category, autologous adipose tissue-derived stem cells (ADSCs) are becoming popular as a result of their easy isolation from the adipose stromal vascular fraction, ease of adipose tissue accessibility, stem cell quality, *in vitro* proliferative potential, and multi-lineage differentiation capacity. The current adipose tissue harvesting procedures introduce non-physiological conditions, which may affect the overall performance of the isolated ADSCs. In this study, we elucidate the differences between ADSCs isolated from adipose tissues harvested within the first 5 minutes of the initial surgical incision ("vascularized" condition) versus those isolated from adipose tissues subjected to local medications and deprived of blood supply as part of routine surgery ("standard" condition).

Methods: Adipose tissues were harvested from adult female patients (n = 3) undergoing breast reconstruction with deep inferior epigastric perforators or anterolateral thigh free flaps according to the Institutional Review Board Approval # 2012-0004. ADSCs isolated from the adipose tissues were cultured and differentiated following Turner et al.[1] Flow cytometry was performed using fluorochrome-conjugated antibodies and analyzed with multicolor Beckman-Coulter Gallios, B5-R1 configuration flow cytometer. Biochemical analysis of cultured ADSCs was performed after 3 days in differentiation media (i.e., day 0 of maturation media (M0)) and 10 days in maturation media (M10). DNA and intracellular triglyceride (TG) content were evaluated using CyQuant DNA assay (Thermo) and triglyceride kit (Sigma). Reverse transcription polymerase chain reaction (RT-PCR) was carried out by droplet digital PCR (BioRad Labs). All assays were performed in triplicates according to manufactures' protocols. *Statistical analyses* were performed using one-way ANOVA with Games-Howell *post-hoc* test. $p \leq 0.05$ was deemed statistically significant.

Results: We used fluorochrome-conjugated monoclonal antibodies of CD31 (to prove the cells are non-hematopoietic in origin), stem cell markers (CD29, CD44, CD59, CD90, CD105), and markers to rule out any adipocyte contamination (CD106, CD117 and CD271). We also analyzed CD36, which is an integral surface protein involved in fatty acid uptake. The isolated cells were negative for CD31, positive for stem cell markers, and negative for adipocyte markers, which confirmed them to be ADSCs (Fig. 1a). Importantly, we found a differential expression for CD36: 17.3 ± 10.1% ADSCs isolated from free-flap versus 35.3 ± 6.7% ADSCs isolated from standard samples were CD36 positive ($p \leq 0.05$). After adipogenic differentiation (Fig. 1b), four out of the five selected stem cell markers (CD29, CD44, CD90, and CD105) showed significant reduction ($p < 0.05$).

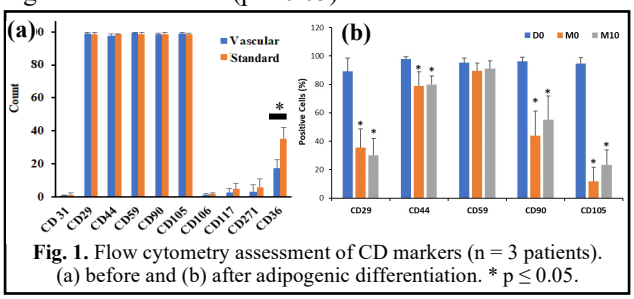

Fig. 1. Flow cytometry assessment of CD markers (n = 3 patients). (a) before and (b) after adipogenic differentiation. * $p \leq 0.05$.

To compare the spheroid formation and adipogenic maturation of ADSCs isolated from vascularized and standard tissues, we cultured 3-D ADSC spheroids atop the elastin-like polypeptide-polyethyleneimine coatings as described previously.[1] These ADSCs initially formed small spheroids (M0), which gradually increased in size during adipogenic maturation (Fig. 2a), achieving a nearly 2-fold larger diameter (~ 8-fold increase in volume) within 10 days (M10). Importantly, the triglyceride content increased almost ~ 6.1-fold for the ADSCs isolated from the vascularized tissues compared to ~ 5-fold for those isolated from the standard tissues (Fig. 2b), indicating a potential pre-disposition of ADSCs' adipogenic capability based on their original tissue harvest condition. The expression level of PPAR-γ in spheroids prepared using the ADSCs isolated from the vascularized and standard tissues were similar, but adiponectin expression in spheroids prepared using the ADSCs isolated from the vascularized tissues was significantly higher compared those prepared from the standard tissues (Fig. 2c).

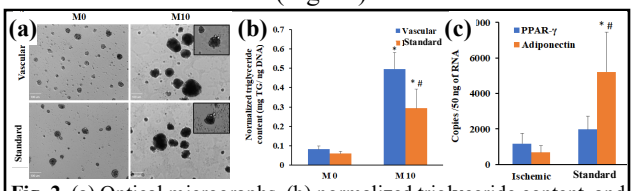

Fig. 2. (a) Optical micrographs, (b) normalized triglyceride content, and (c) PPAR-γ and adiponectin gene expression of spheroids prepared using the ADSCs isolated from the vascularized and standard tissues. * $p \leq 0.05$ for M10 versus M0 values of the same group; # $p \leq 0.05$ between groups on the same day.

Conclusions: The differentiation and maturation of the ADSCs isolated from vascularized and standard tissues were dependent on their original tissue harvest condition. These findings suggest that the metabolic dysfunction imparted due to the non-physiological conditions present during surgery may prompt the ADSCs isolated from standard tissues to a suboptimal adipogenic differentiation. Therefore, more robust ADSCs suitable to establish *in vitro* adipose tissue models should be obtained by harvesting vascularized and non-premedicated adipose tissues.

Funding: NIH R01EB020006; P20GM104357; P20GM121334.

Reference: [1] *Process Biochem. 59:312-320, 2017.*