93
CELLULAR ORIGIN OF GRAFTED ADIPOSE TISSUE: HOST OR DONOR?

Presenter: Kentaro Doi, MD
Authors: Doi K, Kato H, Eto H, Yoshimura K
University of Tokyo

Introduction: Free micro-fat grafting is a promising treatment for soft tissue augmentation/reconstruction but the engraftment process is not yet clearly understood. We recently found that most adipocytes in the graft, except for ones in the superficial layer, die within a few days after grafting and are gradually regenerated from the surface, though the origin of adipocytes remains unclear.

Methods: Inguinal fat tissue was harvested from both an EGFP mouse and a wild type mouse, and cross-transplanted into the subcutis of the head. The mice were killed at 1, 2, 4, 12 and 24 weeks after transplantation. Harvested samples were analyzed with immunohistochemistry for expression of eGFP (donor or host), perillipin (living adipocytes), lectin (vessels and inflammatory cells), MAC2 (macrophage) and Hoechst (nuclei). Whole mount staining with lectin and Hoechst was also performed. In addition, stromal vascular fraction (SVF) cells were isolated from grafted samples and analyzed with flowcytometry and cell culture.

Results: Based on the findings from both cross-transplantation models, most living adipocytes, ASCs and large vessel structures were originated from the donor, although a small number of host-derived adipocytes were confirmed. Based on cell culture, more than 90% of ASCs were from donor. Many host-derived cells start to infiltrate within one week to the deep area where mature adipocyte cannot survive. From two weeks up to 24 weeks, host derived cells become more localized around the oil droplets and were positive for macrophage markers. Whole mount staining showed chimeric capillaries both form host and donor.

Discussions/Conclusions: Our results revealed origin of grafted fat is mainly from donor. However, host derived cells actively engaged in tissue regeneration as macrophages surrounding oil cysts. Some minor host derived cells proven to become ASCs, Adipocytes, large vessel wall structures and capillaries.

94
INTRODUCING NON-ADHERENT PROGENITORS FROM ADIPOSE-DERIVED STEM CELLS (NAPADSCS): PROOF OF STEMNESS AND POSSIBLE FUTURE APPLICATIONS IN REGENERATIVE SURGERY

Presenter: Angelo A. Leto Barone, MD
Authors: Leto Baron AA, Carmisciano M, Giunta G, Toia F, Maggi F, Iovino F, Todaro M, Cordova A, Moschella F
Universita' degli Studi di Palermo Dipartimento di Discipline Chirurgiche ed Oncologiche

Introduction: Mesenchymal Stromal Cells (MSCs) can be isolated from adipose tissue and are currently used in vitro for experimental studies. MSCs are often mislabeled as Adipose-derived Stem Cells (ADSCs) despite presenting a more differentiated phenotype. The isolation and expansion of non-adherent progenitors from adipose-derived stem cells (napADSCs), has been achieved in our laboratory and is currently under investigation. In this study we investigate: a) the proof of stemness of such progenitors, and b) the feasibility of napADSCs adhesion over Integra® for cell colonization and for future differentiation and engineering of semi-synthetic tissues.

Material and Methods: Adipose tissue (20 cc) was extracted from lipoaspirate samples of 15 healthy donors following patients written consent. Following mechanical and enzymatic digestion, samples were plated in stem cell-specific enriched media and in no adherence conditions. Clonal expansion of a single cell was assessed by limiting dilution and asymmetric division was detected by PKH26 staining. Expanded cells were seeded within Integra® in 24-well plates.

Results: NapADSCs represent an upstream line of mesenchymal progenitors compared to more differentiated, adherent, fibroblast-like MSCs. NapADSCs colonies defined as spheroids (polyclonal) and sphere-derived clonogenic cells (monoclonal) were visible in 1-3 weeks and their stemness confirmed in vitro by clonal expansion. NapADSCs adhesion to Integra® was achieved modifying culturing conditions, and was visible in 3-7 days with phenotype change from napADSCs to fibroblast-like MSCs.

Conclusions: Our data suggests that the identification of napADSCs may dissipate the doubts on the stem-cell origin of the more differentiated and commonly used adherent MSCs. The ability of napADSCs to adhere to a clinically available dermal regenerative template, and grow within its three-dimensional structure, may prove useful in regenerative surgery. Further studies are warranted to assess whether such procedure may represent a first preliminary step toward clinical application of semi-synthetic tissues.