Production of Human Endothelialized Adipose Tissues Devoid of Exogenous Biomaterial

Fradette J., Vincent C. and Proulx M.
LOEX, Centre de Recherche FRSQ du CHA universitaire de Québec, and Département de Chirurgie, Université Laval, Québec, Canada

Statement of Purpose: The demand for autologous soft-tissues in reconstructive and plastic surgery is increasing, prompting the need to develop new strategies for adipose tissue engineering. In particular, the actual techniques of fat autografts often point to the lack of adequate revascularization as a cause for the poor long-term success achieved following soft-tissue augmentation. We report the in vitro production by tissue engineering of new human adipose tissue substitutes that are functional, devoid of exogenous biomaterial and enriched with a pre-formed capillary network of endothelial cells. These endothelialized adipose tissues therefore represent good candidates for future clinical applications, as the process of inosculation between the host and preformed capillary networks should promote faster revascularization of thick tissue-engineered constructs.

Methods: Human stromal/stem cells (ASCs) were extracted from lipoaspirated fat and used for their capacity to form conjunctive and adipose tissues using an adapted « self-assembly » culture technique (Vermette M. Biomaterials. 2007;28:2850-2860). This method consists in coaxing cells to produce and organize their own extracellular matrix following serum and ascorbic acid stimulation in vitro, resulting in manipulatable cellular sheets (3.5 cm^2 surface area). By concomitantly inducing adipogenic differentiation, adipocyte-containing sheets are produced. After typically 28 days of culture, the sheets are then assembled into thicker tissues by layering three cell sheets. Addition (at day 21 of culture) of human microvascular endothelial cells onto the reconstructed sheets prior to their assembly into thicker tissues gave rise to endothelialized constructs. Immunohistochemistry for the detection of PECAM-1 (Platelet endothelial cell adhesion molecule-1, Chemicon, Temecula, CA) expression on endothelial cells was performed on tissue cross-sections observed under standard or confocal microscopy. Image analysis was performed using the ImageJ software. Secretion profiles were established after analysis of supernatants using ELISA assays specific to each human molecule examined (R&D Systems). T-Tests and one-way ANOVA were performed to assess statistical significance. A pilot study for tissue implantation of the endothelialized adipose constructs and their controls was performed onto nude mice according to the animal ethic committee. Tissues were grafted subcutaneously onto back muscle and assessed 3, 7 and 14 days after grafting.

Results: Human reconstructed adipose tissues enriched or not with human endothelial cells were produced, as well as their connective tissue counterparts produced from the same ASCs but without adipogenic differentiation. The reconstructed adipose tissues secreted important levels of cytokines and growth factors such as leptin, Ang-1, HGF, and VEGF, as expected for fat tissue. Immunolabelings followed by confocal microscopy analyses revealed a network of PECAM-expressing structures, only within tissues enriched with endothelial cells. Adipocytes within the reconstructed adipose tissues impacted on these PECAM-positive structures by affecting the internal diameter of the structure's lumen seen on tissue cross-sections. When the internal surface of individual capillary-like structures were measured, the mean estimated diameter was 17.9 +/- 4.1 µm (mean +/- SD) for adipose tissues compared to 12.7 +/- 3.2 µm for their respective connective tissues (3 cell populations, 1.4 fold, p=0.0044). This increase in size of capillary-like structures was correlated with an increased secretion of many pro-angiogenic molecules from the adipose tissues, namely a 30-fold increase in leptin levels, 3.8 fold in angiopoietin-1, 1.8 fold in HGF and 1.7 fold in VEGF. Finally, preliminary data of reconstructed human adipose tissues grafted onto nude mice indicate that endotheliazed tissues may be able to promote an accelerated revascularization within the first 14 days after grafting, as assessed by macroscopic and histological analyses of the grafted tissues after resection, as well as immunolabelings for PECAM-expressing structures.

Conclusions: This new model of reconstructed adipose tissues recreates closely the features of human adipose tissue, including functional adipocytes, extracellular matrix components as well as vascularization. Our understanding of the relationships between adipogenesis and angiogenesis will likely benefit from the analysis of these new tissue-engineered substitutes. These tissues will also be useful tools to study adipose tissue metabolic activities under various pharmaco-toxicological treatments in vitro. Finally, these reconstructed 3D tissues constitute promising substitutes for regenerative medicine applications. In particular, the ability to use autologous cells from the patient's own subcutaneous fat depots as well as the absence of animal-derived collagen or synthetic polymer within these tissue-engineered adipose tissues make them unique and natural substitutes.

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