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Production of a new tissue-engineered adipose substitute from human adipose-derived stromal cells

Mélanie Vermette[a], Valérie Trottier[a], Vincent Ménard[a], Lucie Saint-Pierre[a], Alphonse Roy[b], Julie Fradette[a,b,*]

[a]*Laboratoire d'Organogénèse Expérimentale (LOEX), Centre Hospitalier Affilié Universitaire de Québec, Hôpital du Saint-Sacrement, 1050 chemin Sainte-Foy, Québec., Canada*
[b]*Department of Surgery, Faculty of Medicine, Laval University, Québec., Canada G1S 4L8*

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Abstract

Adipose tissue is an accessible and abundant source of mesenchymal stem cells for soft-tissue reconstruction. In an attempt to create a novel, entirely autologous tissue-engineered adipose substitute, we extracted human stromal cells from either lipoaspirated or resected fat, and assessed their capacity to produce a three-dimensional adipose tissue using an adapted "self-assembly" culture methodology. This strategy involved a concomitant induction of adipogenic differentiation whilst ascorbic acid supplementation stimulated the stromal cells to produce and organize their own "biomaterial" in the form of extracellular matrix, forming manipulatable sheets that are then assembled into thicker reconstructed adipose tissues. When compared to resected fat, lipoaspiration-derived cells featured an increased adipogenic potential and the enhanced ability to recreate an adipose substitute in vitro. When viewed by scanning electron microscopy, the appearance of these reconstructed adipose tissues was strikingly similar to subcutaneous fat. Furthermore, these substitutes secreted adipokines and mediated β-adrenergic receptor-stimulated lipolysis, hence reproducing known major biological functions of white adipose tissue. Therefore, our cell-based tissue engineering strategy led to the production of a functional and entirely natural reconstructed adipose tissue, which offers the potential to be used for specific in vitro applications as well as for autologous soft-tissue reconstruction.

Keywords: Adipose tissue engineering; Extracellular matrix (ECM); Mesenchymal stem cell; Cell culture; Scanning electron microscopy (SEM); Autologous cell

1. Introduction

The demand for soft tissue substitutes in reconstructive and plastic surgery is continually increasing. Unfortunately, most current autograft techniques fail to produce long-term satisfactory replacement [1]. This is due in part to the fragility of adipocytes and the lack of appropriate vascularization after grafting [2,3]. Trauma, tumour resection, congenital or acquired anomalies are the main causes justifying the need for adipose substitutes in reconstructive surgery, and tissue engineering strategies are very promising as an alternative therapeutic approach to address the low predictability of autologous fat transplantation. Several groups have pioneered adipose tissue engineering using the commonly used 3T3-L1 murine preadipose cell line [4,5], rat preadipocytes [6,7] or bone-marrow derived mesenchymal stem cells [8–10]. The recent surge of interest in adipose tissue as a source of adult multipotent stem cells (ASCs) [11,12] also generated encouraging results for their use in regenerative medicine [13,14]. Among the stromal cells extracted from adipose tissue, a subpopulation (up to 2%) features stem cell characteristics similar to those of bone marrow-derived mesenchymal cells (up to 0.002%) [15]. Therefore, adipose tissue is considered as an abundant

*Corresponding author. Laboratoire d'Organogénèse Expérimentale (LOEX), Centre Hospitalier Affilié Universitaire de Québec, Hôpital du Saint-Sacrement, 1050 chemin Sainte-Foy, Québec Canada G1S4L8.
Tel.: +1 418 682 7995; fax: +1 418 682 8000.
E-mail address: julie.fradette@chg.ulaval.ca (J. Fradette).

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and practical source of stromal precursor cells with great potential for soft-tissue reconstruction. The adipose tissue necessary for cell sourcing is usually provided via resection or lipoaspiration. Although these two types of procedures have been directly compared for the yield of stromal cells at extraction [16], growth kinetics, as well as the osteogenic and chondrogenic potential of the extracted cells in vitro [17], it is still unclear if these cells perform equally when used for adipose tissue engineering.

In order to reconstruct tissue-engineered adipose substitutes, a variety of natural or synthetic biodegradable polymer scaffolds have been tested in combination with adipocyte precursor cells of animal or human origin. Collagen sponges [18,19], hyaluronic acid-based scaffolds [20], alginate beads [21], polylactic-*co*-glycolic acid (PLGA) [7,22], polytetrafluoroethylene (PTFE) meshes coated with collagen [23], as well as alginate or hyaluronic acid based hydrogels which can be dehydrated and rehydrated to obtain the desired shape [8] have been reported. Despite these efforts, tissue-engineered adipose substitutes have not made it into the clinical realm yet, indicating the need to optimize the current models or to innovate with novel strategies for engineering this peculiar type of tissue.

The present study was undertaken to determine whether or not the production of a novel, entirely human adipose substitute could be achieved using a distinctive cell-based tissue engineering approach. The objective was to evaluate and compare the capacity of stromal cells extracted from lipoaspirated or resected fat to generate functional reconstructed human adipose tissues using an adapted "self-assembly" procedure, during which adipocyte differentiation occurs concomitantly with ascorbic-acid stimulated extracellular matrix (ECM) production from the stromal cells themselves in vitro. Therefore, the resulting adipose substitutes consist of functional adipocytes embedded into their own human ECM scaffold, forming a natural "biomaterial". The structural appearance of the reconstructed adipose tissues was examined by histological analyses and scanning electron microscopy (SEM). The evaluation of their functionality was done by assessing known major biological features of white adipose tissue such as secretion of typical adipokines and β-adrenergic receptor-stimulated lipolysis in vitro.

2. Materials and methods

2.1. Isolation and culture of stromal cells from human adipose tissue

Cells were extracted from adipose tissues of five healthy female patients undergoing cosmetic surgery procedures, following guidelines from Laval University Ethic Board. Tissues were harvested either from lipoaspirations with a 4 mm canulae (LA, $n = 3$) or as resected fat from lipectomy biopsies (LP, $n = 3$). In one case, LP and LA cells were obtained from the same patient (age 33). The mean age of donors was 37.7 ± 9.9 years for LP and 39.0 ± 5.6 years for LA samples. The mean body mass index (BMI) of donors was 23.5 ± 0.4 for LP and 23.6 ± 2.9 for LA samples. The harvested tissues were processed according to standard stromal cell extraction protocols [12,24], generally using 60–70 g of fat per extraction. Adipose tissues were digested with 0.075% collagenase (type 1A, Sigma Chemicals, St.Louis, MO) in Krebs-Ringer Buffer for 60 min at 37 °C followed by 10 min with 0.25% trypsin. Floating adipocytes were discarded and cells from the stromal-vascular fraction were pelleted, rinsed with media, centrifuged and a red cell lysis step in NH_4Cl was done for 10 min at RT. To assess yield reproducibility, a total of eleven stromal cell extractions were performed on LA fat from three patients, and six extractions were performed on excised fat (LP) from three patients. The viable cells obtained were counted using trypan blue exclusion assay and seeded at a density of 8×10^5 cells/cm^2 for in vitro expansion and batch cryopreservation after primary culture (P0). Experiments were performed on cells at passage (P) 3, P6 and P11, that were thawed from P0 and expanded weekly at a density of 8×10^3 cells/cm^2 in expansion media consisting of 1:1 Dulbecco's modified Eagle's medium (DMEM): Ham's F12 medium (H) medium (Invitrogen, Oakville, Ont.) supplemented with 10% foetal calf serum (FCS, HyClone, Logan, UT) and antibiotics (100 U/ml Penicillin and 25 mg/ml Gentamicin (Sigma)). All culture dishes were from Nunc (VWR international, Mississauga, Ont.).

2.2. Production of adipose tissue sheets and conjunctive tissue sheets

Adipose-derived stromal cells were seeded at a density of 1.5×10^5 per well (1.58×10^4/cm^2) in 6-well plates containing a filter-paper anchorage device (Wathman, Fisher Scientific, Ottawa, Ont.) allowing easy manipulation of the adipose sheets while preventing contraction. Confluence was typically reached 3–4 days after seeding. For the entire length of the experiments, media were supplemented with 50 μg/ml (250 μM) ascorbic acid (Sigma) which has been previously demonstrated to stimulate abundant ECM production from smooth muscle cells and dermal fibroblasts [25,26]. In order to produce adipocyte-containing cellular sheets, the stromal cultures were induced 7 days after seeding by supplementing the media with an adipogenic cocktail (100 nM insulin (Sigma), 0.2 nM T3 (Sigma), 1 μM dexamethasone (Sigma), 0.25 mM IBMX (Sigma) and 1 μM rosiglitazone (Cayman Chemical, Cedarlane, Hornby, Ont.) in 3% FCS medium for 3 days, while non-induced cells were treated with 3% FCS expansion medium containing 0.038% DMSO (control media), which is the diluent for IBMX and rosiglitazone. After 3 days, induction medium was substituted by adipocyte medium (expansion media supplemented with 100 nM insulin, 0.2 nM T3 and 1 μM dexamethasone) while non-induced controls were cultured in 10% FCS expansion medium, forming conjunctive tissue sheets devoid of adipocytes. After typically 28 days, manipulatable cellular sheets (3.5 cm^2 surface area) containing or not human adipocytes were ready to be assembled into thicker tissues.

2.3. Production of reconstructed adipose tissues and reconstructed conjunctive tissues

Superposing three adipose sheets produced the reconstructed adipose tissues, while three tissue sheets containing non-differentiated stromal cells formed reconstructed conjunctive tissues. Typically, a strong cohesion between the different layers was obtained by culturing the assembled tissues for 7 additional days in presence of ascorbic acid before analysis.

2.4. Quantification of adipose differentiation

After 14 days of differentiation, Oil Red O (ORO, Sigma) staining of the cytoplasmic droplets of neutral lipids was performed according to a modification from [27,28]. Briefly, cultures were rinsed, fixed with 10% buffered formalin, stained with 0.3% ORO in isopropanol: water (3:2), photographed and extracted with 4% Nonidet (Sigma)/isopropanol for quantification at 520 nm using a SpectraMax Plus spectrometer (Molecular Devices) with SoftmaxPro Ver 4.7.1.