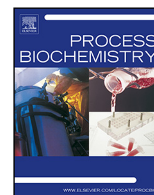
Process Biochemistry 59 (2017) 312–320

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio



Adipogenic differentiation of human adipose-derived stem cells grown as spheroids

Paul A. Turner [a], Bhuvaneswari Gurumurthy [a], Jennifer L. Bailey [b], Carrie M. Elks [b], Amol V. Janorkar [a,*]

[a] Department of Biomedical Materials Science, School of Dentistry, University of Mississippi Medical Center, 2500 N State Street, Jackson, MS, United States
[b] Matrix Biology Laboratory, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA, United States

ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form 4 November 2016
Accepted 3 February 2017
Available online 6 February 2017

Keywords:
3-D cell culture
Spheroids
Adipose tissue model
Elastin-like polypeptide
Cell morphology
Stem cells

ABSTRACT

Understanding the process of adipogenesis is critical if suitable therapeutics for obesity and related metabolic diseases are to be found. The current study presents proof of feasibility of creating a 3-D spheroid model using human adipose-derived stem cells (hASCs) and their subsequent adipogenic differentiation. hASC spheroids were formed atop an elastin-like polypeptide-polyethyleneimine (ELP-PEI) surface and differentiated using an adipogenic cocktail. Spheroids were matured in the presence of dietary fatty acids (linoleic or oleic acid) and evaluated based on functional markers including intracellular protein, CD36 expression, triglyceride accumulation, and PPAR-γ gene expression. Spheroid size was found to increase as the hASCs matured in the adipocyte maintenance medium, though the fatty acid treatment generally resulted in smaller spheroids compared to control. A stable protein content over the 10-day maturation period indicated contact-inhibited proliferation as well as minimal loss of spheroids during culture. Spheroids treated with fatty acids showed greater amounts of intracellular triglyceride content and greater expression of the key adipogenic gene, PPAR-γ. We also demonstrated that 3-D spheroids outperformed 2-D monolayer cultures in adipogenesis. We then compared the adipogenesis of hASC spheroids to that in 3T3-L1 spheroids and found that the triglyceride accumulation was less profound in hASC spheroids than that in 3T3-L1 adipocytes, correlated with smaller average spheroids, suggesting a relatively slower differentiation process. Taken together, we have shown the feasibility of adipogenic differentiation of patient-derived hASC spheroids, which with further development, may help elucidate key features in the adipogenesis process.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Adipose tissue is no longer considered merely a passive reservoir of energy, but an important endocrine organ with systemic homeostatic responsibilities. One of the major stumbling blocks in obesity research continues to be the lack of appropriate *in vitro* multicellular architectures that mimic adipose tissue structure and function found *in vivo*. Planar 2-D monolayer adipocyte cultures do not represent the complex architecture of adipose tissue *in vivo*, while the optimum scaffold material to achieve engineered 3-D adipose tissue remains elusive [1,2]. The creation of *in vitro* models of adipose tissue will allow researchers to test various hypotheses, study altered metabolic pathways due to the disease state, and therefore, facilitate the efforts in understanding adipose pathophysiology.

The International Federation for Adipose Therapeutics and Science (IFATS) has applied the term "adipose-derived stem cells," or ASCs, to pluripotent mesenchymal cells as well as more differentiated adipose progenitor cells found amongst the fibroblastic undifferentiated population of the adipose tissue [3]. A variety of substrate types and materials have recently been investigated for engineering functional adipose tissue by encapsulating ASCs, including decellularized tissue [4], naturally-derived biopolymers [4–7], and degradable synthetic polyesters [6,8,9]. However, due to the restriction placed on the size of the maturing cells embedded directly in such scaffolds, the resulting adipocytes typically remain functionally impaired. 3-D aggregates or "spheroids" of adipocytes display morphology similar to the one found in native adipose tissue (minimum extracellular matrix and closely-packed cells) [10–15]. Benefits of 3-D spheroids over 2-D monolayer cultures include increased adipogenic markers such as triglyceride accumulation as well as expression of adipose-specific genes C/EBP-α, PPAR-γ, and adiponectin [12]. Spheroid organization *in*

* Corresponding author.
E-mail address: ajanorkar@umc.edu (A.V. Janorkar).

http://dx.doi.org/10.1016/j.procbio.2017.02.003
1359-5113/© 2017 Elsevier Ltd. All rights reserved.

vitro prior to implantation has been shown to improve *in vivo* angiogenesis [13] as well as survivability under hypoxic physiological conditions [10]. 3-D spheroid configuration has also been found to enhance pluripotent potential and differential efficacy into multiple mesenchymal cell lines when exposed to appropriate differentiation media *in vitro* [14]. We believe that 3-D spheroids may be advantageous over other encapsulation-based 3-D adipose tissue engineering methods as the growth potential of the 3-D spheroids is not restricted by the exogenous encapsulating substrate material. 3-D ASC spheroids have been fabricated by mechanically-induced suspension [10] and hanging drop [11] techniques. Unfortunately, the suspension culture techniques suffer from declining cellular function over a longer culture period possibly due to the increased shear stress experienced by the cells and the hanging drop culture techniques suffer from logistical drawbacks such as difficulty in providing fresh medium and maintaining the stability of cultures over a longer culture period. To minimize these disadvantages, substrate features such as surface morphology [12], porosity [15], and non-adhesive chemistry including immobilized fibroblast growth factor 2 [13] and chitosan films [14] have been exploited to induce spheroid formation *in vitro*. These approaches, while successfully achieving spheroid formation due to the non-adhesive nature of the substrates, suffer from detachment of the spheroids as the formed spheroids fail to tightly adhere to the substrates.

Our method of 3-D spheroid culture used a surface modification approach and creatively avoided using the size-limiting porous or hydrogel scaffolds, unstable suspension or hanging drop cultures, as well as quick detachment of spheroids from the non-adhesive substrates. Specifically, our research utilized the hASCs from the stromal-vascular fraction from human breast adipose tissue from a donor undergoing elective liposuction or abdominoplasty. hASCs were cultured atop a coating of elastin-like polypeptide- polyethyleneimine (ELP-PEI) copolymer, where the charged PEI segment encouraged spheroid formation, while the biocompatible ELP segment allowed surface attachment of the formed spheroids. To achieve adiposity, we exposed our adipose cultures to a physiologically relevant fatty acid level (500 μM) [16]. We have screened key fatty acids representing clinically and nutritionally relevant classes implicated in influencing adipogenesis; namely, monounsaturated oleic acid (C18:1, OA) and polyunsaturated linoleic acid (C18:2, LA). These differentially treated adipose cultures were then assessed for their growth and adipogenic potential over a 10-day maturation period. 3T3-L1 mouse preadipocytes are unipotent cells committed to adipocyte lineage and have been widely used in adipose functionality studies [17–19]. Therefore, finally, we compared the adipogenic differentiation of hASCs to that of the 3T3-L1 cells.

2. Experimental methods

2.1. Synthesis and characterization of ELP-PEI

Conjugate of ELP with backbone structure Valine-Proline-Glycine-Valine-Glycine (molecular weight = 17,000 Da) and PEI (molecular weight = 800 Da, Sigma-Aldrich, St. Louis, MO) was prepared using carbodiimide chemistry as described elsewhere [20,21]. The phase transition temperatures of ELP and ELP-PEI solutions (5 mg/mL) were determined on 3 replicates (n = 3) using a Cary 100 spectrophotometer equipped with a temperature controller by measuring the absorbance at 350 nm as the solutions were heated from 20 to 80 °C at the rate of 1 °C/min. The primary amine content of 5 independently synthesized ELP-PEI conjugates (n = 5) was measured using the O-phthlaldehyde (OPA) assay (Thermo Scientific, Pittsburgh, PA) according to the manufacturer's protocol.

2.2. Modification of Tissue Culture Polystyrene (TCPS)

ELP-PEI conjugate was adsorbed to 24-well TCPS plates by placing 200 μL of a 5 mg/mL, 5 mol% ELP-PEI solution in deionized water in each well. The plates were incubated at 37 °C for 48 h in a dry incubator to remove all solvent.

2.3. hASC Isolation and maintenance

Our experiments utilized normally discarded breast whole adipose tissue provided during elective procedure from a single unidentified adult female patient in accordance with the protocol approved by the University of Mississippi Medical Center Institutional Review Board (Approval # 2012–0004). Donated adipose tissue was dissected and digested to harvest undifferentiated ASCs for subsequent culturing and differentiation using techniques demonstrated by Flynn [4,22], adapted from methods developed by Hauner et al. [23]. Whole breast adipose tissue was collected and maintained in sterile Na^+/Ca^{++} free PBS, arriving in the lab immediately after extraction. Working under a sterile laminar-flow hood, the tissue was placed in a tissue culture dish, minced into small (~1 mm^3) sections, and washed with PBS to help remove blood, oil, serum, other vascular components, and lymph vessels. The tissue fragments were then digested in Liberase TM collagenase (0.12 AU/mL) in PBS supplemented with 3 mM glucose, 25 mM HEPES, and 20 mg/mL BSA for 20 min at 37 °C in a shaker bath. The tissue was then filtered through sterile layered cotton mesh to remove undigested fragments. Cell media (DMEM, 10% FBS) was then added to the filtrate and the cells allowed to gravity separate. The supernatant was aspirated to remove mature adipocytes and the remaining filtrate centrifuged at 1200 × *g* for 5 min. The cell pellet was suspended in preadipocyte media (DMEM + 10% calf serum). The remaining cells were pelleted again, resuspended in whole media, and filtered through a 60-μm mesh nylon net to remove mature adipocytes, clusters of erythrocytes and endothelial cells, and any remaining tissue and cell aggregates. The remaining stromal-vascular fraction including ASCs was cultured on TCPS dishes in 50:50 DMEM:Ham's F12 with 10% calf serum at 37 °C and 5% CO_2, with regular media changes every 2-3 days. Cells were used for experiments between their 6th and 9th passage.

2.4. hASC Culture and adipogenic differentiation

50,000 hASCs were seeded per well of a 24-well TCPS plate (26,000 cells/cm^2) coated with the ELP-PEI conjugate and allowed to form 3-D spheroids over a 72 h period. To prepare 2-D monolayer culture, 50,000 hASCs were seeded per well of an uncoated 24-well TCPS plate and cultured to confluence over the same 72 h period. hASC differentiation was then carried out for 72 h in media containing 50:50 DMEM:Ham's F12 media supplemented with 1 μM dexamethasone, 0.5 mM IBMX, 0.1 U/mL insulin, 1 μM indomethacin, and 100 units/mL penicillin – 100 μg/mL streptomycin, a similar hormone cocktail demonstrated to induce adipose differentiation [14,24–26].

2.5. Induction of adiposity

Fatty maturation medium was prepared by supplementing the maintenance medium with 2% bovine serum albumin (BSA) acting as the carrier agent and 500 μM of linoleic acid (LA) or oleic acid (OA). Control maturation medium was prepared by supplementing the maintenance medium with 2% BSA and no additional fatty acid. Media were sonicated at 40 °C for 45 min, cooled to 4 °C, and supplemented with 0.2 U/mL insulin, 100 units/mL penicillin, and 100 μg/mL streptomycin. Fatty acid loading efficacy was verified by gas chromatography analysis [20]. hASCs were exposed to control

Download English Version:

https://daneshyari.com/en/article/6452975

Download Persian Version:

https://daneshyari.com/article/6452975

Daneshyari.com