Culture and Analysis of Adipocytes in Two and Three Dimensional Culture Configurations

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Statement of Purpose: Currently in the United States one in three adults are obese, with the CDC reporting 72.5 million adults affected. Obesity related diseases account for $147 billion in health care costs, and the annual mortality rate from this disease exceeds 300,000.[1,2] For effective treatment, obesity must be better understood at the cellular level with respect to metabolic state and environmental stress; however, currently used 2-dimensional (2-D) *in vitro* cell culture does not represent the *in vivo* adipose tissue appropriately due to absence of complex architecture and cellular signaling. 3-D *in vitro* cultures have been reported to have optimal results mimicking the adipose tissue *in vivo*.[3] Previously we have used elastin like polypeptide (ELP) coupled to polyethyleneimine (PEI) to create 3-D liver-tissue constructs.[4,5] The aim of this study is to examine the efficacy of the ELP-PEI conjugate toward creating a 3-D preadipocyte culture system, study their differentiation process when subjected to various concentrations of nutritionally relevant free fatty acids with respect to intracellular triglyceride accumulation, total protein content , and generation of reactive oxygen species.

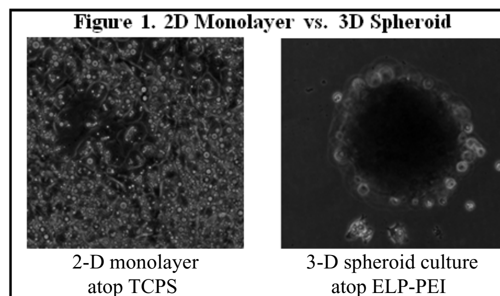
Methods: ELP was conjugated to PEI and ELP-PEI copolymer was coated onto the 24-well TCPS plate as per previous protocol[4,5]. 3T3-L1 mouse preadipocytes were seeded at $5x10^4$ cells per well and were cultured in DMEM with 10% fetal calf serum. Cells were maintained at 37^oC in a humidified atmosphere of 5% CO_2 with fresh medium every 48 hours. Upon confluence and a 2-day growth arrest period, cells were differentiated for 3 days using dexamethasone and IBMX cocktail. After differentiation, cells were provided maintenance medium containing no fat (control), 1-4 mM oleic acid (OA), or 1-4 mM linoleic acid (LA) for five days.

Intracellular Reactive Oxygen Species (ROS) were labled using a ROS kit by following manufacturer's (Invitrogen) protocol and cells were observed continuously under a fluorescence microscope for up to 72 h.

3D and 2D cellular extract was prepared for biochemical analysis by trypsin digestion. After digestion, all aliquots were centrifuged 2 min at 1000 rpm, resuspended in deionized H_2O, and sonicated 30 sec at 10% amplitude. Total triglyceride, total protein, and DNA content were analyzed using standard quantification kits with all assays performed per manufacturers' protocols (Sigma, Thermo Scientific, and Sigma, respectively). Total Protein and triglycerides were normalized to DNA content. All experiments were performed at least in triplicate. Results reported as mean ± 95% confidence intervals.

Results: 3T3-L1 cells on the ELP-PEI surface formed cellular aggregates and subsequently formed spheroids within 72 h with diameters of about 100 μm. (**Figure 1**).

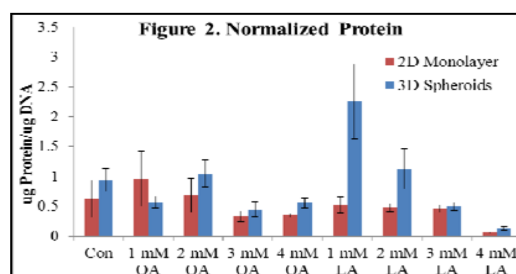
Figure 1. 2D Monolayer vs. 3D Spheroid



2-D monolayer atop TCPS | 3-D spheroid culture atop ELP-PEI

Upon examination of the normalized triglyceride levels, the 2-D culture exhibited no statistically significant difference with respect to the various OA concentrations; however the 2-D cultures had a marked decrease of triglyceride levels upon increased concentration of LA in the maintenance medium (data not shown). The 3-D cultures also had a statistically significant difference with respect to the OA or LA concentrations but to a lesser degree compared to the respective 2D monolayer cultures. Normalized protein levels (**Figure 2**) showed that higher concentrations of OA or LA in the maintenance medium produced lower amounts of protein for both 2-D and 3-D cultures. A possible outlier was 1 mM LA, which will be investigated further. When examining reactive oxygen species, the OA supplementation in the maintenance medium produced lower ROS compared to LA (data not shown). When coupled with the decreased triglyceride levels, the higher ROS activity in the case of LA indicates that LA is more toxic to the cells compared to OA.

Figure 2. Normalized Protein



Conclusions: Our results indicated that the 3-D culture may be a more sensitive modeling technique for *in vitro* adipocyte culture. The adipocytes in 3-D cultures in general produced higher amounts of total protein compared to the respective 2-D monolayer cultures. These studies therefore provide a platform for future evaluation of 3-D *in vitro* adipocyte culture.

References:

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