AN IN VITRO METHOD FOR IDENTIFYING AGENTS WITH LIPOFILLING-LIKE ACTIVITY: PARALLEL ASSESSMENT OF LIPID ACCUMULATION, TOXICITY AND GENE EXPRESSION

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ABSTRACT

An in vitro assay was developed to facilitate the identification and characterization of agents demonstrating lipofilling-like activity. The method involves exposing replicate cultures of primary human pre-adipocytes in 2D culture to a variety of test compounds during cell differentiation. The cultures are then divided into groups for analysis of lipid accumulation using Adipose cell viability using Cell Titer Blue, and collection of RNA for gene expression analysis. Agents for which enhanced lipid accumulation is observed relative to vehicle controls without adverse effects on cell viability are flagged for gene expression analysis to characterize the metabolic pathways affected.

In the initial test of the assay, the effect of three materials purported to enhance lipid accumulation in adipocytes was evaluated. Two of the test agents were found to promote lipid accumulation in differentiating pre-adipocytes as evidenced by Adipose Blue staining which were approximately 3.5 times and 8 times the corresponding vehicle controls, respectively; with no obvious toxicity (Gene expression analysis not performed). In contrast, the third agent failed to enhance lipid accumulation relative to the corresponding vehicle control.

Data obtained from a subsequent test of the method involving the same three agents, along with 12 additional test agents, revealed significant inter-assay variability that is likely related to the use of different batches of primary human pre-adipocytes in each assay run. Despite the need for further assay optimization, the method described here represents an important first step towards developing a reliable in vitro method for identifying and characterizing compounds with lipofilling-like activity for further study.

INTRODUCTION

Human skin aging occurs at different rates, with some individuals showing fewer signs of aging than others, despite environmental exposures. Corrective measures mainly focus on smoothing the surface microtexture, decreasing perceived pigmentation in irregularities, and restoring the appearance of fine lines and wrinkles. New design lines and wrinkles and uneven folds of the skin, the use of filters has come into common use.14 The upper and lower thirds of the face, along with the neck, have received the most attention with procedures now being developed for the microsculpting of facial fillers and stimulators used mainly to replace volume in the aging face with smoothing fat transfer and grafting becoming more common.15,16 Enrichment of grafts for adipocytes derived stem cells is now seen as a step toward better long-term success, but given their multiple potential rates a certain degree of caution may be needed in attempting to stimulate differentiation for enhancement of adipocyte number and lipid accumulation.15,16 In addition to filling in the face, a comprehensive anti-aging approach includes moisturizing with chemicals, laser and microdermabrasion as well as stimulating the restructuring of the extracellular matrix with topical active ingredients and energy sources such as thermal light, ultrasound, and radiofrequencies.17 Methods developed to document the aging face primarily focus on the shape, color, and texture of the facial skin and its thickness.18 However, one aspect of aging facial skin remaining to be adequately targeted is pan-facial subcutaneous lipohypertrophy or the loss of fullness supporting fat layering the dermis in general.19 In this study, we investigated the applicability of standard in vitro cell culture methods for screening topical cosmetic ingredients for use supporting the maintenance or enhancement of adipocyte number and Nordic in the subcutis.

OBJECTIVE

Optimize an in vitro cell culture system for the evaluation of cosmetic ingredients capable of supporting or enhancing facial subcutaneous adipose tissue.

METHODS

CELL, CULTURE, AND COMPOUND TREATMENTS. Primary human facial pre-adipocytes were obtained from individual subjects as adipocytes from Cell Line, Inc. The cells were cultured to confluency in 96-well plates (for lipid accumulation and toxicity assessments) and in 1-well plates (for RNA isolation) using a high-density preconditioning Medium (Zero-Eye, Inc. #1035M) before being differentiated into adipocytes over 16 days by replacing the Preconditioning Medium with Adipocyte Differentiation Medium (Zero-Eye, Cat. #1035M). Test compounds were re-vehicle controls described in the figure below were included in the Adipocyte Differentiation Medium during the first seven days of differentiation. After seven days, the differentiation medium and compounds were removed and replaced with Adipocyte Maintenance Medium (Zero-Eye, Cat. #1035M) and the cells were allowed to continue differentiation for an additional seven days.

ASSAY ENDPOINTS. Cultures treated with each test compound and related vehicles were processed to evaluate lipid accumulation (AdiposeBlue) and compound toxicity (Cell Titer Blue) using replicate cultures for each compound.

For lipid assessment by AdiposeBlue, the medium was removed from the culture and the cells were rinsed with phosphate buffered saline (PBS). PBS (2x50 µL) and AdiposeBlue (50 µL, Lonza, Cat. #70500) were added to each well, mixed, and incubated for 15 minutes at room temperature. Fluorescence was then read for each well using excitation/ emission wave-lengths of 485/535 nm.

For toxicity (lactate dehydrogenase) assessment by Cell Titer Blue, 5 µL of the Cell Titer Blue reagent (Promega, Cat. #A8001) was added to each well containing 250 µL medium. The cultures were then incubated for two hours at 37°C before measuring fluorescence using excitation/emission wave-lengths of 460/590 nm.

RESULTS

LIPID ACCUMULATION. Lipid accumulation was measured in primary human facial preadipocytes differentiated in the presence of three different compounds; A, B, and C. In the presence of compounds A, B, and C, the adipose cells were stained with Oil Red O, and the stained cells were washed with PBS, air-dried, and stained sufficient to the same dye concentration for subsequent examination.

REFERENCES