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Real-time monitoring of 3T3-L1 preadipocyte differentiation using a commercially available electric cell-substrate impedance sensor system

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ABSTRACT

Real-time analysis offers multiple benefits over traditional end point assays. Here, we present a method of monitoring the optimisation of the growth and differentiation of murine 3T3-L1 preadipocytes to adipocytes using the commercially available ACEA xCELLigence Real-Time Cell Analyser Single Plate (RTCA SP) system. Our findings indicate that the ACEA xCELLigence RTCA SP can reproducibly monitor the primary morphological changes in pre- and post-confluent 3T3-L1 fibroblasts induced to differentiate using insulin, dexamethasone, 3-isobutyl-1-methylxanthine and rosiglitazone; and may be a viable primary method of screening compounds for adipogenic factors.

1. Introduction

Label-free live cell monitoring provides an unhindered real-time view of whole cell biology. Real-time monitoring of adipogenesis using electric cell substrate impedance and capacitance based biosensor platforms have been reported for adipogenic human mesenchymal stem cells [1] and the mouse preadipocyte 3T3-L1 model [2], respectively.

In the capacitance based sensor developed by Lee et al. [2] cells were placed between two electrodes and the change in the dielectric constant (ε) was measured. This value is directly proportional to capacitance and is dependent on cell size, cell membrane potential and cellular content [3] making capacitance based sensors ideal to measure the differentiation of cells where gross cellular accumulation or novel biogenesis occurs; case in point, lipid accumulation during adipogenesis. However, lipid accumulation is but one property that can be measured to monitor adipogenesis. The morphology of cells dramatically changes during differentiation processes [4]. These changes in morphology can be measured in real-time using Electric Cell-Substrate Impedance Sensors (ECIS). These impedance based systems measure alternating current impedance differences between a smaller sensing and a larger counter current electrode; adherent cells remain viable and are cultured on the gold sensing electrode thereby passively blocking the current. The electrical impedance which results is registered by the sensor. Impedance is therefore affected by the shape, adhesion, or mobility of the adherent cells [5,6]. Both impedance based and capacitance based biosensors provide insights on what is happening to cells, and ideally an instrument that measures both these parameters would be advantageous.

The 3T3-L1 murine preadipocyte model is viewed as the gold standard to monitor unipotent cell differentiation to mature adipocytes, typically via supplementation with a cocktail of insulin, dexamethasone and 3-isobutyl-1-methylxanthine (INS/DEX/IBMX). The inclusion of the insulin sensitizer, rosiglitazone, has been reported to enhance differentiation [7]. Adipogenesis is typically gauged by end point monitoring by the formation of lipid droplets using Oil Red O staining. As 3T3-L1 differentiation has been shown to be sensitive to cell culture plastics and differentiation cocktail recipes [8], optimization of differentiation protocols through the use of end point assays can be laborious and inaccurate; real-time analysis offers defined benefits for the development of optimal, efficient differentiation protocols. This ultimately leads to more accurate results as a profile of cell growth, arrest or death [9] at any point during the differentiation process can be monitored.

When induced to differentiate, growth-arrested post confluent 3T3-L1 preadipocytes synchronously re-enter the cell cycle and undergo mitotic clonal expansion (MCE), mitotically dividing two to four times before differentiation. The DNA replication process

Abbreviations: INS, insulin; DEX, dexamethasone; IBMX, 3-isobutyl-1-methylxanthine; RTCA, Real-time Cell Analyser; ROSI, rosiglitazone.

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