A common European approach to the regulatory testing of nanomaterials

Standard Operating Procedure

Title	***SOP for High Throughput Cell Impedance Measurement***
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1 Introduction

Cell response to treatment can be evaluated through several parameters, cellular impedance is one of them. The present cellular impedance measurement is made with an xCELLigence MP® (ACEA Bisosciences), is a real time and long term assay, allowing to work with 6x96 wells plates in parallel. Cellular impedance signs global behaviour of the cells since it is an electric signal that includes cell proliferation, morphology and ionic exchanges (at the cytoplasmic membrane interface).

Following exposure to chemical compounds, nanoparticles or genotoxic stress, cell response will be either signed by transient (or not) proliferation arrest, death, volume modification (shrinkage, bubbling, …), or intense ionic pump activity modification.

Depending on the biological effect following exposure, the cell impendence could be either increased, decreased or kept constant.

2 Impedance measurement, High Throughput Screening

2.1 Experimental systems

The experimental systems employed in this study will be: A549, Hep3B, Caki-1 and Calu-3 cell lines.

Experimental systems preparation

Cells are routinely grown as a monolayer in tissue culture grade flasks in a humidified atmosphere at 37oC and 5% CO2/air, and they are sub-cultured when they exceeded 50% confluence (but less than 80%). Cells should be checked regularly for the absence of mycoplasma contamination and only used if none is found.

24 hours before the performance of the assays the cells are seeded in 96 well e-plates (specific plates of the xCELLigence systems, 80% of the wells surface is covered by gold electrodes, that mediate the impedance signal) using a concentration of 2500-15000 cells/well (depending on the experimental system employed). 24 ± 2 hours after seeding, cells are ready for the treatment.

2.2 Measurement of cell impedance

1. Define experimental conditions in the xCELLigence software
    a. Plate repartition, wells with nanoparticles only are needed, in order to evaluate the background. Each conditions is made in at least in four wells by plate.
    b. Frequency of measurement
        i. Every 5min during the first 6h
        ii. Then every 10min until nanoparticles exposure
        iii. Every 5min following exposure
        iv. Then every 10min until the end of experiment (5 days after exposure)
2. Incubate cells with varying concentrations of nanoparticles.
3. After the needed time of exposure (from to 24h to 5 days), data are collected and formatted as following
    a. In the xCELLigence software
        i. Remove instrument errors: remove of the data of wells which can be rejected as instrument readout failure.
        ii. Extract the data to report: extract the cell index at the last good point before exposure start (exposure start – 10min) and 24h after exposure start (the chosen time for NanoReg, a longer time of exposure can be defined) for each well.
        iii. Data are copied in an Excel form for further exploitation
    b. In the Excel form

i. Change time scale: the time of the exposure t=24h is now t=0h in order to get easily the exposure length of each measurement point (subtracting the exact time of the beginning of exposure to the following time points).
ii. The mean of each conditions (at least four wells), and the corresponding standard deviation (SD) are calculated and reported in a graph.
iii. Median and SD of any measurement times can be reported in a table as well.