

Culturing & measurement optimization for improved cell physiology in toxicology applications

INTRODUCTION

Long-term and online monitoring of toxic effects with cell-based systems requires addressing two aspects. First, the stable delivery of data over time and secondly, an adequate sensitivity of the system for the proposed application need to be assured. The composition of the running medium (the media used in the system for the measurement) has shown to be of major importance concerning signal stability during the experiment and sensitivity to toxicants.

In this study the **Bionas Discovery™ 2500 system** was used to investigate the effects of different media compositions on the stability and the sensitivity of V79 and HepG2 cells after the treatment with acetaminophen (APAP). Oxygen consumption, acidification and cell impedance/ adhesion were measured with a multiparametric chip.

MATERIALS & METHODS

Cell culture. V79 and HepG2 cells were purchased from Cell Lines Service (Germany) and cultured in DMEM (5% CO₂) + 4 mM Glutamine + 1 mM Na-pyruvate as the basal medium with varying amounts of glucose and galactose. For measurement, the **Bionas® running medium** (RM, 1mM HEPES) was used with differences in glucose and galactose (see table 1).

Name	Glucose	Galactose	FBS	Buffer
GluCM	25 mM	no	10 %	CO ₂
GluRM	25 mM	no	0.1 %	HEPES
GalCM	0 mM	10 mM	10 %	CO ₂
GalRM	0 mM	10 mM	0.1 %	HEPES

Table 1: Composition of media used for experiments. **GluCM:** Glucose Culture Medium, **GluRM:** Glucose Running Medium, **GalCM:** Galactose Culture Medium, **GalRM:** Galactose Running Medium

Used combinations of Culture Medium and Running Medium (CM/RM) were Glu/Glu, Glu/Gal and Gal/Gal.

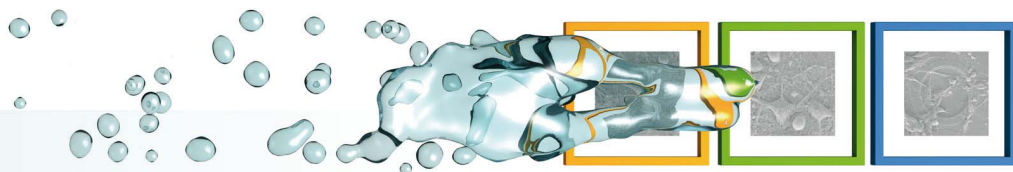
Test Compound. Acetaminophen (APAP, Sigma Aldrich, Germany) was used in varying concentrations from 0.5 - 20 mM. As control, medium with the highest percentage of DMSO was used.

Analysis in the Bionas Discovery™ 2500 system. After receiving a stable baseline with RM, APAP was applied at different concentrations for 24h. The exposure was followed by a regeneration phase and a final killing of the cells by adding Triton-X-100 to the RM. To investigate the behavior of the cellular reactions and to compare the mechanisms in acidification, respiration and cell impedance/ adhesion, dynamic IC₅₀ values were calculated and plotted for each time point. These data sets will show the dynamic behavior of the cellular reactions and allow comparing cellular physiology.

In Figure 1 the acidification, respiration and cell impedance/adhesion of V79 cells in Glu/Gal medium (A, B, C) and HepG2 cells in Gal/Gal medium (D, E, F) are shown as percent of control. In Figure 2 dynamic IC₅₀ values for all media compositions tested with both cell lines are shown. The tables 2 and 3 represent the effect of APAP on the endpoint IC₅₀ values for all combinations of culture and running medium tested.

The most sensitive combination for the V79 cells was the Glu/Gal medium (see Fig. 1: A, B, C). For the HepG2 cells the Gal/Gal medium was the most sensitive (see Fig. 1: D, E, F).





EFFECT OF APAP ON V79 AND HEPG2 CELLS

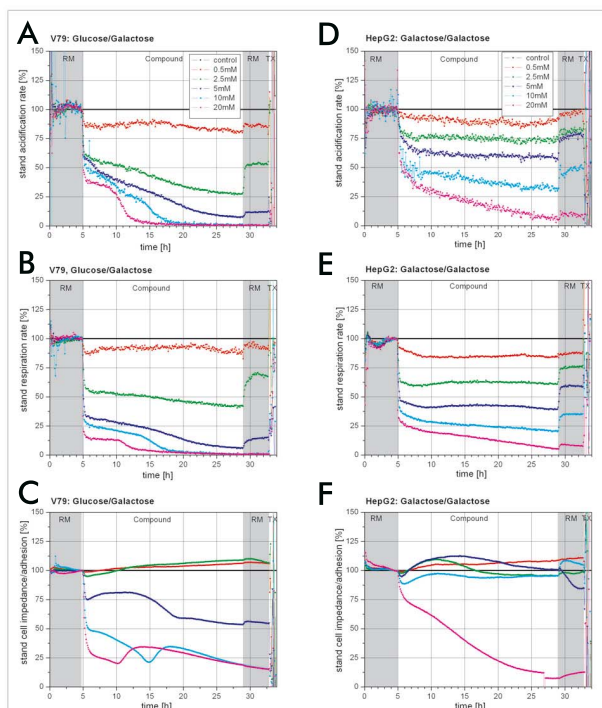


Fig. 1: Acidification, respiration and cell impedance/adhesion of V79 cells in Glu/Gal medium (A, B, C) and HepG2 in Gal/Gal medium (D, E, F).

IC ₅₀ (mM)	Acidification	Respiration	Impedance
Glu/Glu	activation!	3,7	16,0
Glu/Gal	1,7	2,2	5,5
Gal/Gal	3,9	3,9	15,0

Table 2: Effect of APAP on IC₅₀ (24h) values for V79 cells.

CONCLUSION

Culture and experimental settings have a strong influence on dose-dependent toxic effects of APAP. This effect on the respiration and acidification rates as well as on cell impedance/adhesion could be analyzed successfully by the **Bionas Discovery™ 2500 system**.

Running medium containing galactose instead of glucose enhanced selectivity clearly. Regarding sensitivity and stability, Glu/Gal combination for V79 cells and Gal/Gal combination for HepG2 cells were most qualified for the requirements of long-term online monitoring.

These findings lead to the conclusion that the composition of culture and assay medium is of immense importance to guarantee sensitive and highly reproducible results.

DYNAMIC IC50 VALUES OF V79 AND HEPG2 CELLS

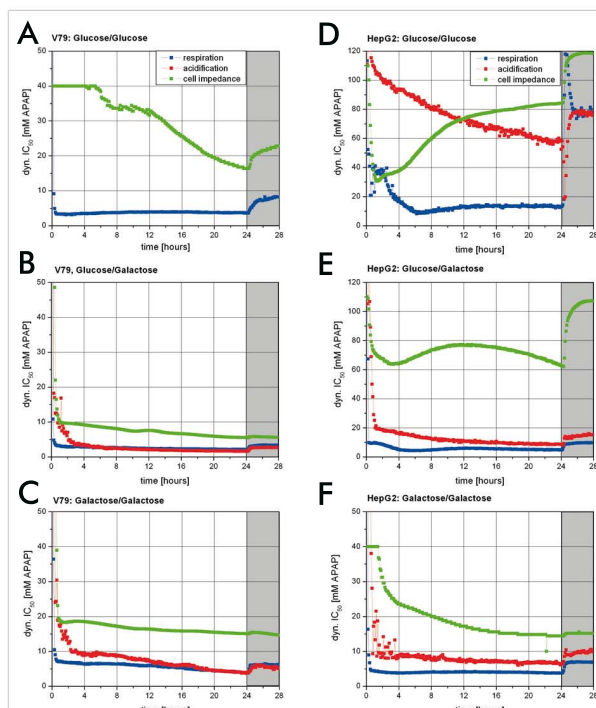


Fig. 2: Dynamic IC₅₀ values for acidification, respiration and cell impedance/adhesion of all media compositions on V79 (A, B, C) and HepG2 (D, E, F) cells.

IC ₅₀ (mM)	Acidification	Respiration	Impedance
Glu/Glu	52,5	13,2	84,1
Glu/Gal	8,7	4,8	62,8
Gal/Gal	6,5	3,8	14,4

Table 3: Effect of APAP on IC₅₀ (24h) values for HepG2 cells.

KEYWORDS

Cell culture optimization, APAP, toxicity, dynamic IC50 value, V79 cells, HepG2 cells

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