Facilitated genotoxicity screenings in expanded human upcyte® keratinocytes

Kathleen Jarnouen[2], Astrid Nörenberg[1], Timo Johannssen[1], Torge Evenburg[1], Meryl Roudaut[2], Julian Bursztyka[2], Pierre-Jean Ferron[2]

[1] upcyte technologies GmbH, Osterfeldstr. 12-14, 22529 Hamburg, Germany
[2] HCS Pharma, 6 Rue Pierre Joseph Colin, 35000 Rennes, France

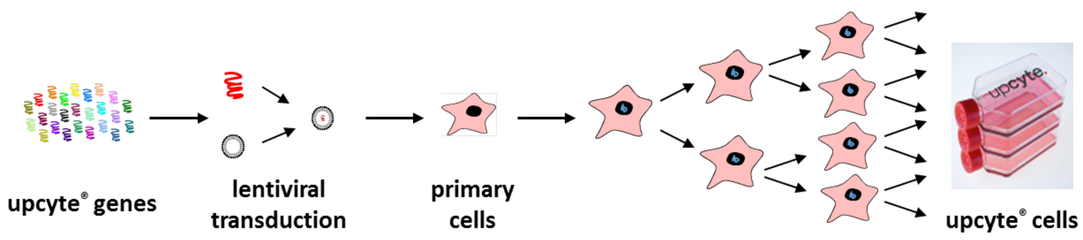
INTRODUCTION

Genotoxicity assessment of pharmaceuticals or active ingredients used in cosmetology is mandatory for registration. In addition to the standard *in vitro/ in vivo* test battery, other assays can be of interest because of their high throughput in the drug discovery stage. Among them, the γH2AX *in vitro* assay was suggested for detecting genotoxic properties of chemicals. The phosphorylation of γH2AX has been demonstrated to be a sensitive marker for DNA double strand breaks (DSB). Likewise, p53-binding protein 1 (53BP1) is an important regulator of the cellular response, promoting the end-joining of distal DNA ends.

Keratinocytes are routinely used for skin toxicity studies. However, large-scale cultures of primary keratinocytes demanded by high throughput approaches face obstacles such as a limited lifetime and *in vitro* differentiation, often compromising reproducibility due to inhomogeneous cultures. Here, we describe the expansion of primary keratinocytes after lentiviral transduction (upcyte® keratinocytes) for large-scale production and their subsequent use in γH2AX/53BP1 genotoxicity screenings.

RESULTS

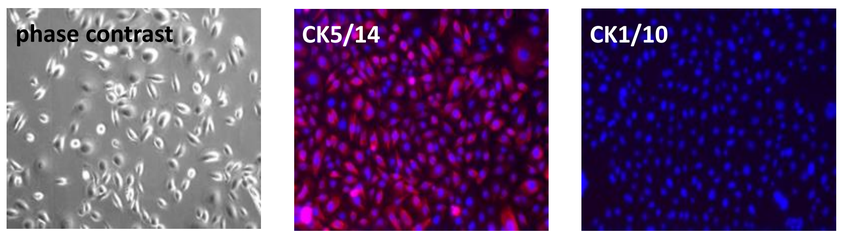
the upcyte® technology



transduction of primary cells with a defined cocktail of lentiviral vectors

Previously, a novel technique was reported which enabled the generation of non-transformed proliferating liver cells from primary human hepatocytes with maintained adult phenotype, so-called "upcyte® cells". upcyte® cells start to grow from primary cells after transduction with a defined cocktail of lentiviral vectors carrying proliferation inducing genes. upcyte® cells have the ability to proliferate for additional cell doublings, depending on the cell type, without losing functional and phenotypic characteristics of mature cells.

morphology & surface markers of upcyte® keratinocytes



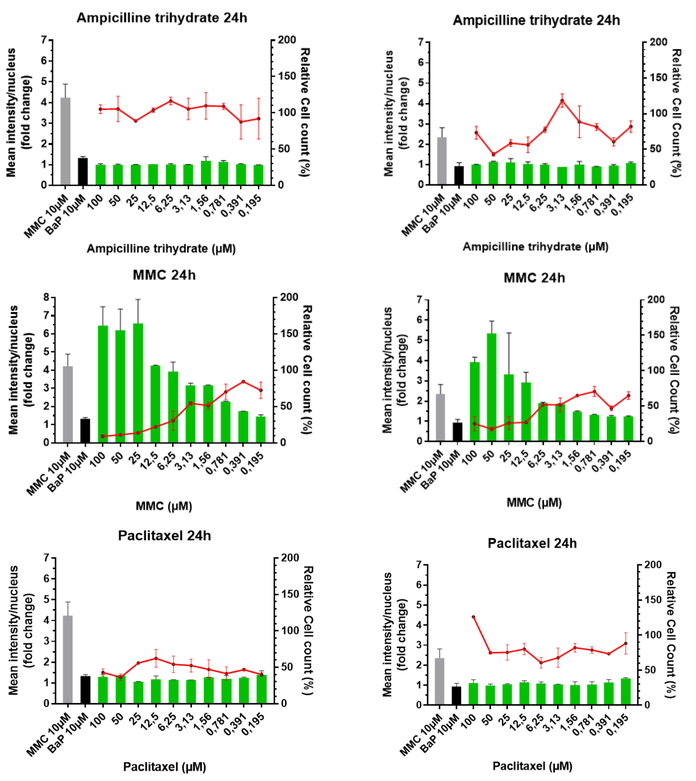
analysis of upcyte® keratinocytes

The morphology of upcyte® keratinocytes is similar to the morphology of primary keratinocytes. upcyte® keratinocytes are analyzed by fluorescence microscopy for the expression of the **cytokeratins 5/14 (red, middle panel), 1/10 (red, right panel)** and counter-stained against **DAPI (blue)**. They are characterized by high expression of the basal marker CK5/14 while lacking expression of the suprabasal markers CK1/10.

suitability of upcyte® keratinocytes for genotoxicity

Mean γH2AX intranuclei intensity after 24 h

upcyte® keratinocytes | NHEK

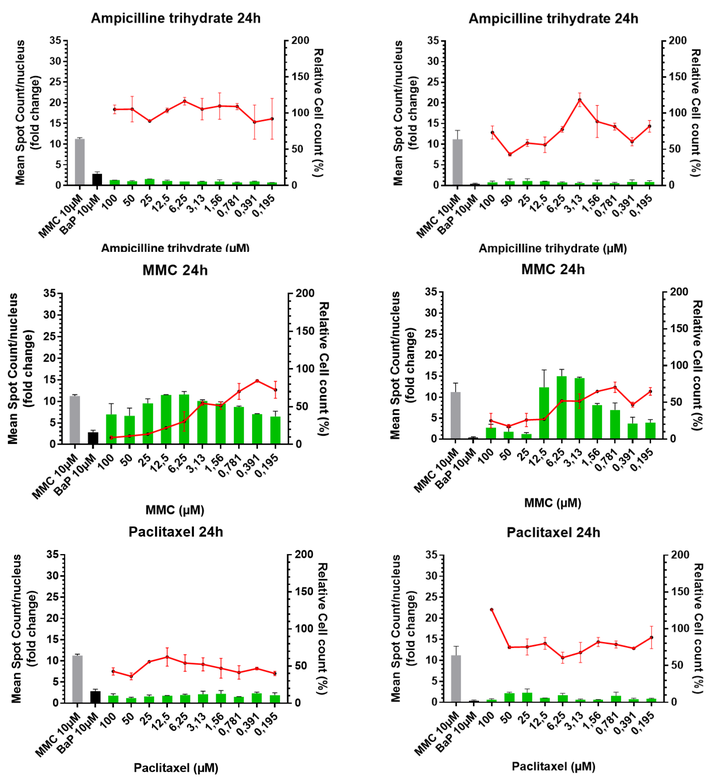


RCC = Relative Cell Count
gH2AX spots = mean intranuclei intensity of the γH2AX staining, expressed as a fold change when compared to control normalized to 1.

To further evaluate suitability of upcyte® keratinocytes for genotoxicity studies, upcyte® keratinocytes and neonatal human epidermal keratinocytes (NHEK) were challenged with 16 model compounds including **non-genotoxic controls** (e.g. ampicilin trihydrate), **clastogens** (e.g. mitomycin C (MMC)) and **aneugens** (e.g. paclitaxel).

Mean γH2AX intranuclei spot count after 24 h

upcyte® keratinocytes | NHEK

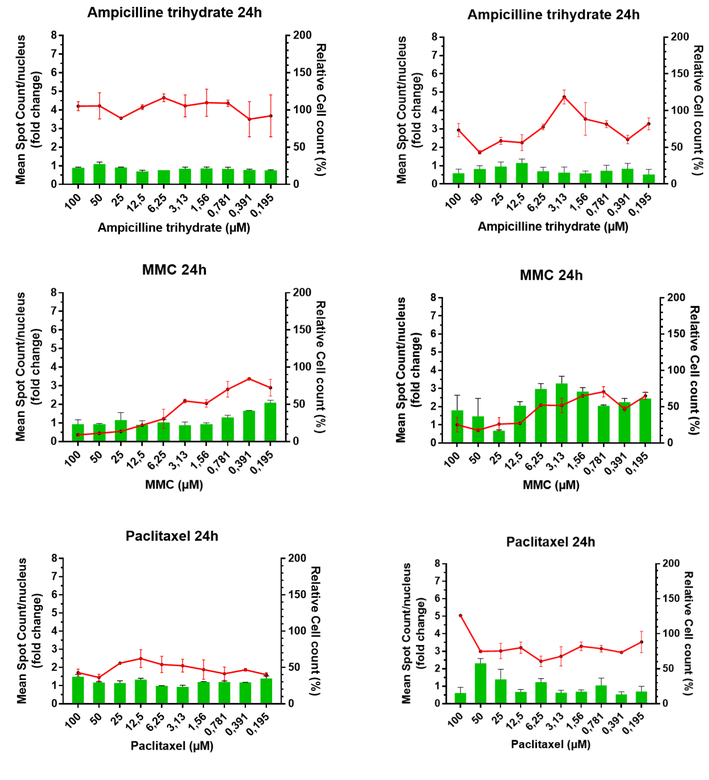


RCC = Relative Cell Count
gH2AX spots = mean intranuclei γH2AX spot count, expressed as a fold change when compared to control normalized to 1.

Intranuclear γH2AX and 53BP1 spots were analyzed using an automated imaging approach. When looking at the γH2AX spot count, upcyte® keratinocytes were comparable to NHEK and allowed accurate identification of clastogenic compounds. In contrast, no increase of γH2AX was observed with non-genotoxic substances.

Mean 53BP1 intranuclei spot count after 24 h

upcyte® keratinocytes | NHEK



RCC = Relative Cell Count
gH2AX spots = mean intranuclei 53BP1 spot count, expressed as a fold change when compared to control normalized to 1.

Interestingly, intranuclei 53BP1 spot counts revealed a base level 2 to 4.8 fold higher in upcyte® keratinocytes when compared to NHEK. This may explain that the fold induction of intranuclear 53BP1 spots after exposure to genotoxic compounds tended to be smaller in upcyte® keratinocytes when compared to NHEK.

SUMMARY & CONCLUSION

In conclusion, fold change of intranuclei γH2AX spot count in upcyte keratinocytes follow the same trends as in NHEK. On the other hand, intranuclei 53BP1 spot count shows a base level 2 to 4.8 fold higher in upcyte keratinocytes when compared to the batch of NHEK used (DMSO 0.5%). Consequently, upcyte® keratinocytes were suitable for genotoxicity studies using the γH2AX assay for DSB assessment. Combined with virtually unlimited cell access, they represent an attractive platform for automated screening approaches such as High Content Imaging.

info@upcyte.com **www.upcyte.com**
pierre-jean@hcs-pharma.com hcs-pharma.com