Could CTA with fluorescent labelling be a reliable alternative method to identify non-genotoxic substances in Food Contact Material extract ?

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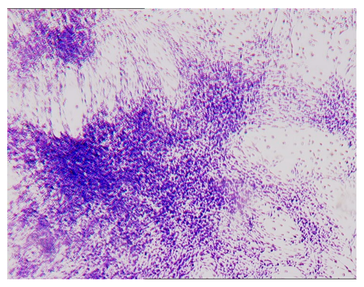
Introduction

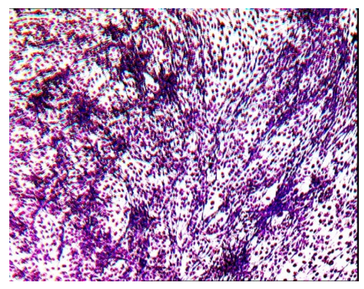
The CTA using Bhas 42 cells, is a 3T3 mouse fibroblast cell line transfected with several copies of the Ras gene allowing a cell transformation process. Indeed the process of cellular transformation is possible because it is a cell already initiated.
This process is described by the formation of foci characterized by :

- deep basophilic staining
- multilayer growth (piling up of cells)
- invasive cell growth into the background monolayer.
- spindle-shaped cells
- random cell orientation

Transformed foci :



Non-transformed foci :



Non-intentionally added substances (NIAS) are chemical compounds that are present in food contact materials (FCMs) and could therefore migrate into food, but they are not added for a technical reason during the production process. Often their presence is not known by the consumer and not even by the producer.

Cell Transformation Assay (CTA) is it able to detect non-genotoxic carcinogen agents with less of test article, inside a complex mix or a Non-Intentionally Added Substances (NIAS) ?

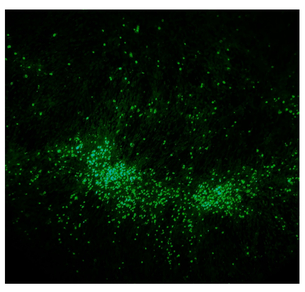
In this work the objective is to minaturize the classical CTA. In a second step, the aim is to establish a more specific and sensitive labeling of transformed foci.
Finally we would like to identify transcriptomics markers impacted by a non-genotoxic cancerigen agent.

Results

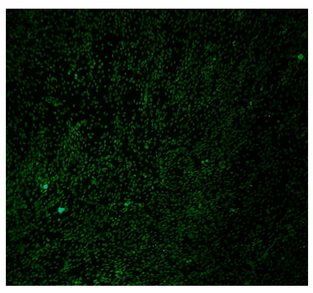
Labelling with BMVC

Identification of the foci is done by an experienced experimenter. This staining allows an easier identification of the foci because it is realized under fluorescence.

Transformed foci

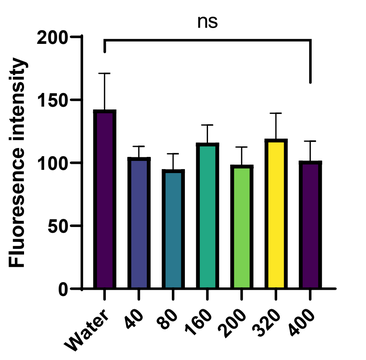


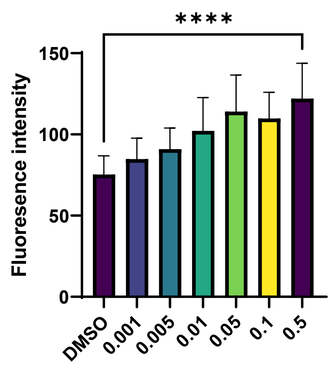
Non-transformed foci

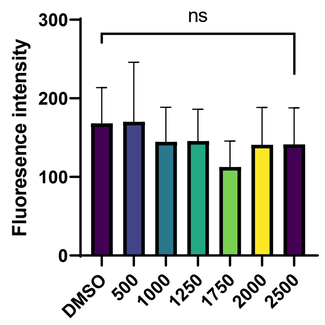


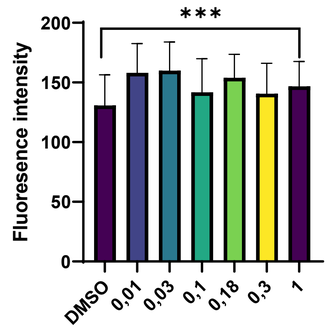
In parallel, a quantitative examination can be performed. The fluorescent properties of BMVC allow a spectrophotometric determination with excitation at 435 nm and emission at 560 nm.

Labelling BMVC cells treated with caffeine, TPA, Cyclophosphamide or Phorbol 12,13-didecanoate :







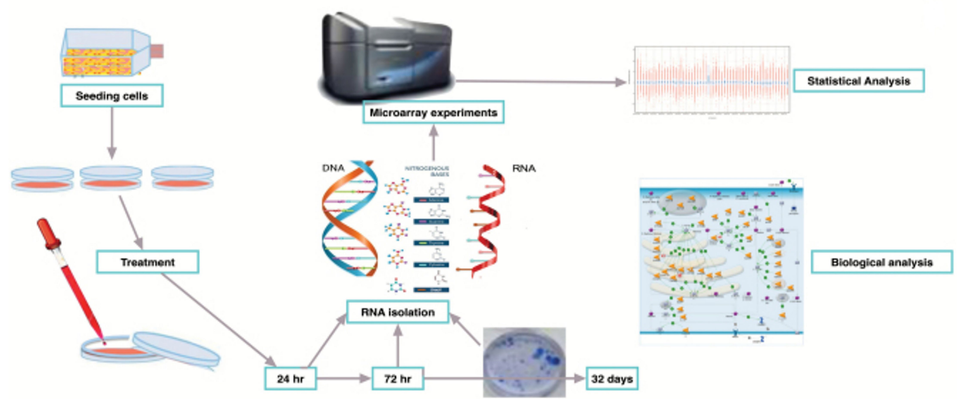


BMVC : 3,6-Bis(1-methyl-4-vinylpyridinium)-carbazole diiodide
TPA : 12-O-tetradecanoylphorbol-13-acetate

Mann-Whitney test,
**** : P value<0,0001, ***: P value<001

Transformics Assay

A transformics assay allows the examination of the transcriptome to target which steps of the cell cycle are impacted by the test product.



Experimental design of the transformics assay *Edited by Mascolo and al, 2018*

Previous publications illustrate its interest:

•TPA and Silice nanoparticles :
➢The selected genes were mainly related to positive regulation of cell proliferation and negative regulation of cell adhesion

•HAPs (2, 4-diaminotoluene, benzo(a)pyrene, 2-acetylaminoflourene, or 3-methycholanthrene) :
➢14 genes regulated in common for the 24h and 32 days groups which show the predictivity of this test

•38 chemicals (tumor promoters, non-tumor promoters, genotoxic carcinogens and food additives) :
➢The cells are treated for 48 hours, resulting in only a list of genes commonly known to be involved in human cancers.

In our laboratory, we would like to combine genomic analysis with BMVC labelling and carry out this analysis specifically on transformed foci.

Conclusion

- The miniaturized 96-well CTA identifies genotoxic and non-genotoxic carcinogens using fewer cells, less medium and, most importantly, less test material.
- Labeling of BMVC on Bhas 42 cells combined with CTA would allow for more accurate and sensitive quantification, in addition to allowing for a non-examiner-dependent quantification.
- The transcriptome analysis allows to identify the impacted cell cycle steps, such as immune response, cell adhesion, apoptosis and cell proliferation.

Outlook

❖Automation of rinsing and identification of foci using a microplate washer and a microplate reader.

❖Use BMVC labeling to identify the potential non-genotoxic carcinogenicity of NIAS.

❖Reduction of FBS variability by using a serum substitute, non-fetal serum or by reducing the proportion of serum in the medium.

❖Identification of non-genotoxic (epigenetic) damage with hypermethylation or hypomethylation as well as with different DNA compactions

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