The use of gap junction intercellular communication bioassays to assess the potential tumorigenic effects of environmental contaminants.

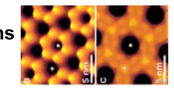
Bailey Delcamp, Abdikeni D. Sharif, Jamie Liebold, Lizbeth Lockwood, Brad L. Upham

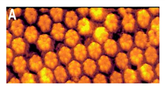
Michigan State University, Department of Pediatrics & Human Development, and the Center for Integrative Toxicology

Introduction

Uncontrolled cell proliferation common to many diseases, such as cancer, involve multiple intracellular signaling (Signal Transduction, ST) pathways, and these ST pathways vary among different types of diseases, including different types and subtypes of cancers, but all these pathways needed for cells to proliferate in tissues must close gap junction channels

Gap Junctions "open"





Gap Junctions "close"

Thus, assessing gap junctional intercellular communication (GJIC) is a great first step in the toxicological assessment of environmental contaminants

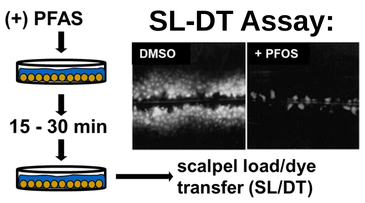
Experimental Methods

Cell Line

WB-F344 rat liver epithelial cells

- Normal diploid, non-tumorigenic **oval cells** that differentiate into hepatocytes and biliary duct cells.
- The cell line was derived from the livers of F344 rats.

GJIC Analysis



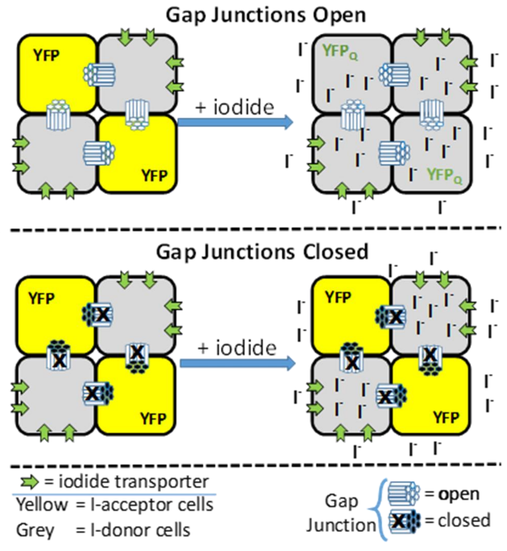
High Throughput Assay:

YFP H148Q/I152L (YFP^{QL}) was developed as a highly sensitive iodide sensor. *(Galietta et al., 2001, FEBS Lett 499: 220-224).*

We transfected this YFP^{QL} into a subset of the WB-F344 cells (YFP), which become the receptor cells.

The SLC26A4 gene encodes for pendrin. This protein transports anions, including chloride, iodide, and bicarbonate, across cell membranes. and was transfected into another subset of WB-F344 cells, which becomes the Iodide transporter donor cells .

- **The addition of iodide initiates the assay where the iodide transporter cells (IT) take up the iodide.**
- **If the gap junction channels are open, then the Iodide is transferred to the YFP cells with a partial quenching of fluorescence.**

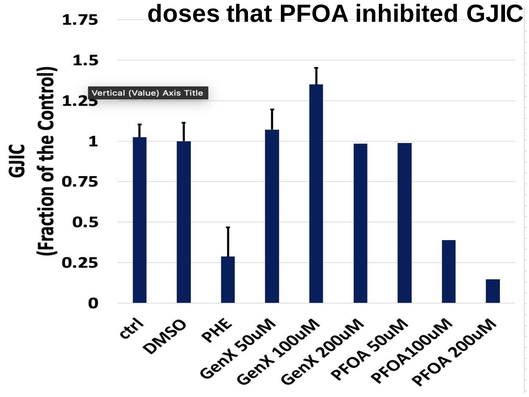


Hypotheses

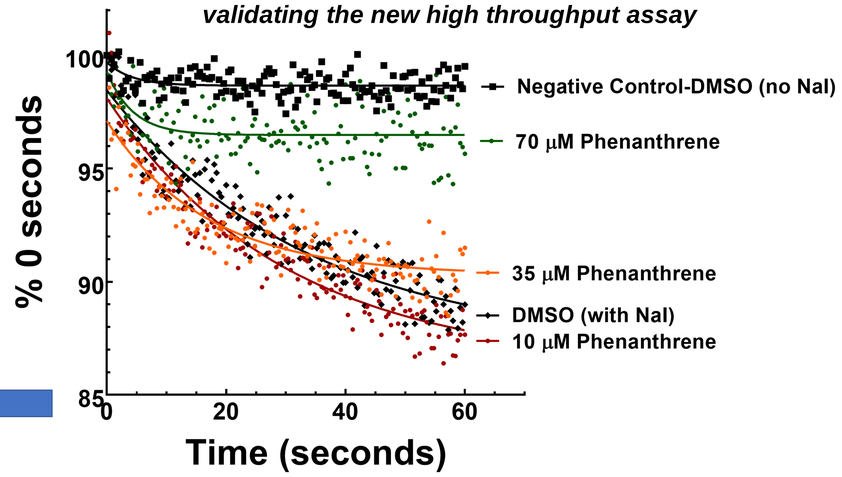
- **GenX, a per-and polyfluoroalkyl substance (PFAS) that manufactures state "it's a safer PFAS", will inhibit GJIC similar to a legacy PFAS, specifically perfluorooctanoic acid (PFOA).**
- **The data from the new high throughput bioassay of GJIC will be similar to the data from the well-established scalpel load – dye transfer assay (SL-DT)**

Results

GenX did not inhibit GJIC at the doses that PFOA inhibited GJIC

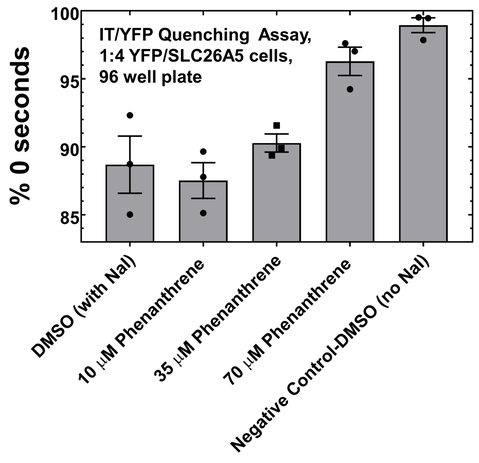


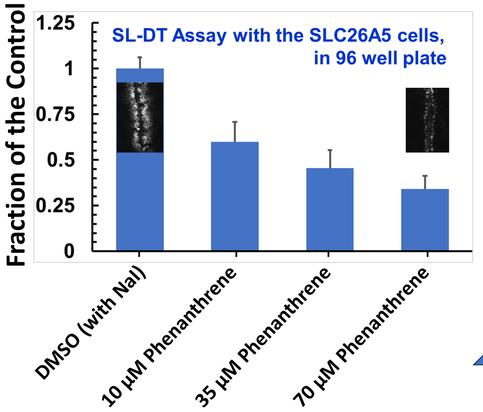
Fluorescence Quenching with the addition of iodide after treatment of the cell mixture with three doses of phenanthrene, a model inhibitor of GJIC, in validating the new high throughput assay

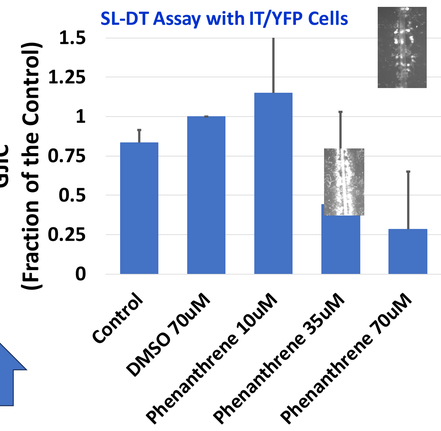


Average of results plotted

Phenanthrene Dose Response







The SL-DT results in both the donor and receptor cells were similar to those of the High Throughput Assay

Note: *For the YFP cells, we had to use cascade blue instead of Lucifer Yellow for the fluorescent tracer*

Conclusions

- **GenX, a PFAS that replaced PFOA and perfluorooctane sulfonic acid (PFOS) in the manufacturing of fluorinated polymers, did not inhibit GJIC at the doses that PFOA did, thus not supporting our original hypothesis.**
- **GenX could be potentially safer than the legacy PFAS's**
- **The new high throughput system (HTS) for assessing GJIC produced similar results to the well-established SL-DT assay using the GJIC model inhibiting compound, phenanthrene.**
- **These results suggest that this new high throughput system has the potential to screen larger numbers of environmental contaminants and drug candidates for effects on GJIC.**

Future Directions

- **Determine the effects of GenX for longer time periods, up to 24 h, on GJIC at non-cytotoxic doses, which will be determined with the neutral red uptake assay.**
- **Begin extensive testing of PFAS using the new high throughput GJIC assay.**

Research supported by the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH) under Award Number R25ES025060, and BRUSH Summer Research program. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH-NIEHS."