Plasma-based Cleaning of Pipette Tips Used in an Automated Cytotoxicity/Cell Proliferation Assay

Introduction

The time to market for new drug candidates (NDCs) is directly related to how quickly screening groups can identify compounds that show high target specificity, low toxicity, oral bioavailability and a significant half-life. Although a wealth of active compounds generally emerge from primary screens (usually biochemical assays), the majority of them do not meet safety and efficacy requirements in whole cells or intact organisms. As a result, high throughput secondary and ADME/Tox screening groups have invested in automated methods for distinguishing 'good' compounds from 'bad' ones earlier and more rapidly in the discovery process. Cytotoxicity and cell proliferation assays remain critical elements in these screening efforts. Because early-stage lead compound development programs require that pharmacological properties are optimized in tandem with therapeutic properties, cell-based toxicology assays are often run in lieu of time-consuming and often misleading in vivo studies.

To meet these demands, a growing number of cell-based assays that reflect normal human responses have been developed. However, these advances alone are not enough to meet the throughput demands of secondary screening and ADME/Tox environments; faster, more accurate and more economical methods for determining cytotoxicity and cell proliferation are required.

Plasma Cleaning of Pipette Tips

The TipCharger™ by IonField Systems™ uses self-contained, low-temperature, atmospheric plasma to clean pipette tips, metal cannula and pin tools associated with automated liquid handlers. Implementation of the TipCharger system provides cleaning equivalent to that of a fresh set of tips, reducing both the direct and indirect costs associated with replacing pipette tips.

In this study, plasma-based pipette tip cleaning efficacy using the TipCharger system was demonstrated using a commercially available assay system for detecting cytotoxicity and cell proliferation (Promega Corporation, Madison WI, CellTiter 96® Aqueous One Solution Cell Proliferation Assay, G3580). Cell viability was via MTS tetrazolium reduction measured by formazan production (Figure 1).

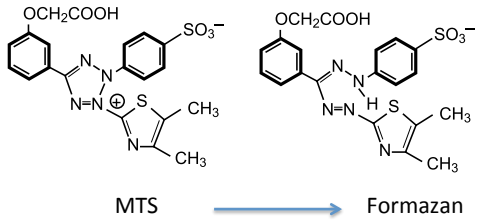


Figure 1. *MTS tetrazolium conversion to farmazan product (Promega Corporation, Madison WI).*

The quantity of formazan product as measured by absorbance at 490nm is directly proportional to the number of living cells in culture. The TipCharger system was shown to be an effective tool for eliminating staurosporine, a Protein Kinase C inhibitor commonly used as a standard cytotoxic agent. Resulting data was compared to historical tip replacement methods.

TipCharger Integration

In this study the TipCharger TC-96 cleaning station was taught as a reagent reservoir in conjunction with a Sciclone ALH 500 (Caliper Life Sciences, Hopkinton MA). Contaminants on the exterior of pipette tips exposed to TipCharger-generated plasma were immediately ionized; contaminants inside pipette tips were removed through a series of aspirate and dispense steps while tips were in the cleaning station.

General Screening Conditions

Trypsinized RBL (rat basophilic leukemia) cells were plated in 96-well tissue culture plates (BD Biosciences #35-6651) at a density of 2.0x105 cells/mL in DMEM (10% FBS, 1% Penicillin/ Streptomycin). The cells were incubated for 16 hours at 37°C, 5% CO2 and 95% humidity.

A staurosporine stock solution (10 mM, 100% DMSO) was plated into wells A2 through A12 of a mother plate and then serially diluted by volume 1:3 in DMSO down the plate (Falcon #353072) to generate a standard curve. DMSO was added to column 1 (A1-H1) as a control. The contents of the mother plate were further diluted 1:33 in an intermediate plate (Falcon # 352191) containing DMEM. Next, tissue culture media used for plating was replaced with fresh medium. The contents of the intermediate plate containing the staurosporine standard curve were transferred to each cell plate and incubated (37°C, 5% CO_2 and 95% humidity) for 24 hours.

Post incubation with staurosporine, 20 μL of CellTiter 96® Aqueous One Solution Reagent was added to each well of the cell plates and incubated for 2 hours. A Spectramax Plus384 (Molecular Device, Sunnyvale CA) at 490 nm was used to measure the conversion of MTS tetrazolium to formazan (Figure 2).

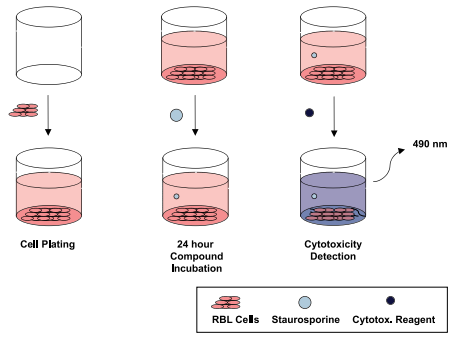


Figure 2. *RBL cell proliferation assay via staurosporine.*

Optimizing TipCharger for Use in Automated Cytotoxicity and Cell Proliferation Assays

Six plates containing RBL cells were generated as previously described. A staurosporine standard curve was generated in an intermediate plate and then replicated into three cell plates.

The same set of tips was then used to aspirate fresh tissue culture medium and added to a fourth cell plate as a means of demonstrating the presence of any residual staurosporine retained in the pipette tips. All cell plates were then incubated under normal tissue culture conditions (37°C, 5% CO_2 and 95% humidity) for 24 hours. All steps associated with the Promega CellTiter 96® Aqueous One Solution Reagent were executed according to the General Screening Conditions section previously.

Each of the three remaining experimental plates were prepared by triturating the remaining contents of the staurosporine intermediate plate with fresh pipette tips, then inserting them into the TipCharger cleaning station for 20 seconds prior to triturating the experimental wells (Table 1). Note- the protocol with and without the TipCharger washes differs only as indicated by the steps indicated in the purple blocks of in table below.

RBL Cytotoxicity Assay	
Component	Volume added or
RBL Cells (2x 10e5 cells/mL)	200 µL
Incubation (37˚C, 5% CO_2	16 hours
Replace media	180 µL
Staurosporine (100 µM, 30	20 µL
w/ wo TipCharger (20 seconds)	20 µL
Incubation (37˚C, 5% CO_2)	24 hours
CellTiter 96® Aqueous One Reagent	20 µL
Incubation (37˚C, 5% CO_2)	2 hours

Table 1. *RBL cytotoxicity assay protocol*

Results

The results show that exposing pipette tips to TipCharger-based atmospheric plasma for only 20 seconds removed staurosporine at concentrations up to 1.0E-5, eliminating the cytotoxic effect upon RBL cells (Figure 3).

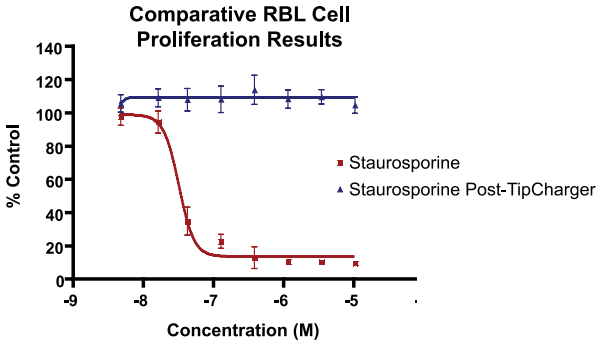


Figure 3. *Pipette tip plasma exposure results for staurosporine induced cytotoxicity.*

Cell plates that received media post-TipCharger failed to demonstrate any effective cytotoxic concentration of staurosporine, while an EC50 of 32nM was observed for non-treated staurosporine. Overall, exposure to plasma revealed no cytotoxic effect and restored pipette tips to a state similar to that of a 'fresh' set. A rinse with deionized H_2O was incorporated in an effort to lower DMSO percentages prior to entering the TipCharger cleaning station, but this rinse step alone was insufficient at eliminating carryover of staurosporine (data not shown).

Summary

Determining and understanding potential cytotoxic properties associated with small molecules and their metabolites are of great importance to drug discovery environments. Cytotoxicity assays are ubiquitous throughout life science industries and play equally important roles in forensics, food safety, and pathology/ toxicology programs. As plasma cleaning produces pipette tips equivalent to fresh, unused tips with no detectable negative impact on the assays evaluated in this study, discovery environments may look to this technology to increase throughput while reducing operational costs.

The implementation of novel technologies like the TipCharger system may help to improve the bottom line of drug discovery environments. Shortening timelines associated with cytotoxic compound identification may enable faster decision making and potentially shorter time-to-market for potential NDCs.

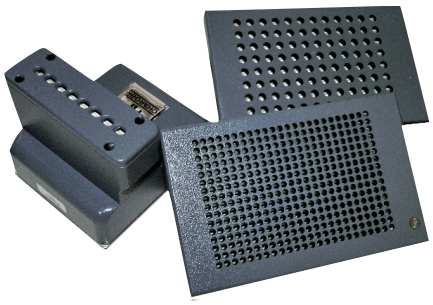
Integrating the TipCharger into Automated Assays Provides the Following Benefits:

Best in Class TipCharger cleans better than any other washing technology - in most applications the TipCharger will clean to background, so there is no difference between plasma cleaning and a new tip.

Cost Benefit TipCharger saves up to 90% on the cost of the disposable tips.

Speed Incorporating the TipCharger System can result in a time savings of 10-30 seconds for every microplate processed or rack of tips cleaned.

Convenience Clear away the clutter and save time: Integrating the TipCharger System eliminates the need to store cases of new pipette tips and dispose of racks of hazardous used tips.



TipCharger Plasma Cleaning Stations
Available in 8, 96, 384 and 1536 channel versions

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About the TipCharger™

The TipCharger Cleaning System replaces existing wash stations and easily integrates with most existing and new automation platforms. The system utilizes a low temperature, atmospheric pressure plasma process that cleans metal and plastic pipette tips and pin tools. Treated surfaces are clean, dry and have uniform surface properties.

The TipCharger cleaning process reduces the incidence of micro-bubble formation and other random surface effects that degrade liquid handling precision and accuracy, even with new disposable tips.

IonField Systems' TipCharger improves the reproducibility of process results, shortens automation cycle times, reduces the number of lost runs, and eliminates environmental waste and liquid handling disposables. The overall result is increased confidence in results and a more effective and productive laboratory operation.

About IonField Systems™

IonField Systems is an advanced technology company focused on the development of best-in-class cleaning methods for life science applications.

We provide comprehensice onsite/ phone support and ongoing services to insure a successful installation and exceptional day-to-day operation of our products.

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