

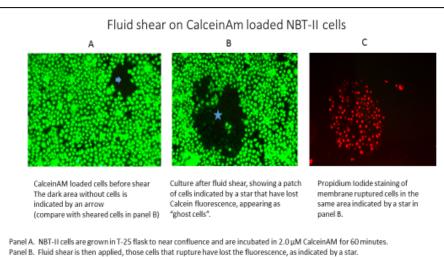
A cell-based method to determine surface heterogeneity on polystyrene used in tissue culture flasks and dishes; *increasing reliability of outcome of cell assays and reducing necessity to repeat cell-based tests*

The Problem

- Scientists in the United States spend \$28 billion each year on basic biomedical research that cannot be repeated successfully. Materials issues made the largest contribution to reproducibility problems, at 36%.^{1,2}
- Polystyrene (PS) is used in all formats of tissue culture including the standard flasks, multiwell plates and Petri dishes.
- We have found that the surface of the PS used for tissue culture is not homogeneous to several strains of cell lines.
 - In particular, when squamous epithelial cells lines when subjected to fluid shear, cells on certain areas of the PS die due to immediate cell membrane rupture.
 - This heterogeneity of the PS surface may be related to the stress pattern on the PS introduced during manufacturing. This
 - stress pattern shows birefringence under polarized light.
- PS used in tissue culture flasks and dishes differ among manufacturers.
- Currently there is no simple analytical method to compare tissue culture wares, resulting in disparity in outcomes.

Unique Attributes of the Tchao Methodology

• The Tchao cell-based method to determine heterogeneity of PS surfaces is quantitative and easy to use.



The method can be used to monitor heterogeneity of PS-based product, as well as changes and improvements introduced in the molding process.

Panel C. Cells are also stained with 5µg/ml Propidium Iodide to show the membrane ruptured cells.

• The Tchao cell-based method is novel, since there is at present no published method to detect heterogeneity on tissue plastic flasks and dishes.

Clinical Applications

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- Elimination or reduction of the heterogeneity on the PS surfaces will render a more uniform PS surface for the end user, and reduce any artifact in experiments due to the heterogeneity of the PS surface.
- Use of the Tchao methodology may reduce the heterogeneity in PS surfaces to less than 5%, thereby increasing reliability of outcome of cell assays and reducing the necessity to repeat cell-based tests.

¹ Freedman, Leonard P. et al, "The Economics of Reproducibility in Preclinical Research," *PLOS Biology*, June 9, 2015.

² Baker, Monya. "Irreproducible Biology Research Costs Put at \$28 Billion Per Year," *Nature News*, June 9, 2015.

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Manufacturer	Catalogue #	Lot #	% Fluid Shear Effect +/- sem (n=3)
Manufacturer A	229331	110723-218	10.5 +/- 0.5
Manufacturer B	3056	09815045	14.1 +/- 0.6
Manufacturer C	3289	22005005	8.7 +/- 0.5
Manufacturer D	353108	5249004	13.6 +/- 1.4
Manufacturer E	690170	08050140	11.3 +/- 0.2
Manufacturer F	136196	136985	20.6 +/- 0.3
Manufacturer G	SC-200262	110423-218	11.8 +/- 0.5
Manufacturer H	831810	6065081	13.4 +/- 1.1
Manufacturer I	90025	20150141	4.5 +/- 1.3

Table 1: Fluid shear effect of NBT-II cells grown on T-25 of various manufacturers



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