Update on the SOT - Colgate Palmolive Grant for Alternative Research:

In Vitro Co-Culture Assay for Identification of Dermal Sensitizers

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ABSTRACT

This research project combined a 3D reconstructed human epidermal (RHE) tissue that is co-cultured with human plasmacytoid Dendritic Cells (pDCs) for use as an *in vitro* Co-Culture dermal sensitization assay. In this assay, RHE tissues were placed at the air-liquid interface above a media suspension of pDC. The tissues are then exposed to test materials, and after 4 hours of incubation together, the RHE tissues and pDC were separately cultured for an additional 20 hours. The RHE media was analyzed for IL-18 release by ELISA, and the pDC were analyzed for changes in CD86 surface expression by flow cytometry. Two non-sensitizing irritants (Lactic Acid and Phenol), along with two weak/moderate sensitizers: Eugenol and Hexylcinnamaldehyde, and two strong sensitizers: 1-Chloro-2,4-Dinitrobenzene and 4-Nitrobenzyl Bromide were assayed. A positive response from the RHE tissues was determined to be a 2-fold increase in IL-18 secretion, and a 1.5 fold increase in CD86 expression on pDC. Tissue viability was measured using the MTT assay. The responses we obtained in both the RHE tissue versus pDC were very consistent. Increases in both secretion of IL-18 and expression of CD86 were detected after exposure to dermal sensitizers. A prediction model was developed in which a sensitizer result for a chemical is defined as either a positive result in the RHE tissue (IL-18) or a positive result in pDCs (CD86). From three individual experiments, and using a 2 x2 contingency table to determine Cooper statistics, we obtained an Accuracy of 100%, 83%, and 83% (89% mean Accuracy). All four of four sensitizers were positively predicted in each experiment (100% Sensitivity).

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INTRODUCTION

We tested the development of a 3D skin model and Dendritic Cell (DC) Co-Culture model to allow communication between skin-like tissues and human-derived Dendritic Cells. **EpiDerm™** (MatTek Corp, Ashland, MA) is a 3D human differentiated epidermal tissue model that closely parallels human skin. EpiDerm™ tissues consist of normal, human-derived epidermal keratinocytes that have been cultured to form a multilayered, highly differentiated model of the human epidermis. EpiDerm™ is mitotically and metabolically active, and ultra-structurally closely parallels human skin. Because EpiDerm™ comprises human keratinocytes, which constitute the majority of epidermal cells found in human skin it is a very relevant model for skin sensitization testing. From our experience we know that the cytokine IL-18 is an essential component of dermal sensitization and that IL-18 is a sensitizer-specific cytokine released from keratinocytes (Gibbs et al., Toxicol Appl Phar 2013 272(2):529-41).

Dendritic Cells (DC) are part of a heterogeneous group of mobile "antigen-presenting" cells. They are responsible for activating T-cells in response to disease and allergen assaults on the body. Based on phenotypic marker expression, cytokine production and anatomical distribution, Dendritic Cells are divided into two subsets of cells: myeloid DC (CD123-/CD11c+) and plasmacytoid DC (CD123+/CD11c-). Human plasmacytoid Dendritic Cells (**pDCs**) have been associated with allergic reactions in the skin (Wollenberg et al., J Invest Dermatol 2002 119(5):1096-102.). To this end, pDCs have been generated and tested for their ability to predict sensitization using surface marker CD86 as an indicator of allergenicity (Ayehunie et al., Toxicology 2009 264(1-2):1-9).

We hypothesized that co-culturing a 3D skin model and pDC model would allow communication between the skin-like tissues and human-derived pDC and that interaction may allow for a more complete assessment of delayed-type hypersensitivity immune reactions of the measured endpoints yielding a more accurate *in vitro* sensitization assay.

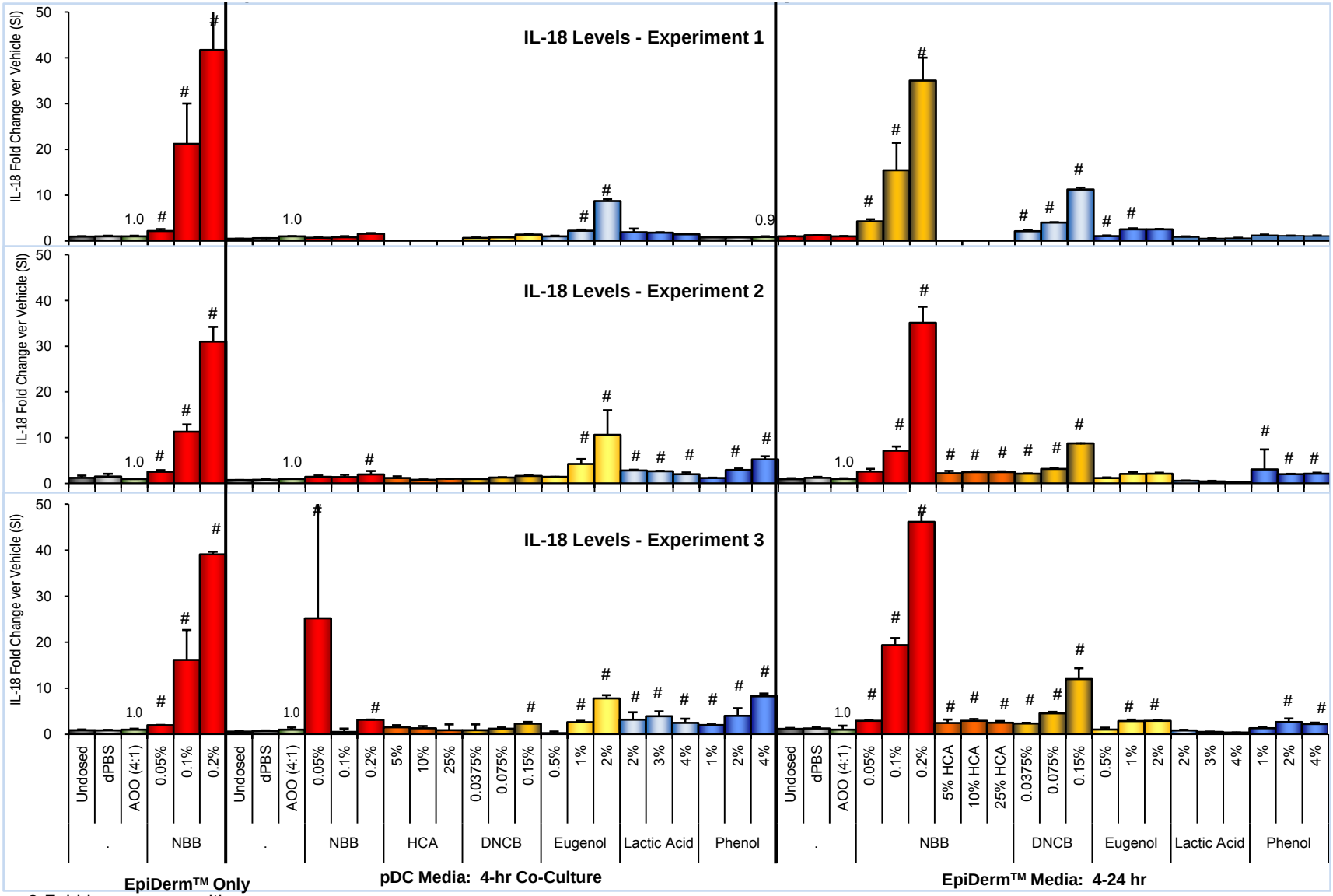
MATERIALS AND METHODS

To establish the 4-hr Co-Culture system, pDC suspended in pDC-MM Medium were added to wells of a 6-well plate. EpiDerm™ tissues were then placed on top of the media. The EpiDerm™ and pDC were dosed and co-cultured together for 4 hours in pDC-MM Media (**4-hr Co-Culture**). After 4 hours the EpiDerm™ was separated and further cultured for an additional 20-hr in EpiDerm™ Media (**4-24 hr**). At 24-hr post-chemical application, tissues were analyzed by the MTT assay for viability with Methyl-thiazolyl-tetrazolium (MTT). IL-18 secretion into the media was measured by a commercially available ELISA kit (MBL, Nagano, Japan). The pDC in the media beneath the EpiDerm™ tissues were analyzed for CD86 expression (directly-conjugated FITC antibody) on living cells (7-AAD viability stain). Acquisition was performed on a BD FACScan flow cytometer and analyzed using BD CellQuest 3.3 software.

IL-18 data from the EpiDerm™ Media (4-24 hr samples) and CD86 data from pDC was used to construct a 2x2 Contingency table for each experiment. A sample was considered positive if it yielded a positive response in either EpiDerm™ tissues (IL-18) or pDC (CD86).

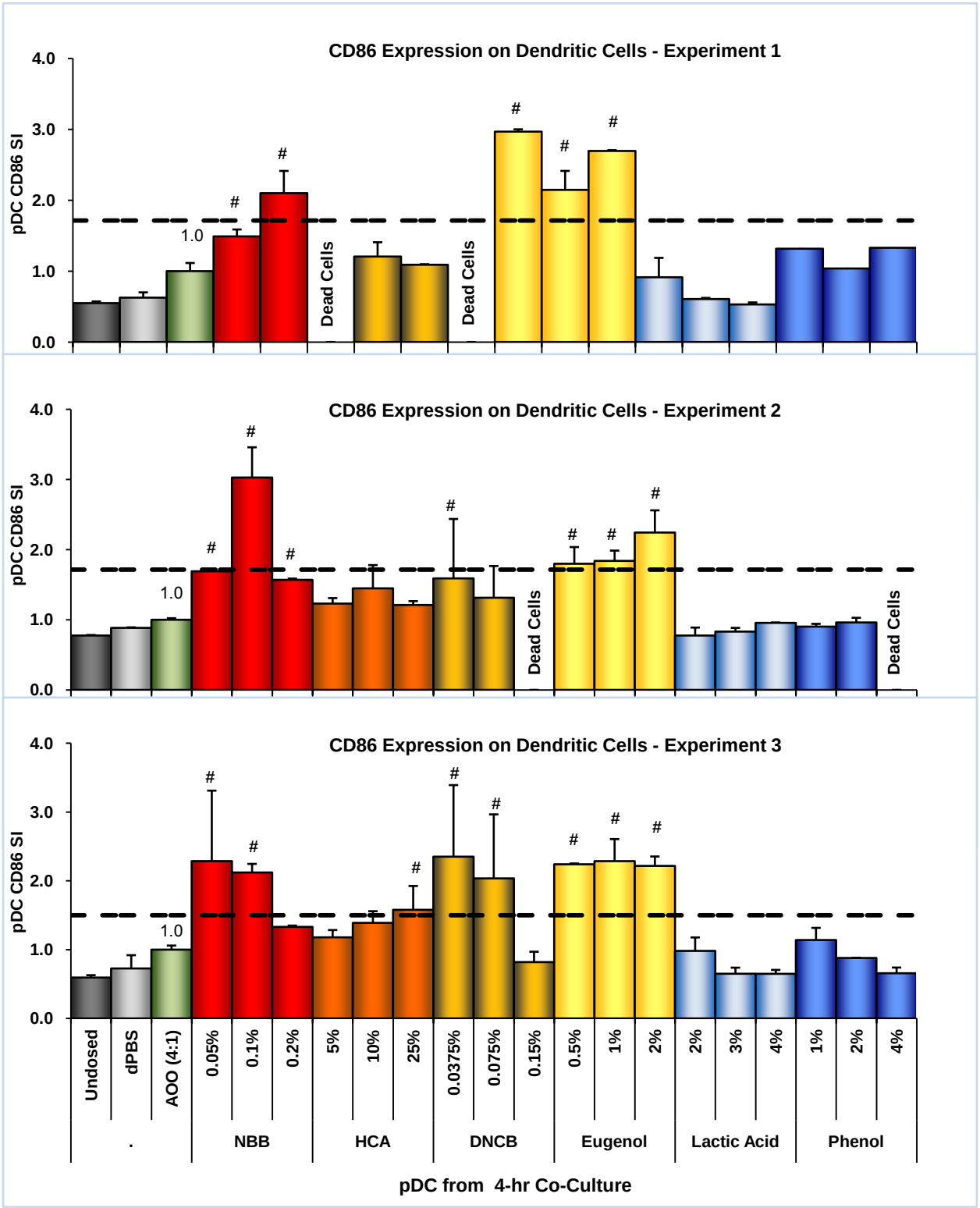
***In Vitro* Co-Culture Assay for Identification of Dermal Sensitizers**

RESULTS



= ≥ 2-Fold Increase = positive response

RESULTS (cont'd)



= ≥ 1.5-Fold Increase = positive response

RESULTS (cont'd)

Experiment 1	Known +	Known –	
Tested +	3	0	3
Tested –	0	2	2
	3	2	5

Experiment 2	Known +	Known –	
Tested +	4	1	5
Tested –	0	1	1
	4	2	6

Experiment 3	Known +	Known –	
Tested +	4	1	5
Tested –	0	1	1
	4	2	6

AVG of the 3 experiments

Accuracy	**89%**
Sensitivity	**100%**
Specificity	**67%**
Positive Predictivity	**87%**
Negative Predictivity	**100%**

CONCLUSIONS

- **An advantage of this Co-Culture test system is that sensitization responses to both keratinocytes (IL-18) and pDC (CD86) can be assessed concurrently.**
- **EpiDerm™ tissues allow for topical application of products in many chemical forms, such as liquids, solids, gels, lotions and powders.**
- **Although the stronger sensitizers NBB and DNCB secrete IL-18 between 4 and 24 hours after dosing, the weaker sensitizer Eugenol secreted higher amounts of IL-18 within 4 hours.**
- **From three independent experiments we obtained a mean accuracy of 89%. Future directions will include testing of additional irritants, sensitizers, mixtures and commercial products.**