

Co-culture of primary human bronchial epithelial cells at the air-liquid interface and THP-1 macrophages to investigate the toxicity of polycyclic aromatic hydrocarbons

Kyle S. Burns 1,2, Audrey G. Biggerstaff 1, Jamie M. Pennington 1 and Susan C. Tilton 1,2,*

1 Department of Environmental and Molecular Toxicology, Oregon State University,
Corvallis, OR 97331, USA; kyle.burns@oregonstate.edu (K.S.B.);
biggerau@oregonstate.edu (A.G.B.);
jamie.pennington@oregonstate.edu (J.M.P.)

2 Superfund Research Center, Oregon State University, Corvallis, OR 97331, USA

* Correspondence: susan.tilton@oregonstate.edu

Supplemental Tables and Figures

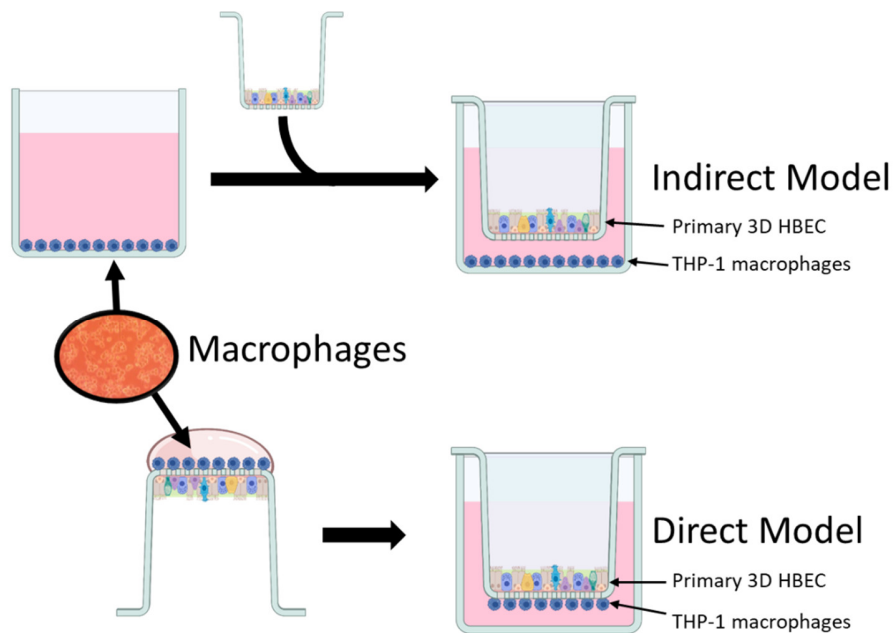
Table S1. Chemical Standards.

Chemical	% of Mixture by molar amount	CAS #	Purity	Supplier
retene	72.8	483-65-8	97%	Santa Cruz Biotechnology
benzo[a]fluorene	14.6	238-84-6	>98%	Santa Cruz Biotechnology
benzo[b]fluorene	7.28	243-17-4	98.1%	AccuStandard
benzo[c]fluorene	3.64	205-12-9	98%	TRC
triphenylene	1.45	217-59-4	99.4%	AccuStandard
benzo[e]pyrene	0.145	192-97-2	99.9%	AccuStandard
benzo[ghi]perylene	0.091	191-24-2	98.9%	AccuStandard

Table S2. Primer sequences.

Gene	Forward (F)/ Reverse (R)	Primer sequence 5'-3'	NCBI Gene ID	Reference
<i>PPIA</i>	F	GCATACGGGTCCTGGCATCTTGTCC	5478	Chang et al., 2019
<i>PPIA</i>	R	ATGGTGATCTTCTTGCTGGTCTTGC	5478	Chang et al., 2019
<i>IL1B</i>	F	ATGATGGCTTATTACAGTGGCAA	3553	Wang et al., 2020
<i>IL1B</i>	R	GTCGGAGATTCGTAGCTGGA	3553	Wang et al., 2020
<i>IL6</i>	F	ACTCACCTCTTCAGAACGAATTG	3569	Wang et al., 2020
<i>IL6</i>	R	CCATCTTTGGAAGGTTCAAGTTG	3569	Wang et al., 2020
<i>CXCL8</i>	F	ACTGAGAGTGATTGAGAGTGGAC	3576	Wang et al., 2020
<i>CXCL8</i>	R	AACCCTCTGCACCCAGTTTTTC	3576	Wang et al., 2020
<i>TNFTNF</i>	F	GAGGCCAAGCCCTGGTATG	7124	Wang et al., 2020
<i>TNFTNF</i>	R	CGGGCCGATTGATCTCAGC	7124	Wang et al., 2020
<i>ALDH3A1</i>	F	TGTTCTCCAGCAACGACAAGG	218	Chang et al., 2019
<i>ALDH3A1</i>	R	AGGGCAGAGAGTGCAAGGT	218	Chang et al., 2019
<i>HMOX1</i>	F	CTCTGAAGTTTAGGCCATTG	3162	Thakor et al., 2011
<i>HMOX1</i>	R	AGTTGCTGTAGGGCTTTATG	3162	Thakor et al., 2011
<i>NQO1</i>	F	GAAGAGCACTGATCGTACTGGC	1728	Chang et al., 2019
<i>NQO1</i>	R	GGATACTGAAAGTTCGCAGGG	1728	Chang et al., 2019
<i>TJP2</i>	F	GGCCTACGACCCAGACTAC	9414	Chang et al., 2019
<i>TJP2</i>	R	ACTCTTCGTTGCTCTGCTTT	9414	Chang et al., 2019
<i>ACTB</i>	F	ATTGCCGACAGGATGCAGAA	60	MaeB et al., 2014
<i>ACTB</i>	R	GCTGATCCACATCTGCTGGAA	60	MaeB et al., 2014
<i>CYP1A1</i>	F	TCGGCCACGGAGTTTCTTC	1543	Chang et al., 2019
<i>CYP1A1</i>	R	GGTCAGCATGTGCCCAATCA	1543	Chang et al., 2019
<i>CYP1B1</i>	F	CCAACCTGCCCTATGTCCT	1545	Chang et al., 2019
<i>CYP1B1</i>	R	CTGGATCAAAGTTCTCCGGG	1545	Chang et al., 2019
<i>CD14</i>	F	CTGGAACAGGTGCCTAAAGGAC	929	Betancur et al., 2022
<i>CD14</i>	R	GTCCAGTGTGAGGTTATCCACC	929	Betancur et al., 2022

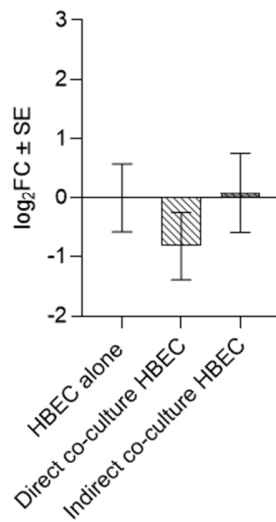
Supplemental Figure S1.



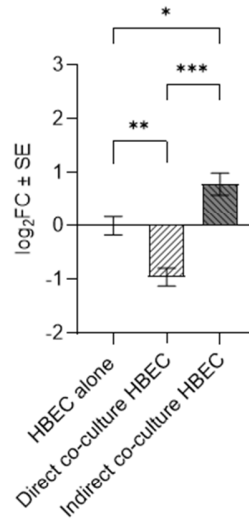
Supplemental Figure S1. Overview of co-culture models. Two co-culture methods were utilized in this study and are referred to as the indirect co-culture model and the direct co-culture model. In the indirect co-culture model (top), THP-1 macrophages were seeded onto the bottom of a cell culture plate well. In the direct co-culture model (bottom), THP-1 macrophages were seeded onto the inverted Transwell® insert. Both models utilized primary HBECs, containing a variety of epithelial subtypes, on the apical side of the Transwell® insert and THP-1 macrophages in the basolateral chamber. HBEC and THP-1 cells were cultured on opposite sides of the Transwell® membrane to simplify analysis and determination of how each cell type (HBEC or macrophage) is contributing to the response to PAH exposure.

Supplemental Figure S2.

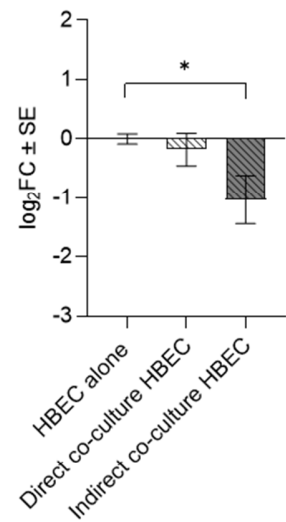
A) CYP1A1



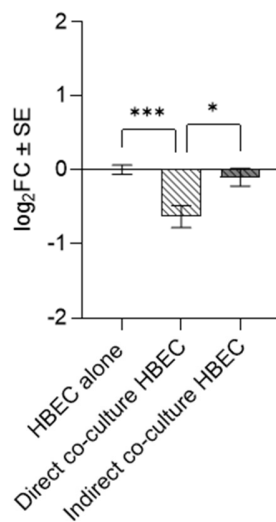
B) CYP1B1



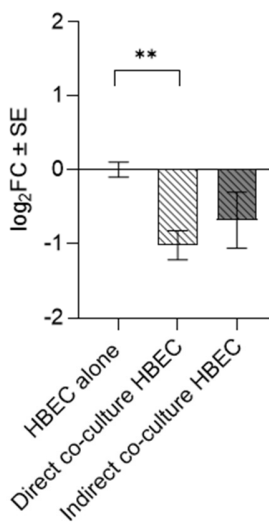
C) ALDH3A1



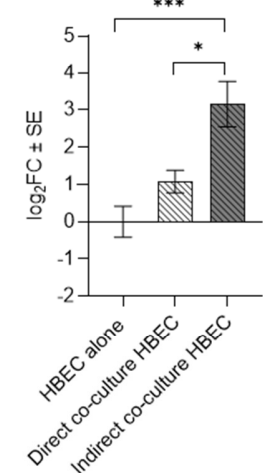
D) HMOX1



E) NQO1



F) TJP2



■ HBEC alone ▨ Direct co-culture ▩ Indirect co-culture

Supplemental Figure S2. Gene expression in HBEC vehicle controls. HBEC alone and in direct and indirect co-cultures were exposed to in 1% DMSO vehicle control and then isolated and evaluated for (A) *CYP1A1*, (B) *CYP1B1*, (C) *ALDH3A1*, (D) *HMOX1*, (E) *NQO1*, and (F) *TJP2* expression. Data points represent the mean response in each platform

normalized to HBEC alone vehicle and presented as the mean $\log_2$ fold change ($\log_2\text{FC}$) normalized to HBEC alone. Error bars represent the standard error (SE) of the means. Statistical significance is indicated by asterisks (*p-adj<0.05, **p-adj<0.01, ***p-adj<0.001, ****p-adj<0.0001; One-way ANOVA followed by Tukey's post-hoc test).