Nanoscale

ARTICLE

The Impact of Tomato Fruits Containing Multi-walled Carbon Nanotube Residues on Human Intestinal Epithelial Cell Barrier Function and Intestinal Microbiome Composition

Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Electronic Supplementary Information (ESI) available:Fig. S1 is Bright field microscopy of T-84 cells after exposure to CNT, Fig. S2 is shematic diagram o fNGS data analysis, Fig. S3-5 deatiled heatmap of top significantly altered genes, Table S1-3 include list of gene classification, Fig. S6 confirmation of NGS data using Real-time PCR, Fig S7-9 PCA based on time, indivudals and doses used. See DOI: 10.1039/x0xx00000x

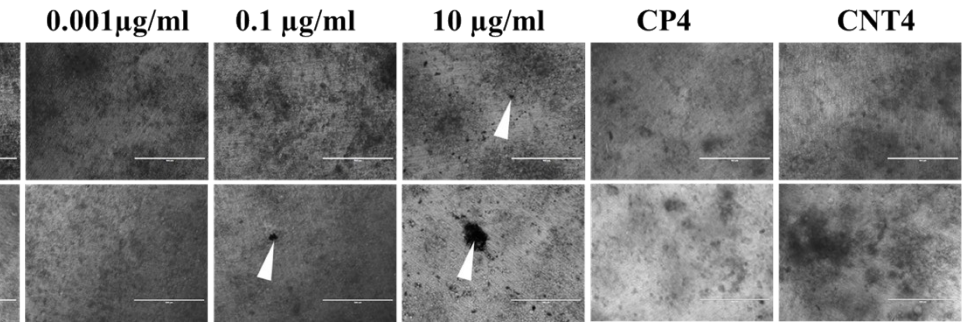

Figure S1. Bright field microscopy of T-84 cells after exposure to CNT at different doses (0.001, 0.1, and 10 µg/ml), control fruits extract (CP4), and CNT-containing fruits (CNT4) during two-time points (1- and 48-hour post-exposures). Arrows showing the agglomeration of CNT after long time exposure. Figures were taken at 20X magnification. Scale bar equal to 100µm.

Figure S2. Schematic diagram of the 4 different analysis considered during the data analysis of RNA Next Generation Sequencing. Analysis 1 included the comparison of gene expression of T-84 cells exposed to CNT (at 3 concentrations) versus the negative (untreated) control. Analysis 2 was a comparison between transcriptome of cells exposed to control fruits versus the negative (untreated) control. Analysis 3 was made by the comparison of gene expression of cells exposed to CNT-containing fruits versus the negative (untreated) control. Analysis 4 included the comparison of transcriptome of two group of cells exposed to control fruits or to fruits containing MWCS2

Classical Analysis			
	up-regulated	Down-regulated	Total
Experimental Study 1			
0.001 µg/ml CNT vs Negative Control	8	11	19
0.1 µg/ml CNT vs Negative Control	8	18	26
10 µg/ml CNT vs Negative Control	97	39	136
Experimental Study 2			
Control fruit vs Negative Control	643	414	1057
Experimental Study 3			
CNT fruit vs Negative Control	129	77	206
Experimental Study 4			
CNT fruit vs Control fruit	500	114	614

Table S1. The number of genes with significantly altered expression in control and CNT-treated T-84 cells. The analysis was performed on the base of Next Generation Sequencing data using Seralogix software. All gene numbers represent significantly perturbed genes, through either up- or down-regulation (p<0.001 and fold change threshold of 0.35).

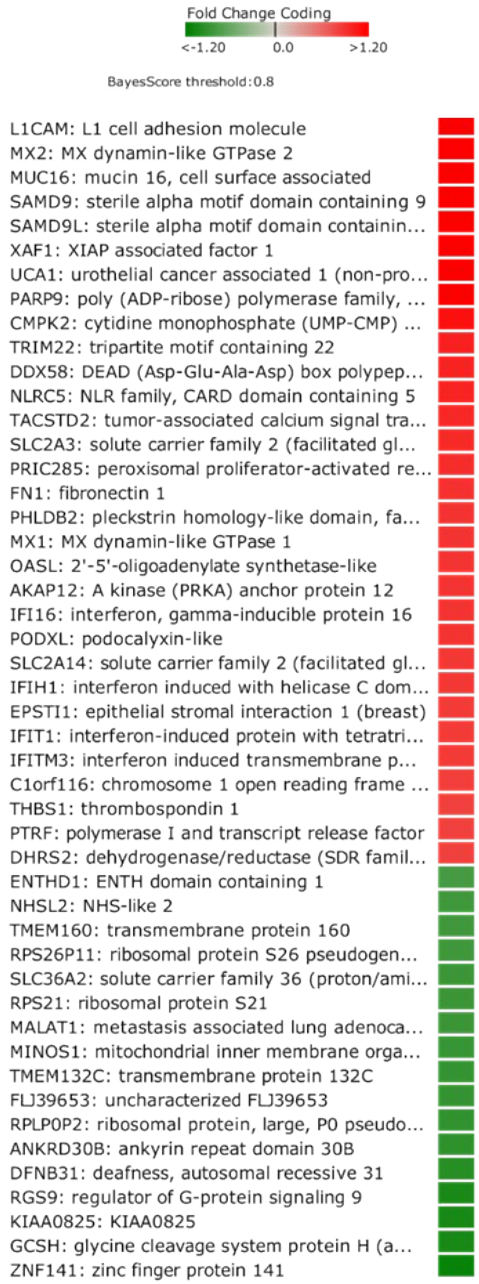

Figure S3. Heat map of the top 48 significantly perturbed genes during exposure of control tomato fruits to T-84 epithelial cells. Tomato fruits extract were exposed at a concentration of 3µg/ml.

Gene expression results are based on the number of RNA-seq transcripts detected, which were normalized and expressed as fold change compared to the untreated control. The fold change threshold score was selected for a 98% confidence. Darker red gradient indicates increased/higher gene expression while darker green gradient indicates suppression/lower of gene expression.

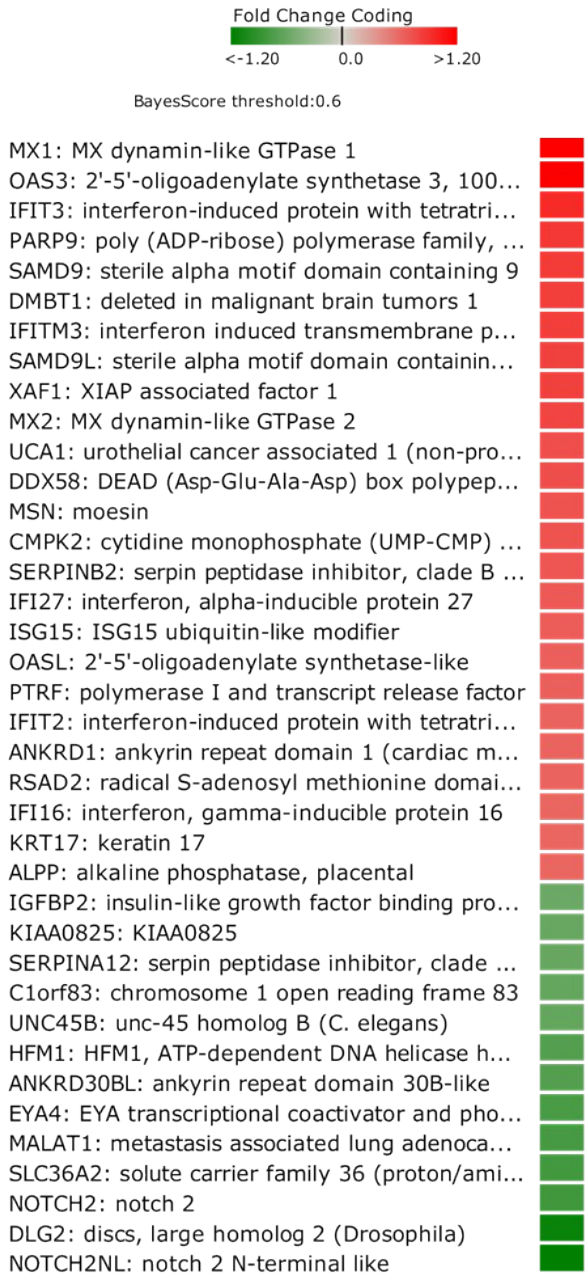

Figure S4. Heat map of the top 38 significantly perturbed genes during exposure of tomato CNT-containing fruitsto T-84 epithelial cells. Tomato fruits extracts were exposed at a concentration of 3µg/ml. Gene expression results are based on the number of RNA-seq transcripts detected, which were normalized and expressed as fold change compared to the untreated control. The fold change threshold score was selected for a 98% confidence. Darker red gradient indicates increased/higher gene expression while darker green gradient indicates suppression/lower of gene expression.

Figure S5. Heat map of the top 76 significantly perturbed genes during exposure of tomato CNT-containing fruits to T-84 epithelial cells. Tomato fruits extracts were exposed at a concentration of 3µg/ml. Gene expression results are based on the number of RNA-seq transcripts detected, which were normalized and expressed as fold change compared to the gene expression of cells exposed to control fruits. The fold change threshold score was selected for a 98% confidence. Darker red

gradient indicates increased/higher gene expression while darker green gradient indicates suppression/lower of gene expression.

Tight junction	
GENE_SYMBOL	GENE NAME
LIMK1	LIM domain kinase 1
ACHE	acetylcholinesterase (Yt blood group)
CLDN2	claudin 2
CTTNBP2	cortactin binding protein 2
EVPL	envoplakin
PPL	periplakin
BCAR1	similar to breast cancer anti-estrogen resistance 1; breast cancer anti-estrogen resistance 1
SYNPO	synaptopodin
ZYX	zyxin

Adherens junction		
	GENE_SYMBOL	GENE NAME
	LIMK1	LIM domain kinase 1
	CTTNBP2	cortactin binding protein 2
	BCAR1	similar to breast cancer anti-estrogen resistance 1; breast cancer anti-estrogen resistance 1
	ZYX	zyxin

Desmosome		
	None	-

Table S2. Classification of genes significantly altered by exposure of CNT to epithelial T-84 cells into three categories: tight junctions, adherens junctions, and desmosomes. The gene identification and classification were performed using DAVID software analysis (DAVID Bioinformatics Resources 6.7, NIAID/NIH).

Tight junction	
GENE_SYMBOL	**GENE NAME**
AMOTL2	angiomotin like 2
CGN	cingulin
CGNL1	cingulin-like 1
CLDN2	claudin 2
SYNPO	synaptopodin

Adherens junction	
GENE_SYMBOL	**GENE NAME**
LMO7	LIM domain 7
LIMK1	LIM domain kinase 1
ACTN1	actinin, alpha 1
CDH3	cadherin 3, type 1, P-cadherin (placental)
CAV1	caveolin 1, caveolae protein, 22kDa
DSP	desmoplakin
ITGA5	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
JUP	junction plakoglobin
MYH9	myosin, heavy chain 9, non-muscle
PXN	paxillin
PVRL1	poliovirus receptor-related 1 (herpes virus entry mediator C)
PVRL2	poliovirus receptor-related 2 (herpes virus entry mediator B)
PVRL4	poliovirus receptor-related 4

SCRIB	scribbled homolog (Drosophila)
BCAR1	similar to breast cancer anti-estrogen resistance 1; breast cancer anti-estrogen resistance 1
SPTAN1	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)
TLN1	talin 1
TLN2	talin 2
TNS4	tensin 4
TRIM25	tripartite motif-containing 25
ZYX	zyxin
Desmosomes	
GENE_SYMBOL	**GENE NAME**
DSP	desmoplakin
EVPL	envoplakin
JUP	junction plakoglobin
PPL	periplakin

Table S3. Classification of genes significantly altered by exposure of control fruits to epithelial T-84 cells into three categories: Cell adhesion, tight junction, and adherens junction. The gene identification and classification were performed using DAVID software analysis (DAVID Bioinformatics Resources 6.7, NIAID/NIH.

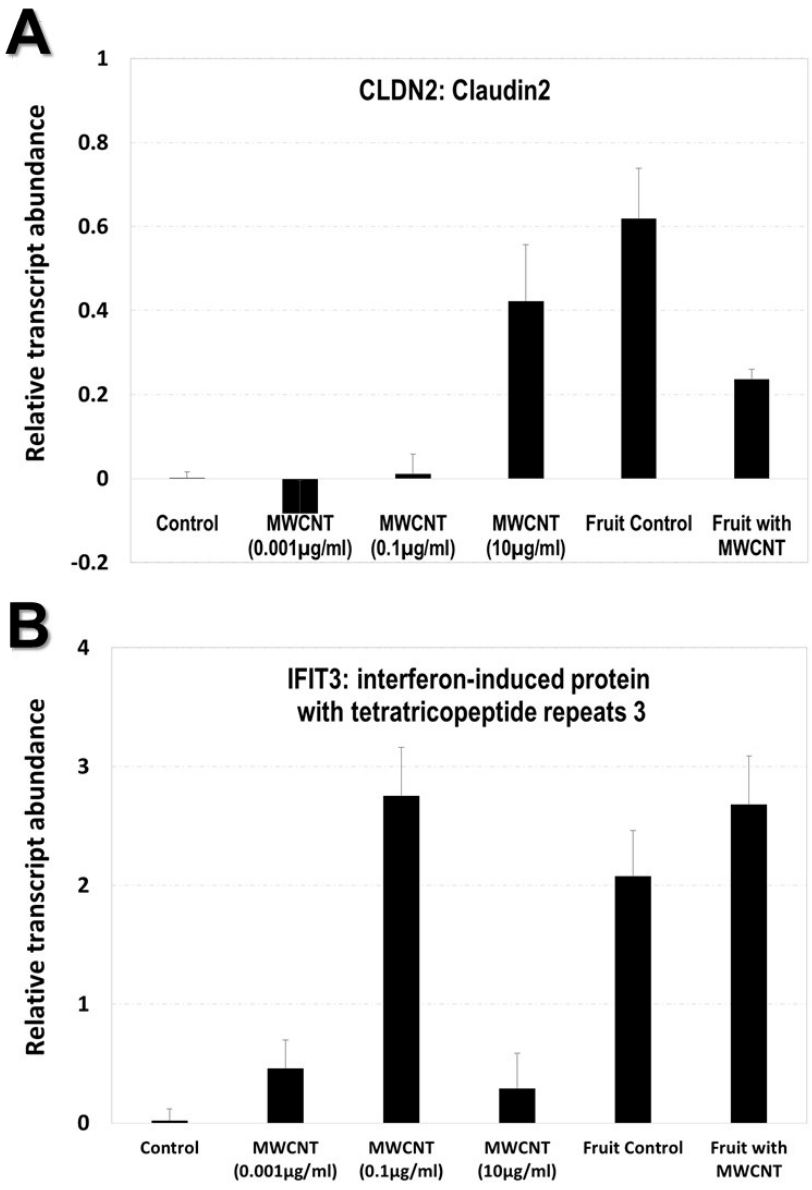

Figure S6. Real-time PCR of selected genes to confirm Next Generation Sequencing results. Genes selected included (A) Claudin2 and (B) interferon-induced protein with tetratricopeptide repeats 3. The expression of transcripts was shown as transcript abundance levels in comparison to the untreated control. Data are presented as the mean of three technical replicates, and two biological replicates (n=6). Error bars represent standard error values.

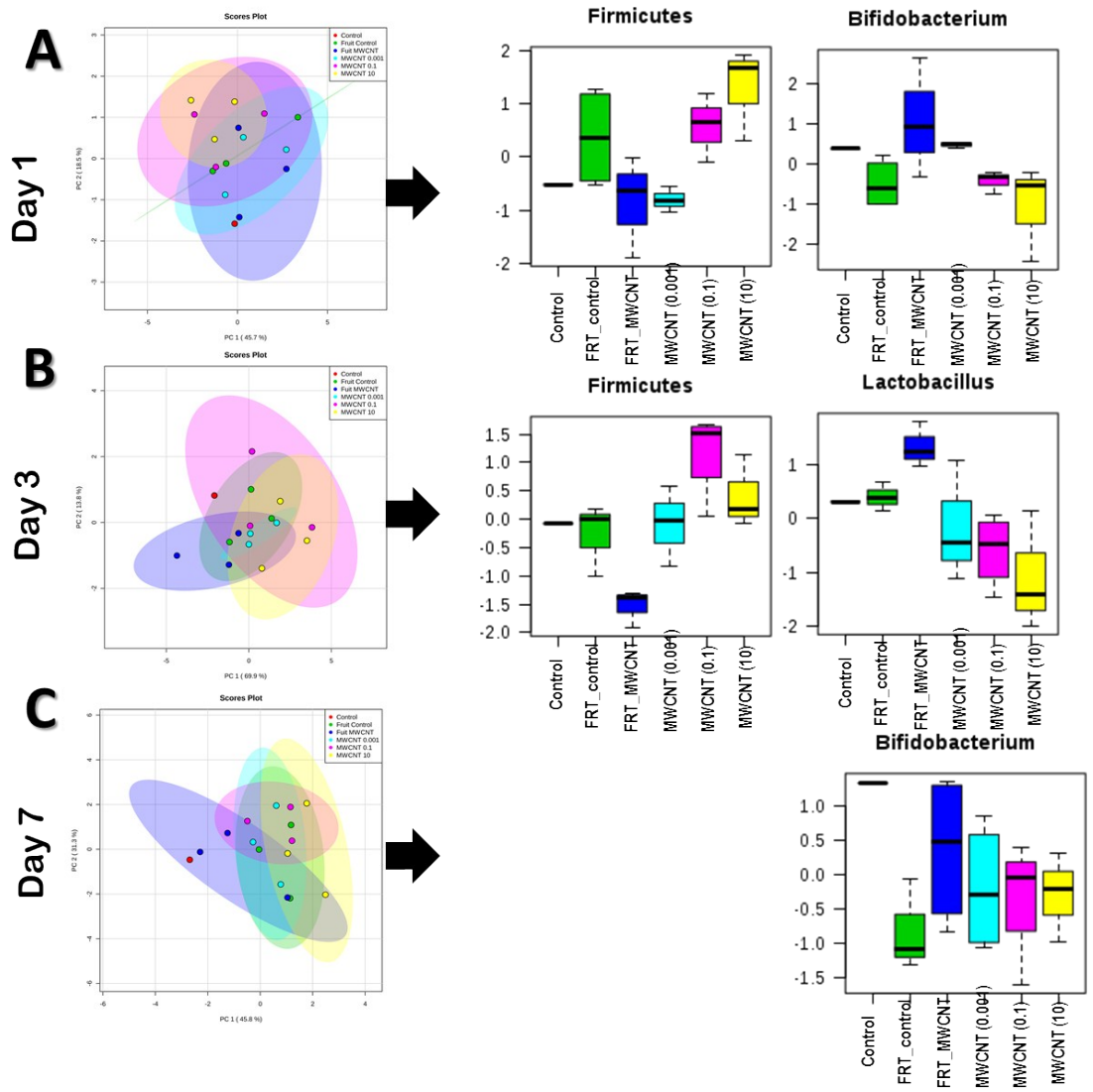

Figure S7. Principle Component Analysis of the different treatments tested on fecal slurry. One color represents each treatment, and each point represents one sample. Each cluster represents one treatment defined by a 95% limit. The analysis was performed for data obtained at day 1 (A), day 3(B) and day 7 (C). During each day, the variables contributing to the most variability in the data are presented.

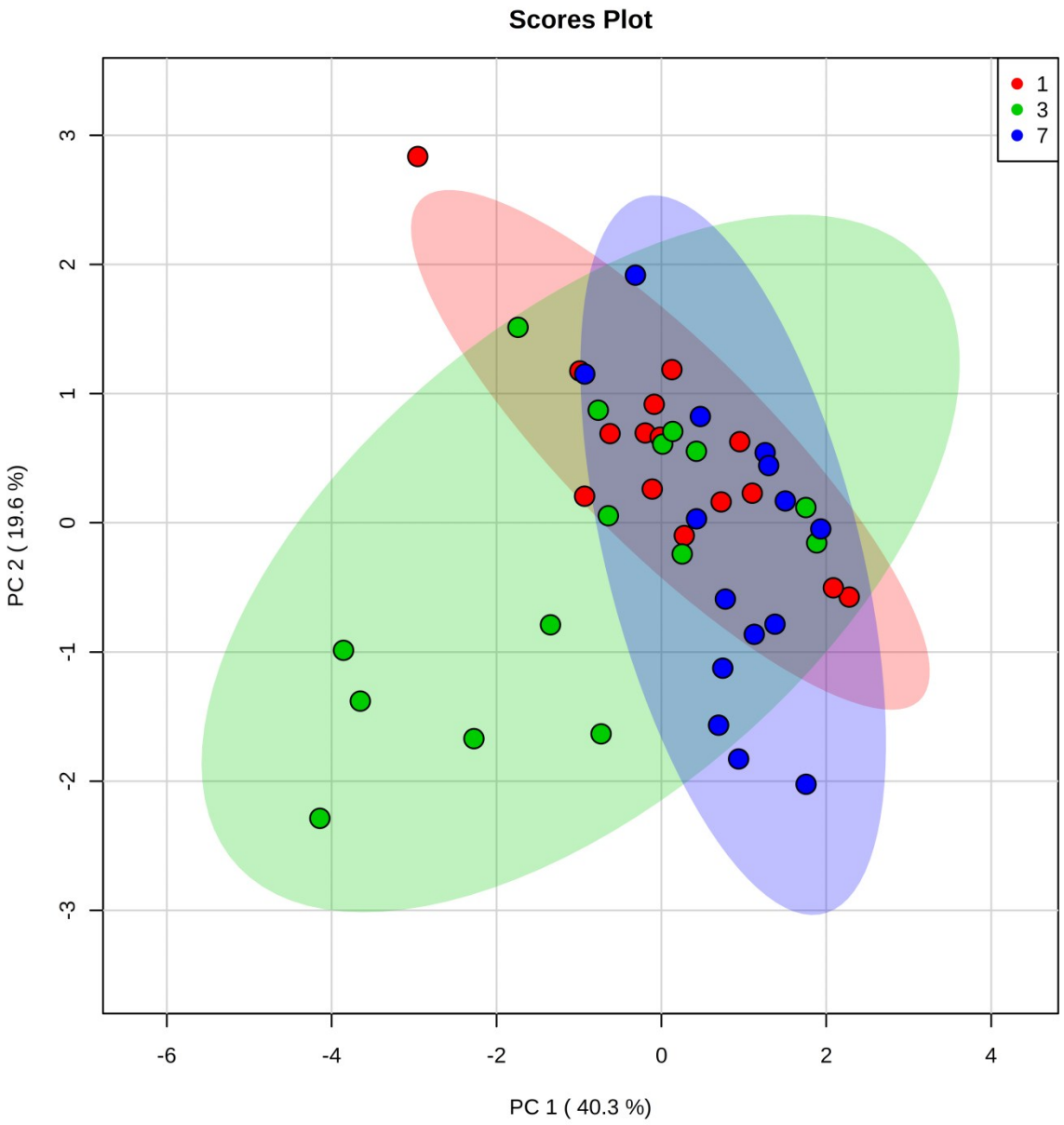

Figure S8. Principle Component Analysis of the different days of sampling. Each day (1, 3, and 7) is represented by one color, and each point represents one treatment. Each cluster represents one day defined by a 95% limit.

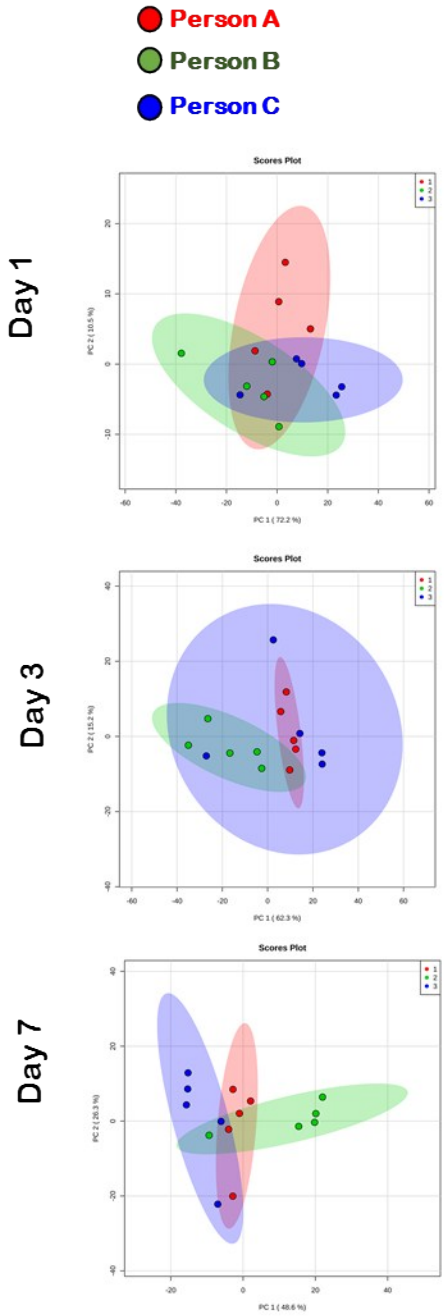

Figure S9. Principle Component Analysis of the individual A, B, and C specimens used during our exposure studies. Each sample is represented by one color, and each point represents one treatment. Each cluster represents one individual sample defined by a 95% limit. The analysis was performed for data obtained at day 1 (A), day 3 (B) and day 7 (C).