Three-dimensional intestinal villi epithelium enhances protection from bacterial infection by inducing MUC17 expression

Supplementary Information

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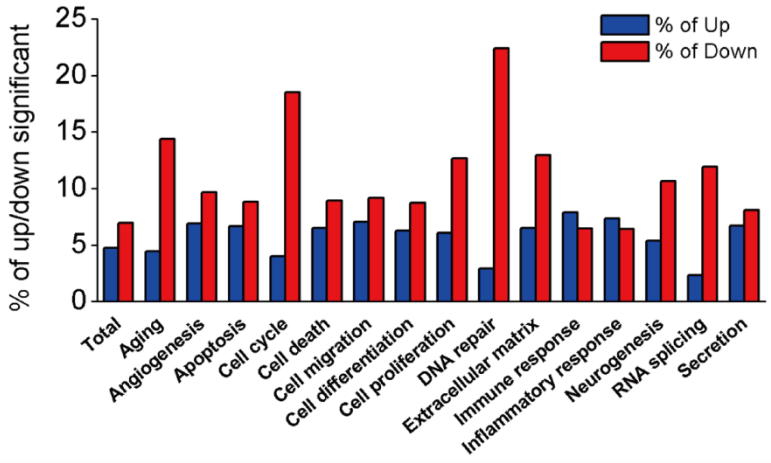
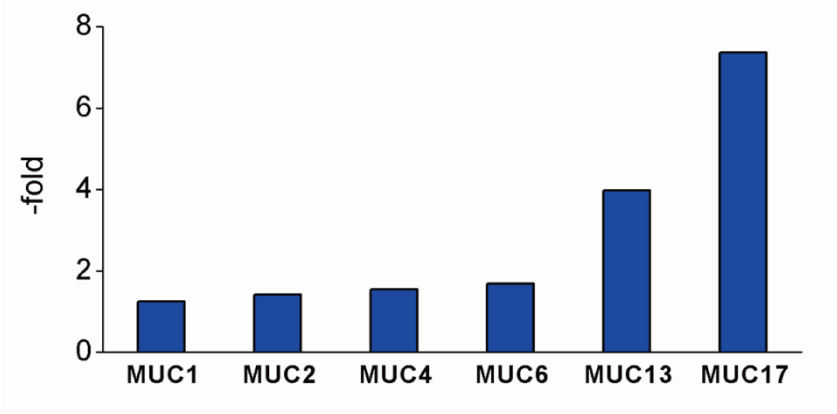
Transcriptome analysis of Caco-2 cells on the 3D villi scaffold

Transcriptome of Caco-2 cells grown in a petri dish and on the 3D villi scaffold was analyzed. Among the 34,127 genes, the number of genes that were up-regulated more than 2-fold was 1,612 (4.7%), whereas the number of genes that were down-regulated more than 2-fold was 2,368 (6.9%). In both cases, p-value had to be less than 0.05 for the increase or decrease in gene expression to be considered statistically significant. The up-regulated groups of genes included genes related to immune response (7.9%), inflammatory response (7.3%), and cell migration (7.0%). The groups of genes that were down-regulated were related to DNA repair (22.4%), cell cycle (18.5%), and aging (14.4%). We further analyzed the expression levels of mucin gene family. The expression level of MUC13 increased 4-fold in the 3D cell culture model compared to the 2D monolayer culture. The expression level of MUC17 increased more than 7-fold, whereas the changes in the MUC1, MUC2, MUC4, and MUC6 expression level were less than 4-fold. The results of transcriptome analysis are summarized in Supplementary Fig. 1.

(a)



(b)

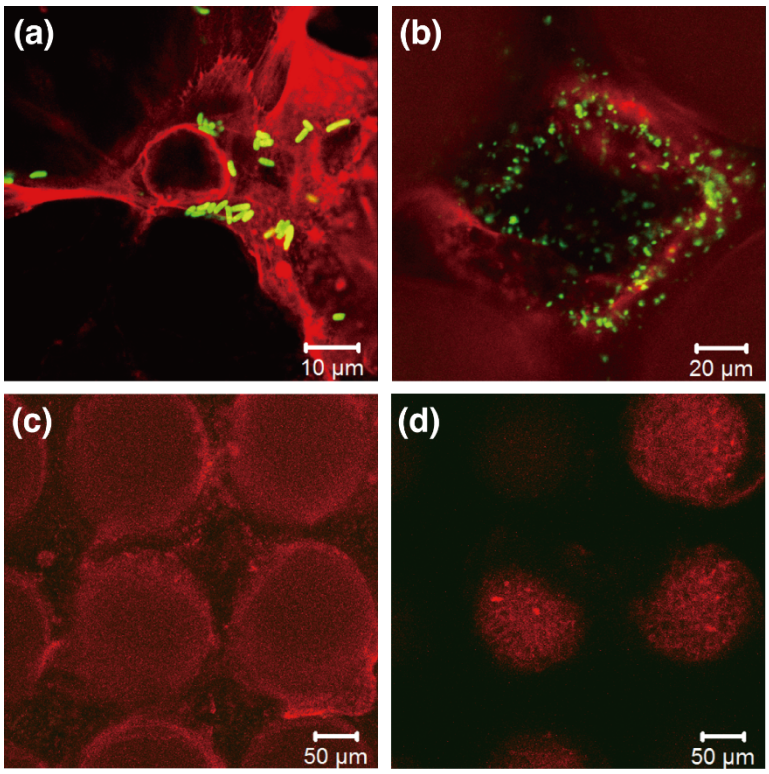


(c)

	Total	Aging	Angiogenesis	Apoptosis	Cell cycle	Cell death	Cell migration	Cell Differentiation	Cell Proliferation	DNA repair	Extracellular matrix	Immune response	Inflammatory response	Neurogenesis	RNA splicing	Secretion
Gene Number	34,127	271	434	1,980	1,728	2,205	995	3,229	1,887	482	540	1,259	560	1,418	386	954
% of Total	100.0	0.8	1.3	5.8	5.1	6.5	2.9	9.5	5.5	1.4	1.6	3.7	1.6	4.2	1.1	2.8
Up Significant	1,612	12	30	132	69	143	70	202	114	14	35	99	41	76	9	64
% of Up Significant	4.7	4.4	6.9	6.7	4.0	6.5	7.0	6.3	6.0	2.9	6.5	7.9	7.3	5.4	2.3	6.7
Dn Significant	2,368	39	42	175	320	197	91	281	239	108	70	81	36	151	46	77
% of Dn Significant	6.9	14.4	9.7	8.8	18.5	8.9	9.1	8.7	12.7	22.4	13.0	6.4	6.4	10.6	11.9	8.1
Total Significant	3,980	51	72	307	389	340	161	483	353	122	105	180	77	227	55	141
% of Total Significant	11.7	18.8	16.6	15.5	22.5	15.4	16.2	15.0	18.7	25.3	19.4	14.3	13.8	16.0	14.2	14.8

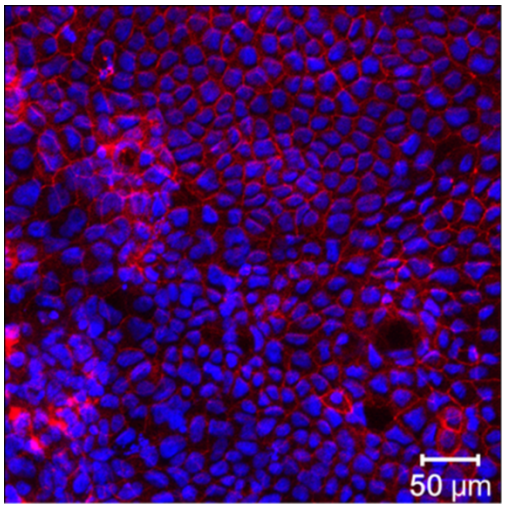
Supplementary Fig. 1 Whole-transcriptome analysis. (a) Fraction of genes that were up- or down-regulated in 3D model compared to 2D model. (b) Fold increase of MUC gene family members. (f) Whole-transcriptome analysis of 3D model in comparison to the 2D model (Significance criteria: 2 fold, p-value < 0.05).

E.coli infection study



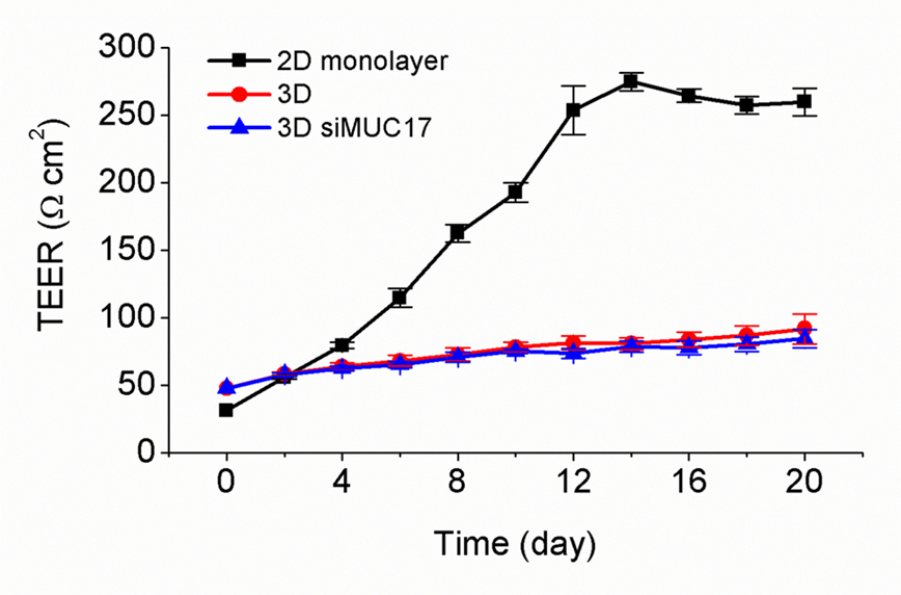
Supplementary Fig. 2 Infectivity of *E. coli* O157:H7 in the 2D and 3D model. Fluorescence image of bacteria-infected Caco-2 cells in (a) 2D culture (Day 4), (b) 3D culture (Day 4, bottom), (c) 3D culture (Day 20, bottom), (d) 3D culture (Day 20, top) (Red: actin, Green: *E. coli* O157:H7).

Occludin expression in 2D Caco-2 cells



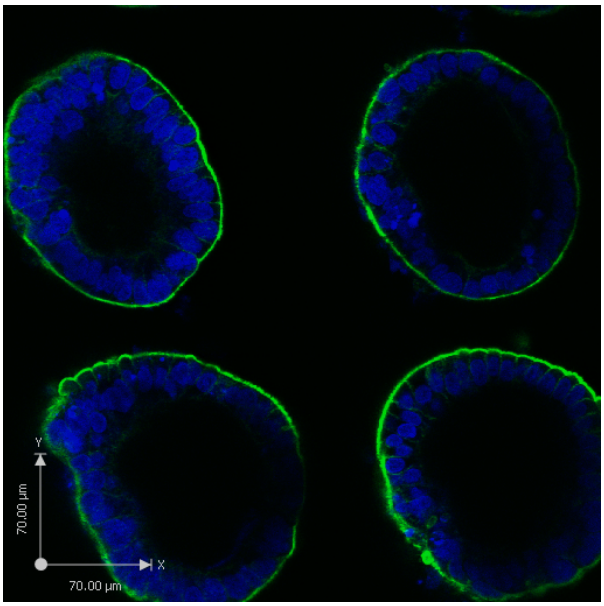
Supplementary Fig. 3 Expression of occludin in 2D monolayer model (Red: occludin, blue: DAPI).

Time course changes of TEER in 2D and 3D Caco-2 models



Supplementary Fig. 4 Time-course change of TEER of Caco-2 cells in 2D monolayer, 3D villi scaffold, 3D villi scaffold treated with siMUC17.

Confocal cross-section images of Caco-2 cells on 3D villi model.



Supplementary Fig. 5 Cross-section image (xy plane) of Caco-2 cells on 3D collagen villi, stained for actin (green) and nucleic acid (blue).