Supplemental Information

Nanostructure-Mediated Transport of Biologics across Epithelial Tissue: Enhancing Permeability via Nanotopography

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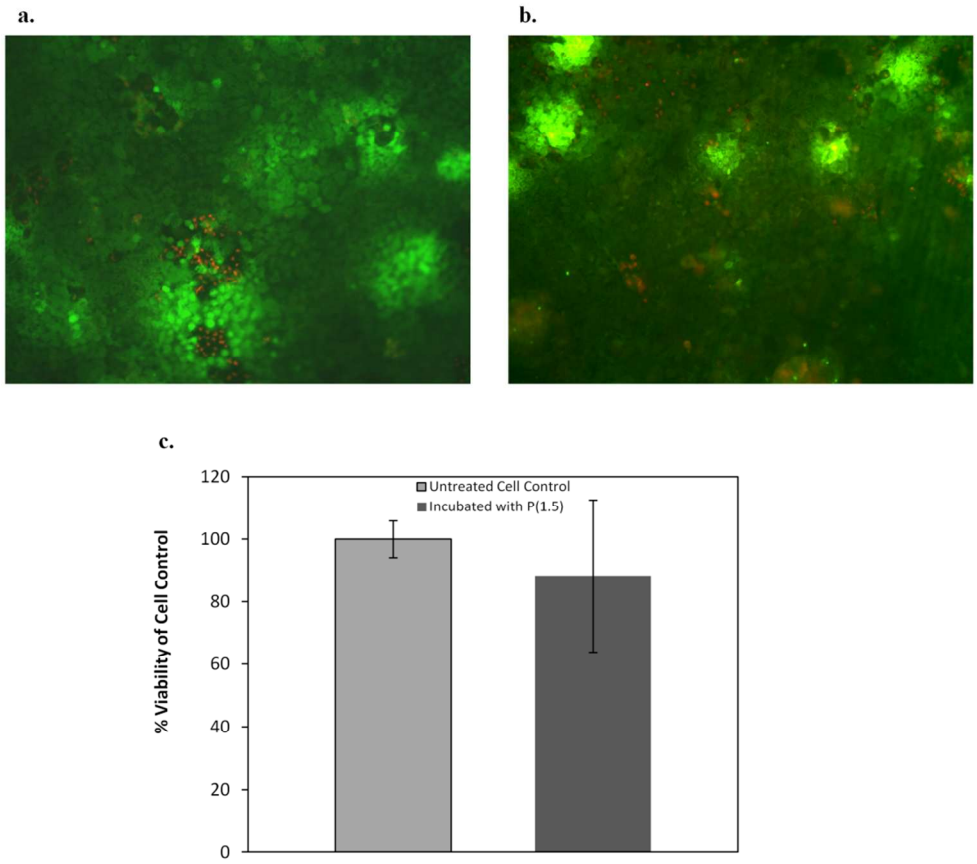

SUPPLEMENTAL FIGURE CAPTION

a A caco-2 monolayer control was stained with CellTracker Green CMFDA (5-Chloromethylfluorescein Diacetate) and propidium iodide to determine the number of live (in green) and dead (in red) cells, respectively. **b** A caco-2 cell monolayer at the same confluency as **a** was incubated with the low aspect ratio nanostructured thin film for 2 hours. The cell viability does not appear to be affected by the nanostructures. **c** An MTT assay was performed on a cell monolayer control and a monolayer that had been incubated with the nanostructured thin film.

The percent viability showed no significant difference between the two groups indicating that the nanostructured thin film does not significantly affect cell viability.

SUPPLEMENTAL INFORMATION

Caco2 cells were seeded at a density of 50,000 cells in a 96-well tissue culture plate. The nanostructured and unimprinted thin films were placed in contact with the cells for 2 hours at 37°C (see Supplemental information). Cell lysis, reverse transcription (Eppendorf), and qPCR (Applied Biosystems, StepONEPlus) were performed using the Fast SYBR Green kit in accordance with the manufacturer's instructions. The experiment was performed in triplicate (n=3) and the evaluation of mRNA expression by qPCR was also performed in triplicate. The expression of GAPDH (forward 5' CTCTCTGCTCCTCCTGTT CG-3', reverse 5'-GCCCAATACGACCAAATCC-3'), ZO-1 (forward 5'- TGTGAGTCCTTCAGCTGTGG-3', and reverse 5'- TTTCCTGCTCAACTCCTTCG-3'), ocln (forward 5'-ACCGAATCATTATGCACCAAGC-3', reverse 5'- AGATGGCAATGCACATCA CAA-3'), MLCK (forward 5'- CCCGAGGTTGTCTGGTTCAAA-3', reverse 5'-GCAGGTGTACTTGGCATCGT-3'), and FAK (forward 5'- GGCCTGCTTTGGATTCCG C-3', reverse 5'- CAGTGAACCTCCTCTGACCG-3') were analyzed.