Visualizing host-microbe interactions in cancer using 3D perfusion culture

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Background and Significance

The human gut microbiome, which is the population of microorganisms that are harbored in the digestive tract, includes up to 100 trillion bacteria in the average adult. These microbes are generally thought to play a beneficial role for the intestine host, but the presence of certain pathobionts can predispose individuals to inflammatory bowel diseases and colorectal cancer development. One such bacterium is E. coli NC101, a genotoxic bacterium that harbors the pks gene island, which produces the genotoxin colibactin causing DNA damage and eventually colorectal cancer. The genotoxic ability of this bacterium is dependent upon cell-to-cell contact. Here, we present 3D printed E. coli NC101 and rat intestinal epithelial cells (IEC-6) in a perfusion imaging plate, enabling visualization of the cytotoxic effects of the bacteria in real time using confocal microscopy, in combination with flow cytometry for cell cycle arrest (a surrogate marker of DNA damage). Visualizing these interactions allows us to understand how E. coli NC101, an adherent invasive bacterium, targets healthy epithelial cells in a physiologically relevant printed structure, which closely mirrors the actual form of epithelial cells in the human gut.

Methods

Construction of Δpks Mutant E. coli

The mutant Δpks strain was constructed using the lambda red recombinase system which replaced the pks gene island with a kanamycin resistance cassette which was then removed through flp flanking sites. Transformants were selected by plating and the mutation confirmed by PCR.

3D Printing of IEC-6 cells and Infection with E. coli NC101 or mutant strains

A 5% dispersion of green fluorescent IEC-6 cells (rat colonic intestinal epithelial cells) was printed in a series of 3 concentric rings within a 2 mm diameter 500 µm above the glass coverslip within each well of the imaging plate. Red fluorescent E. coli NC101 or Δpks mutant bacterial strains were then hand-printed at a distance of 1 mm above the epithelial cell layer at an MOI of 100. The infection was allowed to proceed for 18 hours during which timelapse image collection was performed using a Nikon A1R Confocal Microscope. Cells were then collected for flow cytometric analysis.

Flow Cytometry Analysis for Cell Cycle Arrest

Following 18 hours of infection, the IEC-6 cells were harvested from the plate and fixed in paraformaldehyde. The cells were stained with propidium iodide and run on a BD LSR Fortessa flow cytometer. Cell cycle analysis was performed using FlowJo software.

Conclusion

Our 3D perfusion culture model of bacterial interaction with host epithelial cells accurately reflects the DNA damage capability of E. coli NC101 seen in mouse models and human patients.

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Figure 1. Schematic diagram of 3D perfusion imaging plate.

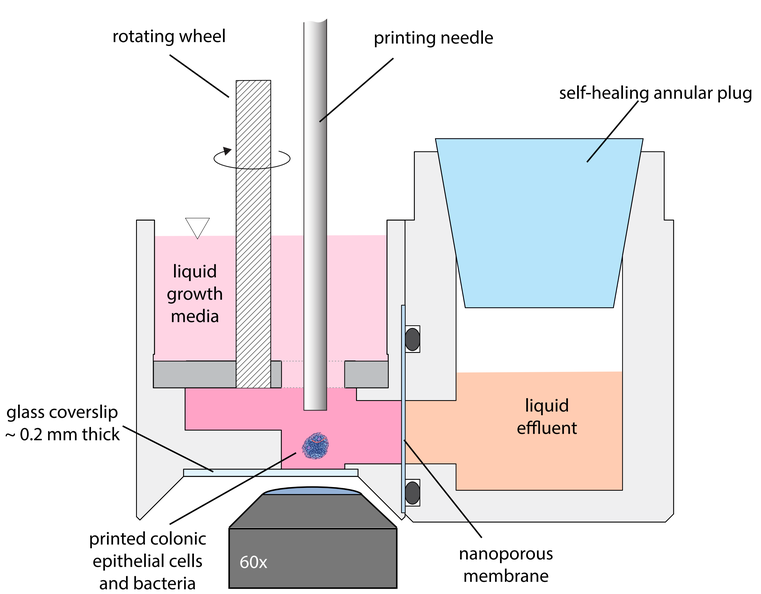

Figure 2. Bacterial infection of a 3D-printed epithelial cell layer

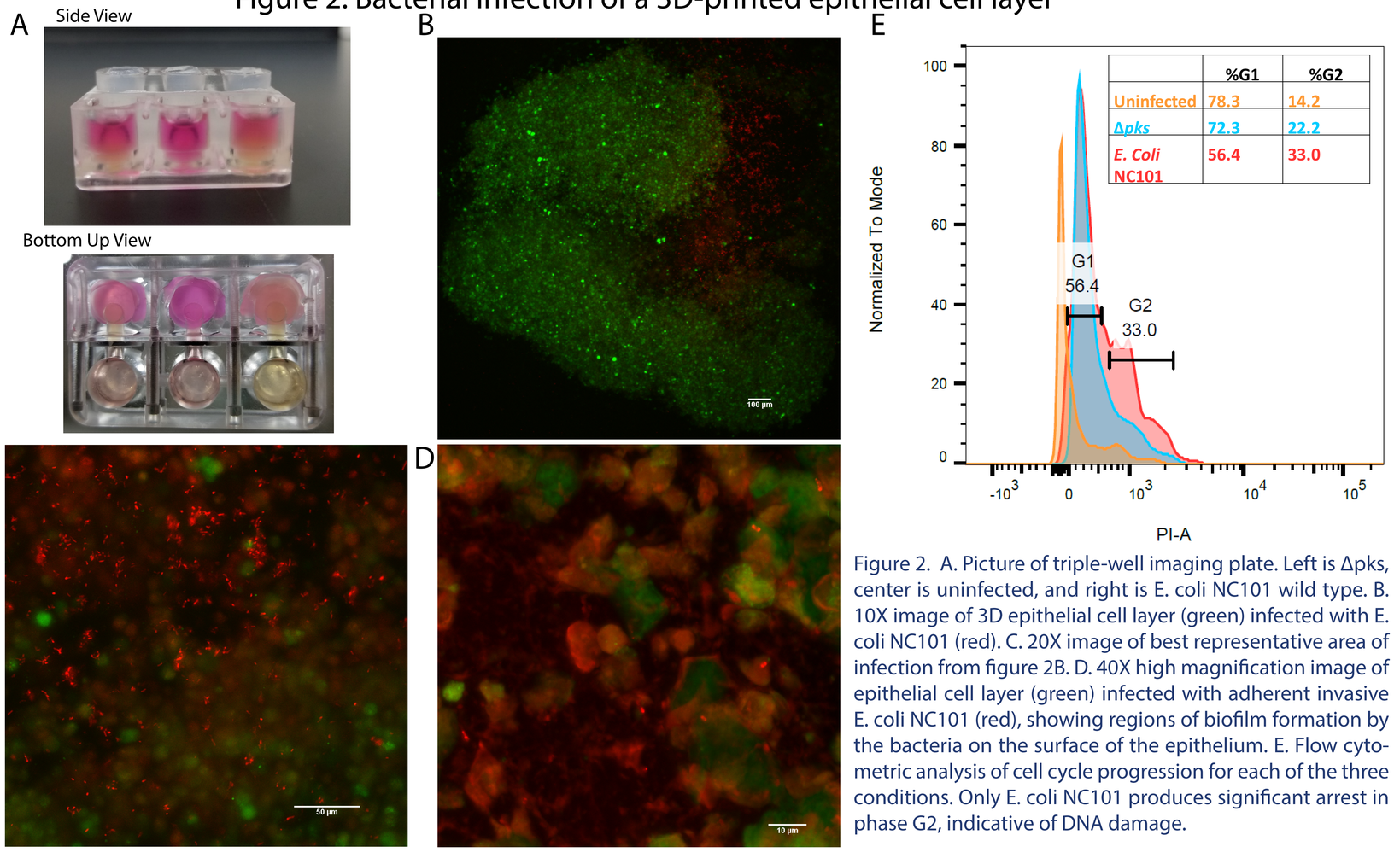

Figure 2. A. Picture of triple-well imaging plate. Left is Δpks, center is uninfected, and right is E. coli NC101 wild type. B. 10X image of 3D epithelial cell layer (green) infected with E. coli NC101 (red). C. 20X image of best representative area of infection from figure 2B. D. 40X high magnification image of epithelial cell layer (green) infected with adherent invasive E. coli NC101 (red), showing regions of biofilm formation by the bacteria on the surface of the epithelium. E. Flow cytometric analysis of cell cycle progression for each of the three conditions. Only E. coli NC101 produces significant arrest in phase G2, indicative of DNA damage.