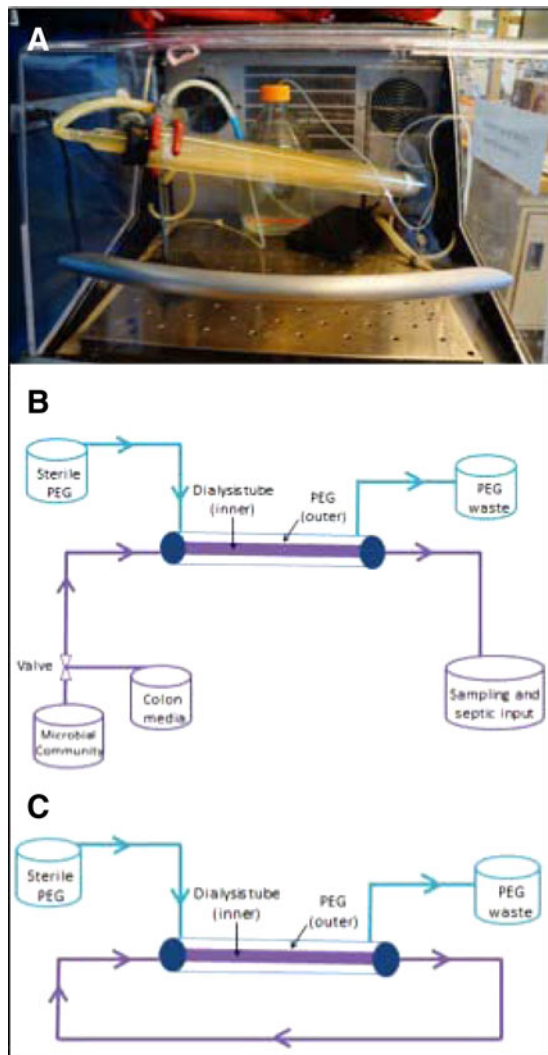


Supplementary Data

Microbial Community and Model Colon

A human colon was replicated by using a custom-built reactor (Marcus *et al.*, 2013) that represented conditions inside a proximal colon (Nugent *et al.*, 2001) by maintaining the reactor at pH 5.5–7 and 37°C inside an incubator (Barnstead MaxQ 4000; Thermo Scientific, Asheville, NC). The dialysis tube containing the microbial community and colon media

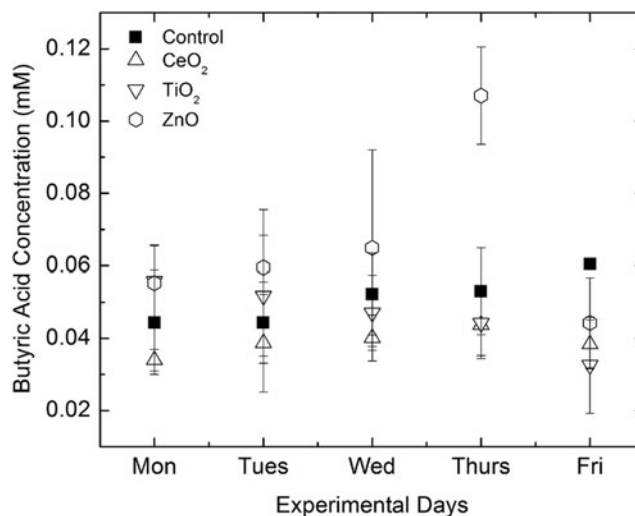


SUPPLEMENTARY FIG. S1. Visual schematics of model colon system. (A) is a photograph of the model colon system inside an incubator. Photo courtesy of A. Taylor. (B) is a diagram of the system during the Monday AM set-up and all feedings. The microbial community is added only on Monday AM. During all feedings, the colon media is added. Colon media contains the nanoparticles during the appropriate exposure conditions. Both PEG waste and colon waste are located during the feedings. Not mentioned in the work, an additional study has used the colon waste as an input for a model septic system (Marcus *et al.*, 2013; Taylor and Walker, 2014). (C) is the system during nonfeeding times. The colon media circulates in a closed loop system.

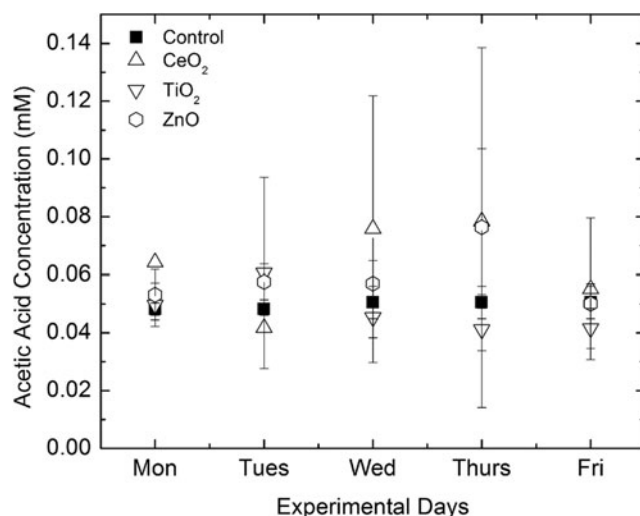
inside the reactor was encompassed by a polyethylene glycol (PEG) mixture, and the PEG was kept at a steady flow throughout the duration of the experiments as previously described (Jiménez-Vera *et al.*, 2008). To mimic normal adult feedings, 100 mL of sterile colon media was pumped into the dialysis tubing thrice per day, 6 h apart, with a rate of 5 mL/min (Jiménez-Vera *et al.*, 2008). The colon media is neither anaerobic nor oxygen depleted (Jiménez-Vera *et al.*, 2008).

Nanoparticle Testing

To simulate conditions in the stomach and the potential of a low pH to alter the NPs physical-chemical properties before entering the intestines, additional controls monitored changes to the NPs as a function of pH. These control tests were conducted in tests outside of the colon reactor. Controls were conducted in sterile 1,000 mL lidded flasks with 500 mL of sterile colon media. Controls consisted of the following combinations: colon media only or colon media with one of the NPs at the following concentrations: 0.01 μg ZnO NP/L, 0.01 μg CeO₂ NP/L, or 3 mg TiO₂ NP/L. Controls were also conducted with and without the microbial community. Controls with the microbial community were inoculated in the same manner and proportion as the colon reactor. Samples were collected from the batch tests at 0, 30, 60 min, and 24 h using a range of pH 2–8 at 0.5 increments (Fallingborg, 1999; Kim and Rhee, 2012; Mojaverian, 1996) and were measured in triplicate for the following tests: zeta potential and hydrophobicity. These tests were performed in an identical manner to the washed bacteria cell samples from the colon effluent. Multiple control tests were conducted over a range of pH 2–8 at 0.5 increments.



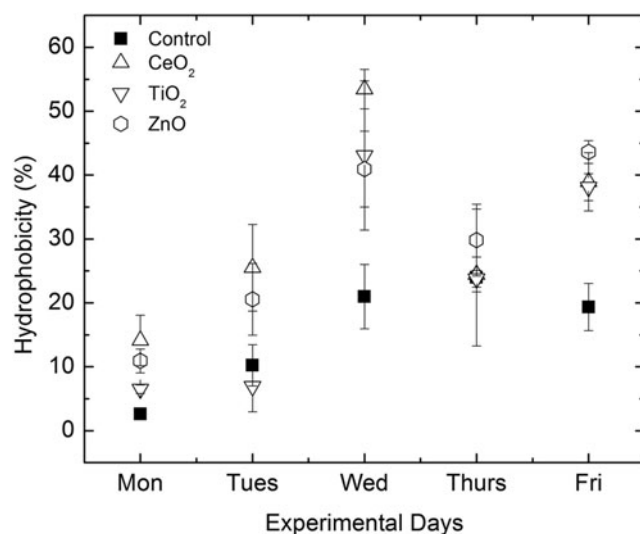
SUPPLEMENTARY FIG. S2. Comparison of the production of butyric acid over the 5-day-long experiments in the model colon in the presence and absence of TiO₂, CeO₂, and ZnO NPs. SCFA samples were collected twice a day for a minimum of 2 weeks and analyzed in triplicate with GC-FID. Each data point is an average of data that was collected over the course of two experimental runs (two 5-day-long experiments) with samples in triplicate collected twice a day. Error bars indicate standard deviation.



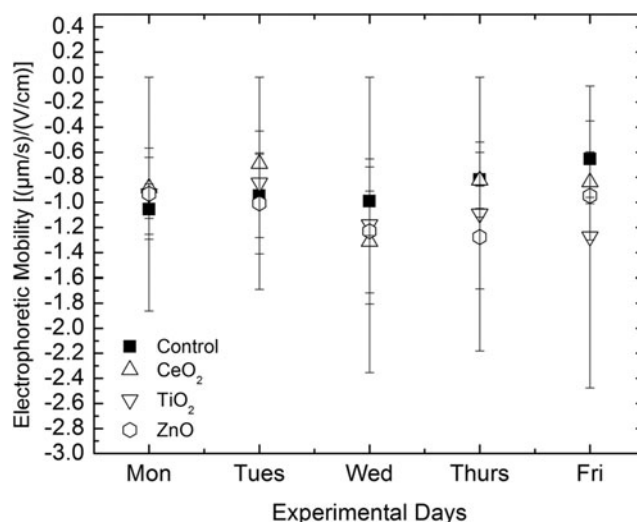
SUPPLEMENTARY FIG. S3. Comparison of the production of acetic acid over the 5-day-long experiments in the model colon in the presence and absence of TiO₂, CeO₂, and ZnO NPs. SCFA samples were collected twice a day for a minimum of 2 weeks and analyzed in triplicate with GC-FID. Each data point is an average of data that was collected over the course of two experimental runs (two 5-day-long experiments) with samples in triplicate collected twice a day. Error bars indicate standard deviation.

Phenotypic Characterization of Microbial Community

Cell concentration of the washed colon effluent cells was determined using a cell counting chamber (Gong *et al.*, 2011), (BuerkerTürk chamber; Marienfeld Laboratory Glassware, Lauda-Königshofen, Germany) and a light microscope (Fisher Scientific Micromaster, Pittsburgh, PA). EPS (extracellular polymeric substance) was measured as total protein and total sugar by a multi-step process: fixation of cells in 0.22%

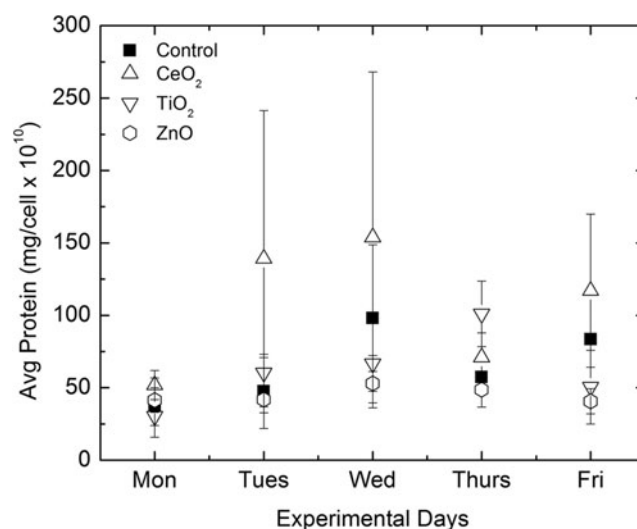


SUPPLEMENTARY FIG. S4. Hydrophobicity of the bacterial cells is displayed as a function of NP exposure in the model colon during the two runs of the 5-day-long experiments. Data are displayed per experimental day. Hydrophobicity was measured twice a day in triplicate. All measurements were made on washed cells from the colon effluent. Error bars indicate standard deviation.

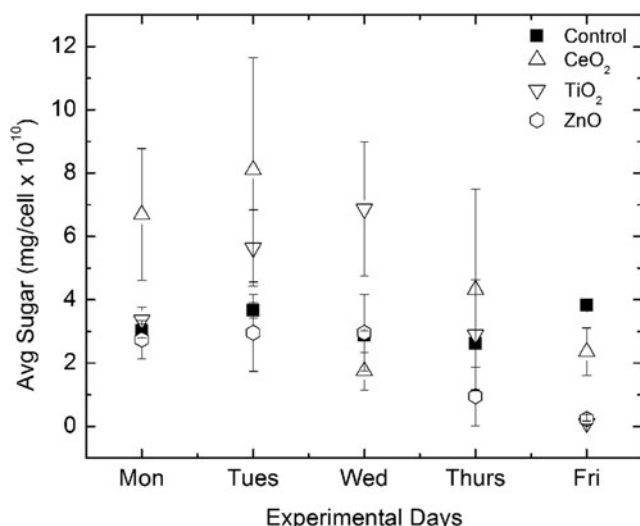


SUPPLEMENTARY FIG. S5. Electrophoretic mobility (EPM), an indicator of the relative surface charge, of the bacterial cells was measured in triplicate twice a day during the course of two runs of the 5-day-long experiments. All measurements were made on washed cells from the colon effluent. Error bars indicate standard deviation.

formaldehyde solution, separation via centrifugation, lyophilization, and, finally, colorimetric measurements following published methods (Gong *et al.*, 2009). The extraction process used here analyzes the EPS bound to the cell rather than the free EPS found in the colon effluent, which is removed via centrifugation. A zeta potential analyzer (ZetaPALS; Brookhaven Instruments Corp., Holtsville, NY) measured the EPM (electrophoretic mobility) of the washed cells (Tazehkand *et al.*, 2008). A microbial adhesion to hydrocarbon (MATH) test quantified partitioning of cells into the hydrocarbon versus the KCl solution (electrolyte phase) and gave a measure of relative



SUPPLEMENTARY FIG. S6. The average protein content of the extracellular polymeric substance (EPS) was measured in triplicate once a day during the course of the 5-day-long experiments for a minimum of two runs (two 5-day-long experiments). EPS samples were collected once a day from the colon effluent and analyzed for sugar and protein content in triplicate. Error bars indicate standard deviation.



SUPPLEMENTARY FIG. S7. The average sugar content of the extracellular polymeric substance (EPS) was measured in triplicate once a day during the course of the 5-day-long experiments for a minimum of two runs (two 5-day-long experiments). EPS samples were collected once a day from the colon effluent and analyzed for sugar and protein content in triplicate. Error bars indicate standard deviation.

hydrophobicity (Walker *et al.*, 2005a, 2005b). Cell size was established by capturing images taken of the washed bacteria in the KCl solution using a phase-contrast microscope (Fisher Scientific Micromaster), camera (Westover Scientific Digital MCD Model 2200), and imaging software (MicronUSB2 v. 1.09). Images containing $n > 50$ cells were then processed with Matlab (version 7.11.0.584 R2010b; The MathWorks, Inc., Natick, MA) in which cell radius, length, and width were confirmed (Marcus *et al.*, 2012a, 2012b). Many current studies in the literature have successfully used bacterial and enzymatic characterization tests in complex media with the presence of NPs (Priester *et al.*, 2013, 2014; Adeleye *et al.*, 2014; Ge *et al.*, 2014; Hong *et al.*, 2015). Due to the estimated low concentrations of NPs present in the bacterial cell solution before the washing and centrifugation steps ($\sim 0.0005 \mu\text{g ZnO}$ or $\text{CeO}_2/45 \text{ mL}$ washed bacterial solution, and $0.015 \text{ mg TiO}_2/45 \text{ mL}$ washed bacterial solution), it is therefore highly unlikely that NPs caused interference with tests.

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