Published in final edited form as:

Biomed Microdevices. 2016 June ; 18(3): 51. doi:10.1007/s10544-016-0076-9.

Microfluidics Meets Metabolomics to Reveal the Impact of *Campylobacter jejuni* Infection on Biochemical Pathways

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Abstract

Microfluidic devices that are currently being used in pharmaceutical research also have a significant potential for utilization in investigating exposure to infectious agents. We have established a microfluidic device cultured with Caco-2 cells, and utilized metabolomics to investigate the biochemical responses to the bacterial pathogen *Campylobacter jejuni*. In the microfluidic devices, Caco-2 cells polarize at day 5, are uniform, have defined brush borders and tight junctions, and form a mucus layer. Metabolomics analysis of cell culture media collected from both Caco-2 cell culture systems demonstrated a more metabolic homogenous biochemical profile in the media collected from microfluidic devices, compared with media collected from transwells. GeneGo pathway mapping indicated that aminoacyl-tRNA biosynthesis was perturbed by fluid flow, suggesting that fluid dynamics and shear stress impacts the cells translational quality control. Both microfluidic device and transwell culturing systems were used to investigate the impact of *Campylobacter jejuni* infection on biochemical processes. Caco-2 cells cultured in either system were infected at day 5 with *C. jejuni* 81-176 for 48 hours. Metabolomics analysis clearly differentiated *C. jejuni* 81-176 infected and non-infected medias collected from the microfluidic devices, and demonstrated that *C. jejuni* 81-176 infection in microfluidic devices impacts branched-chain amino acid metabolism, glycolysis, and gluconeogenesis. In contrast, no distinction was seen in the biochemical profiles of infected versus non-infected media collected from cells cultured in transwells. Microfluidic culturing conditions demonstrated a more metabolically homogenous cell population, and present the opportunity for studying host-pathogen interactions for extended periods of time.

Keywords

Microfluidic; metabolomics; *Campylobacter jejuni*; Caco-2 cells; host-pathogen interaction; infectious disease model

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1 Introduction

While microfluidic devices (μF) are being developed for use in predicting drug efficacy and toxicity (Mahler et al., 2009, Esch et al., 2011, Sung et al., 2011, Huh et al., 2013, Huh et al., 2012, Kim et al., 2012, Kim and Ingber, 2013), chemical exposure (Shintu et al., 2012, Prot et al., 2012), biomolecular interactions (Javanmard et al., 2010) and detection (Mok et al., 2014), nanoparticle exposure (Esch et al., 2014, Mahler et al., 2012), and in the field of nutrient research (Ramadan et al., 2013), there has been little effort to date to develop these models for the study of infectious disease. Microfluidic cultures of different organs have been reported in the literature including lung (Sellgren et al., 2014, Huh et al., 2010), kidney (Jang et al., 2013, Baudoin et al., 2007), liver (Ouattara et al., 2012, Prot et al., 2011, Kang et al., 2015, Zhang et al., 2008), intestinal tract (Kim et al., 2012, Kim and Ingber, 2013, Mahler et al., 2009), placenta (Lee et al., 2015) and combinations of multiple organs (Esch et al., 2014, Maschmeyer et al., 2015). Studies have shown that fluid dynamics in μF impacts cell biology compared to traditional stagnant culture conditions. Flow rate has been shown to increase the growth rate and glucose consumption in Madin-Darby Canine Kidney (MDCK) cells (Baudoin et al., 2007). Human kidney proximal tubular epithelial cells morphology show that fluid flow increases cell height and cilia formation as well as expression of Na/K-ATPase and aquaporin 1 proteins (Jang et al., 2013). For hepatoma HepG2/C3A cells, transcriptomic analysis showed that 1,025 genes were statistically differentially expressed as a results of fluid flow, showing that gene expression of cytochromes P450, 1A1/2, 2B6, 3A4, 3A5 and 3A7 were all upregulated in μF (Prot et al., 2011). In the study transcriptomic analyses were followed up with biotransformation assays which confirmed the upregulation of CYP1A1/2 activity as well as several phase II enzymes including sulfotransferases, UDP-glucuronyltransferase and phase III transporters (Prot et al., 2011). Previous reported studies in the literature have also demonstrated a relationship between fluid shear stress and cell adhesion (Lu et al., 2004), as well as force-induced cellular focal adhesion translocation (Mack et al., 2004) in endothelial cells. The literature strongly suggests that fluid dynamics, including mechanical and shear forces, impacts cell physiology and biomolecule interactions. In the case of human intestinal epithelial cells, Caco-2, cultured under fluid flow have been reported to be taller and have higher transepithelial electrical resistance (TEER) compared to Caco-2 cells cultured in stagnant transwell plates (Huh et al., 2012, Chi et al., 2015), as well as a higher cytochrome CYP3A4 activity (Kim and Ingber, 2013), and mucus production (Chi et al., 2015). The microfluidic cell culture model presented here is also designed to facilitate metabolomics analysis of cellular responses.

Metabolomics is a systematic study of small-molecules and the unique biochemical fingerprints that specific cellular processes leave behind as a result of the metabolism in a biological system. Metabolomics analysis is a powerful discovery tool to identify metabolites that are important for differentiating, for example, non-infected and infected cell systems, and can provide unique insight into host-pathogen interaction and progression of infection. Metabolomics analyses of cell media have been reported previously on microfluidic culture of HepG2-C3A and MDCK cells using a polydimethylsiloxane (PDMS) μF comprised of multiple chambers and channels (Ouattara et al., 2012, Shintu et al., 2012).

In the study analyzing media from HepG2/C3A cells cultured in μ F or petri dishes, metabolomics showed a clear differentiation of the samples in a multivariate analysis using orthogonal partial least squares discriminant analysis (OPLS-DA); indicating that cells cultured under fluid flow had higher glucose, glutamine and ethanol consumptions compared to cells grown in stagnant cultures, while stagnant culture conditions caused higher cellular consumption of lactate (Shintu et al., 2012). Another study of HepG2/C3A cells combining metabolomics profiling and metabolic flux analysis showed that cells cultured in μ F had similar glycolytic activity as cells cultured in petri dishes, while exhibiting higher TCA cycle activity which were supported by transcriptional activity of hypoxia-regulating factor-1 (Ouattara et al., 2012). Metabolomics analysis in the field of infectious diseases have been used to uncover host-pathogen interactions (Milner et al., 2015, Jung et al., 2015, Sanchez and Lagunoff, 2015, Villar et al., 2015), antibiotic susceptibility (Lobritz et al., 2015), and find potential strategies and targets for prevention, control, and therapeutics (McGarvey et al., 2009). Metabolite profiling of the liver, spleen, and serum from mice infected with the bacterial pathogen *Orientia tsutsugamushi* showed that infection caused decreased energy production and deficiencies in both remethylation sources and glutathione, as well as acceleration of uncommon energy production pathways (i.e. excess fatty acid and protein oxidation in the host) (Jung et al., 2015). A systems biology approach to understand the host-pathogen interaction between the bacteria *Anaplasma phagocytophilum* and tick cells integrated metabolomics, transcriptomics and proteomics data and demonstrated novel aspects of the co-evolution between host and pathogen (Villar et al., 2015). These studies illustrate the strength of integrating metabolomics analysis in understanding host-pathogen interaction.

Infectious diseases continue to be a major burden on global health, resulting in illness, post-infectious complications and millions of deaths each year, and novel approaches such as metabolomics analysis add important insight in host-pathogen interaction when exploring potential strategies and targets for prevention, control, and therapeutics. Yet, studying infectious diseases to improve prevention strategies are challenged by the shortcomings of the present *in vivo* and *in vitro* models. A major obstacle in the study of host-pathogen infectious disease interactions includes the need for mimicking fluid dynamics, nutrient flux and the presence of a mucus layer. In the commonly used stagnant co-culture models, the bacterial growth rate often does not allow for studying the progression of infection over several days because the bacteria will outcompete the mammalian cells in an environment of limited nutrients. While stagnant *in vitro* models are beneficial for high throughput-screening, these traditional experimental setups do not reflect the high bacterial adhesion and invasion for *Campylobacter jejuni* observed in human infections (Friis et al., 2005, Mills et al., 2012). Few studies have explored the possibility of co-culturing mammalian and microbial cells in μ F (Kim et al., 2012, Chi et al., 2015). Kim and co-workers successfully co-cultured the commensal *Lactobacillus rhamnosus* GG (LGG) as a representative bacterial strain of the microbiome, with Caco-2 cells under microfluidic conditions for up to a week, without compromising the epithelial cell viability (Kim et al., 2012). Compared to stagnant culture conditions where LGG caused TEER to drop near zero within 24 hours, while in microfluidic co-culture the TEER of Caco-2 cells increased (Kim et al., 2012). In another study by Chi and colleagues, Caco-2 cells were also cultured in a microfluidic system with

Salmonella typhimurium for 3 h, and found that significantly fewer bacteria adhered and invaded Caco-2 cells in μ F compared to transwell (TW) cultures (Chi et al., 2015). To address the need for studying the onset and progression of infectious diseases we developed a microfluidic gut model to study *C. jejuni* infection. Campylobacteriosis is believed to cause over two million cases of foodborne diarrhea in the US annually, resulting in approximately 100 deaths (CDC, 2013), but it is a more serious infection in developing countries among young children, reflected in the reported 8.8% fatality rate among children younger than five in a study from Kenya (WHO, 2013). Campylobacteriosis can cause post-infectious complications including joint or muscular symptoms (including reactive arthritis) (Hannu et al., 2002, Loch and Krogfelt, 2002, Hill Gaston and Lillicrap, 2003, Hannu et al., 2004, Ajene et al., 2013, Tuuminen et al., 2013) and Guillain-Barre Syndrome (an immune-mediated demyelinating polyneuropathy of peripheral nervous system), which, after the decline in polio cases, became the most common cause of acute flaccid paralysis (Jackson et al., 2013, Nyati and Nyati, 2013). Despite the considerable morbidity and socioeconomic cost of campylobacteriosis, many aspects of host-pathogen interaction are not fully understood due to the lack of suitable small animal models (Newell, 2001). To our knowledge this is the first time metabolomics analysis has been used study host-pathogen interaction in a microfluidic device.

In this study μ F cultured with Caco-2 cells were established and used to study physiological and biochemical responses to the bacterial pathogen *C. jejuni*. The use of this model system demonstrated physiologically relevant evaluations of the host-pathogen interaction in terms of the important metabolic perturbations *C. jejuni* cause in this model cell line of the human gut. An improved understanding of how the pathogen impacts host metabolism under these conditions will improve future studies focused on evaluating infection prevention and treatment strategies.

2 Materials and Methods

2.1 Cell culturing

Human intestinal epithelial Caco-2 cells (ATCC HTB-37) were purchased from (ATCC, Manassas, VA) and cultured in Dulbecco's Modified Eagle's Medium (ATCC, Manassas, VA), with 10% fetal bovine serum (Gibco, Life Technologies, Grand Island, NY), 1% non-essential amino acids (ATCC, Manassas, VA) and 100 U penicillin/streptomycin (Invitrogen, Life Technologies, Grand Island, NY). Cells were maintained at a concentration of 3×10^4 cell/mL and passaged twice a week. At the passage proceeding microfluidic cell culturing, cells were seeded at 1×10^4 cells/mL. Caco-2 cells were used in experimental cultures at passage number 15-30.

2.2 Microfluidic device and transwell cell cultures

A 100 mm TW polycarbonate membrane with 0.4 μ m pores (Corning, Tewksbury, MA) was sandwiched between apical and basolateral microfluidic polystyrene (PS) channels (Ibidi LLC, Verona, WI). The μ F were connected to silicon tubing (Cole-Parmer, IL) and 8 mL glass vials containing growth media. The apical and basolateral channels measure 400 μ m in height, 5 mm wide and 45 mm long. The membrane was pre-coated with a solution of 50

µg/mL extracellular matrix proteins (BD, Sparks, MD) and 300 µg/mL collagen type I (Gibco, Life Technologies, Grand Island, NY) to increase cell attachment to the surfaces (Kim et al., 2012). Caco-2 cells were seeded at 1×10^5 cells/cm² in the apical channel and allowed to attach for 2 hours before flow at 0.04 dynes/cm² (32 µL/min) was started using a peristaltic pump (Masterflex, Vernon Hills, IL). Fresh media was added every 48 hours, unless otherwise stated, by replacing 3 mL of the 5 mL total volume recirculating in the microfluidic device.

Transwell (TW) cultures were performed in 24-well polystyrene plates with 0.4 µm pores (Corning, Tewksbury, MA). TW plates were also seeded at a concentration of 1×10^5 cells/cm². TEER was measured using an Epithelial Voltammeter EVOM2 with STX2 electrode (World Precision Instruments, Sarasota, FL). Media were replaced every 48 hours unless described otherwise.

2.3 Bacteria

The *Campylobacter jejuni* model strains 81-176 (ATCC BAA-2151) (Hu and Kopecko, 1999, Korlath et al., 1985) were purchased from ATCC (ATCC, Manassas, VA), and cultured on trypticase soy agar (BD, Sparks, MD) with 5% defibrinated sheep blood (BD, Sparks, MD) at 37°C in 5% CO₂. *C. jejuni* is a microaerophilic bacteria, but was cultured under the same conditions as the Caco-2 cells to facilitate the co-culture.

2.4 Infection in microfluidic and transwell cell culture systems

Caco-2 cells were cultured in either µF or TW for 5 days before being infected with *C. jejuni*. Growth media were replaced with antibiotic free media 24 hours before *C. jejuni* 81-176 were introduced to the µF or TW Caco-2 cultures. *C. jejuni* 81-176 were harvested from a 2 day 5% blood trypticase soy agar plate and washed in antibiotic free media. Inoculum were adjusted to OD₂₆₀=0.1 (app. 1×10^8 CFU/mL). Caco-2 cultures were infected for 48 hours under flow in µF or stagnant culture conditions in TW. Growth media were replaced after 24 hours.

2.5 Adhesion and invasion assay

Adhesion and invasion of *C. jejuni* 81-176 in µF or TW Caco-2 cultures were evaluated using the total association and gentamicin protection assay (Elsinghorst, 1994). For invasion, gentamicin sulfate (Sigma, St. Louis, MO) in a final concentration of 250 µg/mL was added to the media for 2 hours. Both culture systems were washed in 1X PBS, and 0.1% Triton X-100 (Sigma, St. Louis, MO) were added to the apical channel for 5 min. Serial dilution of the Caco-2 cell lysate containing the viable *C. jejuni* 81-176 was plated out to determine the CFU/mL of bacteria.

2.6 Lactate dehydrogenase assay

Manufacturer protocol was followed to measure release of lactate dehydrogenase (LDH) (TOX7, Sigma-Aldrich, St. Louis, MO). Briefly, 100 µl of media from the apical compartment of the TW plate or collected from the µF cell cultures were analyzed in triplicate- to assess cell viability as a function of membrane integrity.

2.7 Microscopy

Caco-2 cells cultured either in μ F or TW devices were fixed using a 4% paraformaldehyde for 30 min, followed by a 5 min 0.05% TX-100 permeabilization on ice. Primary antibodies 5 μ g/mL ZO-1 mouse monoclonal antibodies (Life Technologies, Grand Island, NY) or 10 μ g/mL MUC2 mouse monoclonal antibodies (Abcam, Cambridge, MA) in 1% BSA (Sigma, St. Louis, MO) were added and incubated on ice for 1 hour. After washing the secondary antibody 2 μ g/mL Alexa Fluor® 488 Goat Anti-Mouse IgG (Life Technologies, Grand Island, NY) in 1% BSA (Sigma-Aldrich, St. Louis, MO) at room temperature for 1 hour. Nuclei were labeled using 1:500 Hoechst 33342 staining (Life Technologies, Grand Island, NY) and actin filaments were stained using 1:30 Phalloiden-rhodamine (Life Technologies, Grand Island, NY) in a 20 min incubation. Reflective fluorescence imaging of Caco-2 cultures on non-transparent polycarbonate membranes was done using a Zeiss Observer Z1 3D microscope with three fluorescence channels. To access bacteria viability, bacteria were stained using LIVE/DEAD® BacLight™ Bacterial Viability Kit (Life Technologies, Grand Island, NY) following the protocol provided by the manufacture, and imaged with Zeiss Observer Z1 using a 40X objective. Darkfield Enhanced Microscopy (CytoViva, Auburn, AL) was conducted by placing 5 μ L of cell culture media between a coverslip and objective glass (VWR, Radnor, PA) and imaged using the oil 100X objective.

2.8 Metabolomics analysis of growth media

Cell media were collected from 13 TW plates (5 non-infected, 8 infected) and 19 μ F (6 non-infected, 13 infected) 7 days after the Caco-2 cultures were initiated. The media samples were stored at -80°C . An aliquot of each media sample was thawed on ice and prepared for metabolomics analysis, as previously described (Sumner et al., 2009, Sumner et al., 2010b, Sumner et al., 2010a, Pathmasiri et al., 2012, Snyder et al., 2015). Each of the individual media sample was extracted with 50% acetonitrile (in water) and aliquot of the supernatant was lyophilized to dryness overnight. The dried samples were reconstituted in 700 μ L D_2O (containing 0.5 mM DSS and 0.6 mM imidazole). In addition, a total pool was created with 25 μ L aliquots of each sample and were prepared in triplicate identical to the individual media samples. Metabolomics data were acquired for each of the individual study samples and the three pooled samples. ^1H NMR spectra were acquired on a Bruker Avance III 700 MHz NMR spectrometer (located at Department of Biochemistry, NC State University) using a 1D NOESY presaturation pulse sequence (noesypr1d). NMR spectra were pre-processed using ACD 1D NMR Processor 12.0 (ACD Labs, Toronto, CA) for metabolomics analysis. NMR data was binned (0.03 – 9.00 ppm), excluding water (4.83 – 4.85 ppm) using intelligent binning width of 0.04 ppm and 50% looseness factor. In a subsequent analysis, the bins associated with lactate were also removed (1.28 – 1.34 ppm, 4.07 – 4.12 ppm). Integrals of each of the bins were normalized to total integral of each of the spectrum.

Normalized binned NMR data were mean-centered and Pareto-scaled and prior to multivariate analysis. Multivariate data analysis methods (e.g. principal component analysis [PCA] and OPLS-DA) were used to reduce the dimensionality and to enable the visualization of the separation of the study groups (SIMCA 13, Umetrics, Umeå, Sweden). The PCA scores plots were inspected to ensure that the phenotypic pooled samples were tightly clustered in the center of phenotypic groups and that pool of all of the individual

study samples clustered in the center of all samples, a quality control method that is widely used in metabolites studies (Chan et al., 2011). Loadings plots and variable influence for the projections (VIP) plots from OPLS-DA were inspected, and bins that had a VIP > 1.0 with a jack-knife confidence interval that did not include 0 were determined to be important to differentiating the study groups. Chenomx NMR Suite 7.7 Professional software (Edmonton, Alberta, Canada), which has a concentration library of approximately 350 compounds, was used to match the signals in the identified bins to VIP metabolites. Statistical analyses were performed using SAS 9.4 (SAS, Cary, NC). Statistical tests for continuous variables were conducted using a two-sided exact Wilcoxon Rank Sum test. Metabolic pathway and network analysis were performed by using MetaCore® module in GeneGo software (GeneGo, St. Joseph, MI), a curated database of human protein-protein, protein-DNA interactions, transcription factors, signaling and metabolic pathways, disease and toxicity, and the effects of bioactive molecules.

All of the metabolomics data are available for download at the NIH Common Fund Metabolomics Data Repository and Coordinating Center at the University of California at San Diego (Dr. Shankar Subramaniam, PI) under studies ST000124 and ST000125 (<http://www.metabolomicsworkbench.org/>).

3 Results and discussion

3.1 The impact of fluid flow on cell physiology

To investigate the role of fluid flow on Caco-2 cell morphology, fluorescence microscopy was used to compare three physiological parameters between μ F or TW cultures after 5 days; brush borders, tight junctions and mucus layer (Fig. 1). Brush border formation was imaged using phalloidin staining of actin filaments, since each microvilli structure is supported by these filaments. Fluorescence microscopy showed that Caco-2 cells cultured in μ F had well defined brush border, whereas Caco-2 cells from TW showed sporadic microvilli formation (Fig. 1a-d). Imaging the presence and integrity of tight junctions were done by visualizing the intracellular tight junction protein ZO-1, and showed that tight junctions were defined and intact for Caco-2 cells in μ F, but not for Caco-2 cells cultured in TW (Fig. 1e-h). Mucus layer was visualized with anti-MUC2 and showed a higher presence of mucin 2 in Caco-2 cells cultured under microfluidic flow compared to TW (Fig. 1i-l). When comparing the intensity for MUC2 stained images μ F cell cultures had a higher intensity than TW (Fig. 1m).

In the μ F established in this study, the Caco-2 cells have polarized at day 5, and were uniform, had well defined brush borders and tight junctions, and mucus and were as good or better when compared with our results for Caco-2 cells cultured in a traditional TW. These findings are supported by previously reported studies that found; i) the brush border enzyme cytochrome of the polarized Caco-2 cells are more active in μ F compared to TW (Kim and Ingber, 2013), ii) TEER was higher for Caco-2 cells cultured in μ F compared to TW (Kim et al., 2012), and iii) MUC2/GAPDH expression in Caco-2 cells cultured in μ F were magnitudes higher compared to TW (Chi et al., 2015). These three cell morphological parameters all suggested that the fluid dynamics significantly impact cell biology.

3.2 The impact of fluid flow on endogenous metabolism

To explore the impact of fluid flow on Caco-2 cellular biochemical profiles, metabolomics was used to analyze media collected at day 7 from μ F or TW Caco-2 cell cultures. Differences in the metabolic profiles of media collected from each of the TW cultures compared with the media from each of the μ F was observed in the PCA analysis of the broad spectrum metabolomics data (Fig. 2a, μ F (blue) and TW (green); each media sample is defined by a colored circle). As observed in Fig. 2a, the individual media samples from the μ F are tightly clustered while the media samples from the TW are dispersed indicating that there is much more variability in the biochemical profiles of the TW media than in the μ F media. OPLS-DA (Fig. 2b) was used to determine the metabolites that best differentiated the media from the two type of culturing systems and are listed in Table 1. Metabolites found to differentiate media collected from μ F Caco-2 cultures compared to TW cultures were 2-hydroxy-3-methylvalerate, alanine, glucose, glutamate, isoleucine, leucine, lysine, N-acetylaspartate, pyroglutamate, tyrosine, and valine. Pathway mapping of these metabolites using GeneGo (MetaCore) analysis indicates that aminoacyl-tRNA biosynthesis ($-\log(p\text{-value}) > 10$) is most perturbed by fluid flow (compared with stagnant TW culture) involving the metabolites alanine, isoleucine, leucine, lysine, tyrosine and valine, which suggests that fluid flow impacts translation of proteins.

The role of the aminoacyl-tRNA biosynthesis pathway in cells is to match amino acids with tRNAs containing the corresponding anticodon and functioning as a translational quality control of proteins synthesis (Ibba and Soll, 2000, Ling et al., 2009). Determining the functional differences in aminoacyl-tRNA biosynthesis between μ F and TW cell cultures on Caco-2 cell biology is an area for future investigation. However, the fact that the variability of μ F cell culture media samples are much less than the variability observed in TW cultures, indicates an overall more homogenous metabolism and protein synthesis for cells cultured in μ F. All together the morphological and metabolomics results indicate that fluid flow has a significantly positive impact on both cell physiological parameters, but also cellular metabolism.

3.3 *Campylobacter jejuni* infection in μ F and TW systems

The potential of μ F in the areas of host-pathogen interaction and infectious diseases has not been explored. μ F cultured with human intestinal epithelial cells have been used as GI models to investigate oral drug administration (Mahler et al., 2009, Sung et al., 2011, Cianferoni, 2013), and emerging efforts have been reported in nutrition analysis (Ramadan et al., 2013). Here, a μ F Caco-2 culture was used to study campylobacteriosis, and the results were compared to interactions studied in TW Caco-2 culture. The virulence factors adhesion and invasion of *Campylobacter* spp. are traditionally tested *in vitro* using the well-established total association and gentamicin protection assay (Elsinghorst, 1994). Where bacterial strains are introduced to an established cell culture for 3 hours to determine total bacterial association, and to determine bacterial invasion followed by 2 hours of gentamycin treatment. However, a *Campylobacter* infection takes 2-3 day to manifest itself (Black et al., 1988). In this study *C. jejuni* model strain 81-176 were co-cultured for 48 hours with Caco-2 cells to study the host-pathogen interaction during onset and progression of infection

focusing on changes in the biochemical profile of cell culture media using metabolomics analysis to understand how infection might impact cellular biochemistry.

To evaluate possible impact on Caco-2 viability due to *C. jejuni* 81-176, LDH levels were measured in the media (Fig. 3). LDH did not increase significantly as a response to *C. jejuni* infection, but there was an overall increase in LDH level in TW at both 24 and 48 hours of infection compared to non-infected cell cultures. Interestingly, the levels of LDH in media from TW cultures were 4-8-fold higher than microfluidic cell culture media. Changes in TEER values for Caco-2 cells as a response to infection were measured for TW cultures, and showed that the non-infected cell layer TEER increased steadily from day 5 to day 7 for non-infected control cells (916 ± 253 at day 5, $1,052 \pm 364$ at day 6, and $1,232 \pm 402$ at day 7). For Caco-2 cells in TW the TEER decreased after infection to 782 ± 264 and 772 ± 370 after 24 and 48 hours of *C. jejuni* 81-176 infection, respectively.

For TW cultures the total association and gentamicin protection assay were used to determine cellular association and invasion of *C. jejuni*. The association of *C. jejuni* 81-176 to Caco-2 cells in TW culture were 2.9×10^8 CFU/mL and the invasion was 3.5×10^4 CFU/mL. Under fluid flow conditions, the *C. jejuni* is viable, but not culturable, which was previously reported by Ica et al., 2012 (Ica et al., 2012). Ica and co-workers found that *C. jejuni* monoculture under fluid flow maintained viability, but not culturability, however, when co-cultured with *Pseudomonas aeruginosa*, *C. jejuni* regained its culturability (Ica et al., 2012). In the study presented here viability of *C. jejuni* in μ F cultures were established using live/dead stain and darkfield microscopy. The responses in Caco-2 cell physiology to *C. jejuni* 81-176 were studied using fluorescence microscopy, and revealed a separation of tight junctions between neighboring cells, and formations of 'holes' in the brush border in microfluidic cultures (Fig. 4). These 'holes' were also observed in TW cultures, but the separation of tight junctions were not possible to study, since the tight junction formation was not well developed at day 7 in the TW culturing system.

Metabolomics analyses were conducted on media collected from non-infected and *C. jejuni* 81-176 infected μ F and TW Caco-2 cell cultures (Fig. 5). The metabolomics profiles of the media from the non-infected and infected cells from the μ F could be differentiated (Fig. 5a). The non-infected media samples cluster more tightly than the media from the infected samples, consistent with expected variability introduced by exposure to *C. jejuni* 81-176, and the variation of bacterial count observed from the infected TW. In contrast, metabolomics could not be used to differentiate the infected and non-infected cells from TW devices due to a high degree of variability (Fig. 5b). The metabolites identified in the media on the μ F cell cultures that best distinguish non-infected and infected cell cultures include; 2-hydroxy-3-methylvalerate, 3-methyl-2-oxocalerate, acetate, fatty acids, glucose, glutamate, isoleucine, leucine, malonate, methionine, N-acetylaspartate, phenylalanine, pyroglutamate, pyruvate, t-methylhistidine (Table 2). The metabolic pathways most perturbed after 48 hours of infection by *C. jejuni* 81-176 in μ F cultures were leucine, isoleucine and valine metabolism ($-\log(p\text{-value}) > 5.5$, involving the metabolites leucine, isoleucine, and valine), and glycolysis and gluconeogenesis ($-\log(p\text{-value}) > 4.5$, involving the metabolites pyruvate and glucose). Metabolites identified in the cell culture media could originate from both mammalian cells and bacteria, and when comparing metabolomics

analysis between non-infected and infected cultures, would be expected to contribute to the separation of samples.

Metabolomics analysis of cell media collected from the *C. jejuni* 81-176 infected and non-infected μ F clearly differentiated the media, demonstrating that *C. jejuni* 81-176 infection in μ F impacts branched-chain amino acid metabolism, glycolysis, and gluconeogenesis. While there was not an observable separation between non-infected and infected cell cultures metabolites in TW, good separation was found between infected μ F and infected TW cultures. This could indicate that Caco-2 cellular response to the presence of a pathogen is different based on the culturing conditions, and that the metabolism of the *C. jejuni* is affected by the fluid flow as well as it was shown here how the metabolism of Caco-2 cells are.

C. jejuni cannot catabolize glucose and other hexose sugars because they lack transporters and the key glycolytic enzyme 6-phosphofructokinase (Parkhill et al., 2000). The main substrates for *C. jejuni* are amino acids (Wright et al., 2009, Liu et al., 2012, Howlett et al., 2014), as reviewed by Stahl et al (Stahl et al., 2012). The preferred amino acids for *C. jejuni* in a sequential manner, are serine, aspartate, asparagine, and glutamate, although this might vary between strains (Stahl et al., 2012). *C. jejuni* 81-176 has been shown to exhibit expanded ability to utilize amino acids compared to the other model strain *C. jejuni* 11168 (Hofreuter et al., 2008). Studies of *C. jejuni* 11168 show a substrate switch at stationary growth phase, and an acetate switch mechanism whereby bacteria changes from acetate excretion to acetate uptake (Wright et al., 2009). These reported studies in the literature regarding *C. jejuni* metabolism, suggest that the two most perturbed pathways found here between the non-infected and infected μ F devices (leucine, isoleucine and valine metabolism, and glycolysis and gluconeogenesis) is due to differences in Caco-2 response to *C. jejuni* and not differences exerted on the bacterial metabolism. The short chain fatty acid, acetate, is initially produced and secreted as a byproduct in several of *C. jejuni*'s primary metabolic pathways, but in later stages of growth acetate can also be used as a carbon source by the bacteria if more preferable substrates are exhausted (Stahl et al., 2012). In this study acetate levels were increased between non-infected and *C. jejuni* 81-176 infected cultures which could be due to normal bacterial metabolism.

4 Conclusion

In summary the study presented here demonstrates that fluid dynamics has an important impact on cell physiology, enabling more biologically relevant investigations *in vitro*. Microfluidic cell culture models using Caco-2 cells were shown to have uniform and defined brush borders, tight junctions, and mucin layers, making them suitable for the study of host-pathogen interactions. Because of fluid flow, the use μ F also enables more biologically relevant investigation of host-pathogen interactions *in vitro*. Metabolomics analysis further demonstrates that the μ F cell culture had a more homogenous metabolism, and the fluid dynamics affects the aminoacyl-tRNA biosynthesis in both mitochondria and cytoplasm of the cells. The study also demonstrates the potential utility of combining μ F and metabolomics analysis in the field of infectious disease research.

Acknowledgements

This study was supported by RTI International internal research funding and National Institute of Diabetes and Digestive and Kidney Diseases (1U24DK097193-01, Dr. Susan Sumner, PI). The authors are grateful to Andrew Novokhatny and Zachery Acuff for providing analytical and statistical assistance with the metabolomics data analysis. The metabolomics data are available at the NIH Common Fund Metabolomics Data Repository and Coordinating Center at the University of California at San Diego (Dr. Shankar Subramaniam, PI) under studies ST000124 and ST000125.

References

- Ajene AN, Fischer Walker CL, Black RE. Enteric pathogens and reactive arthritis: a systematic review of *Campylobacter*, salmonella and *Shigella*-associated reactive arthritis. *J Health Popul Nutr*. 2013; 31:299–307. [PubMed: 24288942]
- Baudoin R, Griscom L, Monge M, Legallais C, Leclerc E. Development of a renal microchip for in vitro distal tubule models. *Biotechnol Prog*. 2007; 23:1245–53. [PubMed: 17725364]
- Black RE, Levine MM, Clements ML, Hughes TP, Blaser MJ. Experimental *Campylobacter jejuni* infection in humans. *J Infect Dis*. 1988; 157:472–9. [PubMed: 3343522]
- Cdc.. *Campylobacter jejuni* - Pathogens & Protocols. 2013. [Online]. <http://www.cdc.gov/pulsenet/pathogens/campylobacter.html>: Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED)
- Chan EC, Pasikanti KK, Nicholson JK. Global urinary metabolic profiling procedures using gas chromatography-mass spectrometry. *Nat Protoc*. 2011; 6:1483–99. [PubMed: 21959233]
- Chi M, Yi B, Oh S, Park DJ, Sung JH, Park S. A microfluidic cell culture device (muFCCD) to culture epithelial cells with physiological and morphological properties that mimic those of the human intestine. *Biomed Microdevices*. 2015; 17:9966. [PubMed: 26002774]
- Cianferoni A. Food Allergy. *Curr Pharm Des*. 2013
- Elsinghorst EA. Measurement of invasion by gentamicin resistance. *Methods Enzymol*. 1994; 236:405–20. [PubMed: 7968625]
- Esch MB, King TL, Shuler ML. The role of body-on-a-chip devices in drug and toxicity studies. *Annu Rev Biomed Eng*. 2011; 13:55–72. [PubMed: 21513459]
- Esch MB, Mahler GJ, Stokol T, Shuler ML. Body-on-a-chip simulation with gastrointestinal tract and liver tissues suggests that ingested nanoparticles have the potential to cause liver injury. *Lab Chip*. 2014; 14:3081–92. [PubMed: 24970651]
- Friis LM, Pin C, Pearson BM, Wells JM. In vitro cell culture methods for investigating *Campylobacter* invasion mechanisms. *J Microbiol Methods*. 2005; 61:145–60. [PubMed: 15722140]
- Hannu T, Kauppi M, Tuomala M, Laaksonen I, Klemets P, Kuusi M. Reactive arthritis following an outbreak of *Campylobacter jejuni* infection. *J Rheumatol*. 2004; 31:528–30. [PubMed: 14994400]
- Hannu T, Mattila L, Rautelin H, Pelkonen P, Lahdenne P, Siitonen A, Leirisalo-Repo M. *Campylobacter*-triggered reactive arthritis: a population-based study. *Rheumatology (Oxford)*. 2002; 41:312–8. [PubMed: 11934969]
- Hill Gaston JS, Lillicrap MS. Arthritis associated with enteric infection. *Best Pract Res Clin Rheumatol*. 2003; 17:219–39. [PubMed: 12787523]
- Hofreuter D, Novik V, Galan JE. Metabolic diversity in *Campylobacter jejuni* enhances specific tissue colonization. *Cell Host Microbe*. 2008; 4:425–33. [PubMed: 18996343]
- Howlett RM, Davey MP, Paul Quick W, Kelly DJ. Metabolomic analysis of the food-borne pathogen : application of direct injection mass spectrometry for mutant characterisation. *Metabolomics*. 2014; 10:887–896. [PubMed: 25177231]
- Hu L, Kopecko DJ. *Campylobacter jejuni* 81-176 associates with microtubules and dynein during invasion of human intestinal cells. *Infect Immun*. 1999; 67:4171–82. [PubMed: 10417189]
- Huh D, Kim HJ, Fraser JP, Shea DE, Khan M, Bahinski A, Hamilton GA, Ingber DE. Microfabrication of human organs-on-chips. *Nat Protoc*. 2013; 8:2135–57. [PubMed: 24113786]

- Huh D, Leslie DC, Matthews BD, Fraser JP, Jurek S, Hamilton GA, Thorneloe KS, McAlexander MA, Ingber DE. A human disease model of drug toxicity-induced pulmonary edema in a lung-on-a-chip microdevice. *Sci Transl Med*. 2012; 4:159ra147.
- Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting organ-level lung functions on a chip. *Science*. 2010; 328:1662–8. [PubMed: 20576885]
- Ibba M, Soll D. Aminoacyl-tRNA Synthesis. *Annu Rev Biochem*. 2000; 69:34.
- Ica T, Caner V, Istanbulu O, Nguyen HD, Ahmed B, Call DR, Beyenal H. Characterization of mono- and mixed-culture *Campylobacter jejuni* biofilms. *Appl Environ Microbiol*. 2012; 78:1033–8. [PubMed: 22179238]
- Jackson BR, Zegar JA, Lopez-Gatell H, Sejvar J, Arzate F, Waterman S, Nunez AS, Lopez B, Weiss J, Cruz RQ, Murrieta DY, Luna-Gierke R, Heiman K, Vieira AR, Fitzgerald C, Kwan P, Zarate-Bermudez M, Talkington D, Hill VR, Mahon B. Binational outbreak of Guillain-Barre syndrome associated with *Campylobacter jejuni* infection, Mexico and USA, 2011. *Epidemiol Infect*. 2013;1–11.
- Jang KJ, Mehr AP, Hamilton GA, Mcpartlin LA, Chung S, Suh KY, Ingber DE. Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment. *Integr Biol (Camb)*. 2013; 5:1119–29. [PubMed: 23644926]
- Javanmard M, Babrzadeh F, Davis RW. Microfluidic force spectroscopy for characterization of biomolecular interactions with piconewton resolution. *Appl Phys Lett*. 2010; 97:173704. [PubMed: 21103190]
- Jung J, Jung Y, Gill B, Kim C, Hwang KJ, Ju YR, Lee HJ, Chu H, Hwang GS. Metabolic responses to *Orientia tsutsugamushi* infection in a mouse model. *PLoS Negl Trop Dis*. 2015; 9:e3427. [PubMed: 25569562]
- Kang YB, Sodunke TR, Lamontagne J, Cirillo J, Rajiv C, Bouchard MJ, Noh M. Liver sinusoid on a chip: Long-term layered co-culture of primary rat hepatocytes and endothelial cells in microfluidic platforms. *Biotechnol Bioeng*. 2015
- Kim HJ, Huh D, Hamilton G, Ingber DE. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip*. 2012; 12:2165–74. [PubMed: 22434367]
- Kim HJ, Ingber DE. Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus differentiation. *Integr Biol (Camb)*. 2013; 5:1130–40. [PubMed: 23817533]
- Korlath JA, Osterholm MT, Judy LA, Forfang JC, Robinson RA. A point-source outbreak of campylobacteriosis associated with consumption of raw milk. *J Infect Dis*. 1985; 152:592–6. [PubMed: 4031557]
- Lee JS, Romero R, Han YM, Kim HC, Kim CJ, Hong JS, Huh D. Placenta-on-a-chip: a novel platform to study the biology of the human placenta. *J Matern Fetal Neonatal Med*. 2015:1–9. [PubMed: 26365622]
- Ling J, Reynolds N, Ibba M. Aminoacyl-tRNA synthesis and translational quality control. *Annu Rev Microbiol*. 2009; 63:61–78. [PubMed: 19379069]
- Liu X, Gao B, Novik V, Galan JE. Quantitative Proteomics of Intracellular *Campylobacter jejuni* Reveals Metabolic Reprogramming. *PLoS Pathog*. 2012; 8:e1002562. [PubMed: 22412372]
- Lobritz MA, Belenky P, Porter CB, Gutierrez A, Yang JH, Schwarz EG, Dwyer DJ, Khalil AS, Collins JJ. Antibiotic efficacy is linked to bacterial cellular respiration. *Proc Natl Acad Sci U S A*. 2015; 112:8173–80. [PubMed: 26100898]
- Locht H, Krogfelt KA. Comparison of rheumatological and gastrointestinal symptoms after infection with *Campylobacter jejuni/coli* and enterotoxigenic *Escherichia coli*. *Ann Rheum Dis*. 2002; 61:448–52. [PubMed: 11959770]
- Lu H, Koo LY, Wang WM, Lauffenburger DA, Griffith LG, Jensen KF. Microfluidic Shear Devices for Quantitative Analysis of Cell Adhesion. *Anal. Chem*. 2004; 76:5257–5264. [PubMed: 15362881]
- Mack PJ, Kaazempur-Mofrad MR, Karcher H, Lee RT, Kamm RD. Force-induced focal adhesion translocation: effects of force amplitude and frequency. *Am J Physiol Cell Physiol*. 2004; 287:C954–C962. [PubMed: 15189816]

- Mahler GJ, Esch MB, Glahn RP, Shuler ML. Characterization of a gastrointestinal tract microscale cell culture analog used to predict drug toxicity. *Biotechnol Bioeng.* 2009; 104:193–205. [PubMed: 19418562]
- Mahler GJ, Esch MB, Tako E, Southard TL, Archer SD, Glahn RP, Shuler ML. Oral exposure to polystyrene nanoparticles affects iron absorption. *Nat Nanotechnol.* 2012; 7:264–71. [PubMed: 22327877]
- Maschmeyer I, Lorenz AK, Schimek K, Hasenberg T, Ramme AP, Hubner J, Lindner M, Drewell C, Bauer S, Thomas A, Sambo NS, Sonntag F, Lauster R, Marx U. A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. *Lab Chip.* 2015; 15:2688–99. [PubMed: 25996126]
- Mcgarvey PB, Huang H, Mazumder R, Zhang J, Chen Y, Zhang C, Cammer S, Will R, Odle M, Sobral B, Moore M, Wu CH. Systems integration of biodefense omics data for analysis of pathogen-host interactions and identification of potential targets. *PLoS One.* 2009; 4:e7162. [PubMed: 19779614]
- Mills DC, Gundogdu O, Elmi A, Bajaj-Elliott M, Taylor PW, Wren BW, Dorrell N. Increase in *Campylobacter jejuni* invasion of intestinal epithelial cells under low-oxygen coculture conditions that reflect the in vivo environment. *Infect Immun.* 2012; 80:1690–8. [PubMed: 22354027]
- Milner JJ, Rebeles J, Dhungana S, Stewart DA, Sumner SC, Meyers MH, Mancuso P, Beck MA. Obesity Increases Mortality and Modulates the Lung Metabolome during Pandemic H1N1 Influenza Virus Infection in Mice. *J Immunol.* 2015
- Mok J, Mindrinos MN, Davis RW, Javanmard M. Digital microfluidic assay for protein detection. *PNAS.* 2014; 111:2110–2115. [PubMed: 24449893]
- Newell DG. Animal models of *Campylobacter jejuni* colonization and disease and the lessons to be learned from similar *Helicobacter pylori* models. *Symp Ser Soc Appl Microbiol.* 2001:57S–67S.
- Nyati KK, Nyati R. Role of *Campylobacter jejuni* infection in the pathogenesis of Guillain-Barre syndrome: an update. *Biomed Res Int.* 2013; 2013:852195. [PubMed: 24000328]
- Ouattara DA, Prot JM, Bunescu A, Dumas ME, Elena-Herrmann B, Leclerc E, Brochot C. Metabolomics-on-a-chip and metabolic flux analysis for label-free modeling of the internal metabolism of HepG2/C3A cells. *Mol Biosyst.* 2012; 8:1908–20. [PubMed: 22618574]
- Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Chillingworth T, Davies RM, Feltwell T, Holroyd S, Jagels K, Karlyshev AV, Moule S, Pallen MJ, Penn CW, Quail MA, Rajandream MA, Rutherford KM, Van Vliet AH, Whitehead S, Barrell BG. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature.* 2000; 403:665–8. [PubMed: 10688204]
- Pathmasiri W, Pratt K, Collier D, Lutes L, Mcritchie S, Sumner S. Integrating metabolomic signatures and psychosocial parameters in responsivity to an immersion treatment model for adolescent obesity. *Metabolomics.* 2012; 8:1037–1051.
- Prot JM, Aninat C, Griscom L, Razan F, Brochot C, Guillouzo CG, Legallais C, Corlu A, Leclerc E. Improvement of HepG2/C3a cell functions in a microfluidic biochip. *Biotechnol Bioeng.* 2011; 108:1704–15. [PubMed: 21337338]
- Prot JM, Bunescu A, Elena-Herrmann B, Aninat C, Snouber LC, Griscom L, Razan F, Bois FY, Legallais C, Brochot C, Corlu A, Dumas ME, Leclerc E. Predictive toxicology using systemic biology and liver microfluidic "on chip" approaches: application to acetaminophen injury. *Toxicol Appl Pharmacol.* 2012; 259:270–80. [PubMed: 22230336]
- Ramadan Q, Jafarpoorchekab H, Huang C, Silacci P, Carrara S, Koklu G, Ghaye J, Ramsden J, Ruffert C, Vergeres G, Gijss MA. NutriChip: nutrition analysis meets microfluidics. *Lab Chip.* 2013; 13:196–203. [PubMed: 23184124]
- Sanchez EL, Lagunoff M. Viral activation of cellular metabolism. *Virology.* 2015; 479-480:609–18. [PubMed: 25812764]
- Sellgren KL, Butala EJ, Gilmour BP, Randell SH, Grego S. A biomimetic multicellular model of the airways using primary human cells. *Lab Chip.* 2014; 14:3349–58. [PubMed: 25000964]
- Shintu L, Baudoin R, Navratil V, Prot JM, Pontoizeau C, Defernez M, Blaise BJ, Domange C, Pery AR, Toulhoat P, Legallais C, Brochot C, Leclerc E, Dumas ME. Metabolomics-on-a-chip and predictive systems toxicology in microfluidic bioartificial organs. *Anal Chem.* 2012; 84:1840–8. [PubMed: 22242722]

- Snyder RW, Fennell TR, Wingard CJ, Mortensen NP, Holland NA, Shannahan JH, Pathmasiri W, Lewin AH, Sumner SCJ. Distribution and Biomarker of Carbon-14 Labeled Fullerene C60 ([¹⁴C(U)]C60) in Pregnant and Lactating Rats and their Offspring after Maternal Intravenous Exposure. *Journal of Applied Toxicology*. 2015 In press.
- Stahl M, Butcher J, Stintzi A. Nutrient acquisition and metabolism by *Campylobacter jejuni*. *Front Cell Infect Microbiol*. 2012; 2:5. [PubMed: 22919597]
- Sumner S, Snyder R, Burgess J, Myers C, Tyl R, Sloan C, Fennell T. Metabolomics in the assessment of chemical-induced reproductive and developmental outcomes using non-invasive biological fluids: application to the study of butylbenzyl phthalate. *J Appl Toxicol*. 2009; 29:703–14. [PubMed: 19731247]
- Sumner SCJ, Fennell TR, Snyder RW, Taylor GF, Lewin AH. Distribution of carbon-14 labeled C60 ([¹⁴C]C60) in the pregnant and in the lactating dam and the effect of C60 exposure on the biochemical profile of urine. *J. Appl. Toxicol*. 2010a; 30:354–360. [PubMed: 20063269]
- Sumner SJ, Burgess JP, Snyder RW, Popp JA, Fennell TR. Metabolomics of urine for the assessment of microvesicular lipid accumulation in the liver following isoniazid exposure. *Metabolomics*. 2010b; 6:238–249. [PubMed: 21057652]
- Sung JH, Yu J, Luo D, Shuler ML, March JC. Microscale 3-D hydrogel scaffold for biomimetic gastrointestinal (GI) tract model. *Lab Chip*. 2011; 11:389–92. [PubMed: 21157619]
- Tuuminen T, Lounamo K, Leirisalo-Repo M. A Review of Serological Tests to Assist Diagnosis of Reactive Arthritis: Critical Appraisal on Methodologies. *Front Immunol*. 2013; 4:418. [PubMed: 24363655]
- Villar M, Ayllon N, Alberdi P, Moreno A, Moreno M, Tobes R, Mateos-Hernandez L, Weisheit S, Bell-Sakyi L, De La Fuente J. Integrated metabolomics, transcriptomics and proteomics identifies metabolic pathways affected by *Anaplasma phagocytophilum* infection in tick cells. *Mol Cell Proteomics*. 2015
- Who. The Global View of Campylobacteriosis - Report of Expert Consultation - Utrecht, Netherlands, 9-11 July 2012. WHO Document Production Services; Geneva, Switzerland: 2013.
- Wright JA, Grant AJ, Hurd D, Harrison M, Guccione EJ, Kelly DJ, Maskell DJ. Metabolite and transcriptome analysis of *Campylobacter jejuni* in vitro growth reveals a stationary-phase physiological switch. *Microbiology*. 2009; 155:80–94. [PubMed: 19118349]
- Zhang MY, Lee PJ, Hung PJ, Johnson T, Lee LP, Mofrad MR. Microfluidic environment for high density hepatocyte culture. *Biomed Microdevices*. 2008; 10:117–21. [PubMed: 17682945]

Fig. 1.

Comparison of brush boarder (a-d), tight junctions (ZO-1) (e-h), mucin 2 (MUC2) (i-l) expression, and in Caco-2 cells grown in TW (a, b, e, f, i and i) or μF (c, d, g, h, k, and l) conditions at 5 days. (m) showed the difference in intensity of MUC2 between μF (black line) and TW (gray line) which shows the higher intensity of MUC2 staining in μF at 5 days.

Fig. 2. Metabolomics analysis of growth media from μ F (blue) and TW (green) showed a good clustering of μ F, but not TW in both a) unsupervised (PCA) and b) supervised (OPLS-DA) multivariate analyses. Each media sample is defined by a colored circle.

Fig. 3.

LDH released from Caco-2 cells cultured in μ F and TW devices. LDH release at 24 and 48 hours of *C. jejuni* 81-176 infection.

Fig. 4.

Caco-2 cells in μ F (a-b) non-infected and (c-d) infected with *C. jejuni* 81-176, showing that (c) 81-176 disrupted tight junctions, white arrows, and (d) created irregularities or 'holes' in the brush border as indicated with white arrows.

Fig. 5.

Metabolomics analysis on cell culture media from non-infected or *C. jejuni* 81-176 infected Caco-2 cells grown in μ F or TW. Supervised OPLS-DA plot showed good separation between non-infected and infected in (a) μ F (non-infected in red, and *C. jejuni* infected in yellow), but not in (b) TW (non-infected in blue, and *C. jejuni* infected in green). Each media sample is defined by a colored circle.

Table 1

Library matched metabolites important in distinguishing Caco-2 cell media in μ F from Caco-2 cell media in TW.

Caco-2 cells: μ F vs TW	VIP Range	P-Value Range*	Fold Change Range**
Alanine	4.2	0.004	(-1.3)
Glucose (2 bins)	2.4 - 2.8	0.052 - 0.082	0
Glutamate	5.3	0.126	1.6
Isoleucine, Valine	2.6	0.052	(-1.1)
Leucine, Isoleucine, Valine, 2-Hydroxy-3-methylvalerate	5.2	0.004	(-1.2)
Lysine	3.2	0.004	1.2
Pyroglutamate	1.1	0.052	1.1
Pyroglutamate, Glutamate, N-Acetylaspartate	2.3	0.052	(-1.1)
Tyrosine	3.5	0.329	(-1.1)
Unknown (6 bins)	1.0 - 2.3	0.052 - 0.762	(-1.0) - 1.7
Valine	1.5	0.052	(-1.1)

* Exact Wilcoxon Rank Sum Test.

** Positive fold change means the peak intensity of μ F is higher than TW.

Table 2

Library matched metabolites important to distinguishing media from *C. jejuni* 81-176 infected and non-infected Caco-2 cells grown in μ F.

μ F: <i>C. jejuni</i> 81-176 infected vs non-infected	VIP Range	P-Value Range *	Fold Change Range **
Acetate	2.8	<.001	1.7
Fatty acids	1.0	0.087	(-1.2)
Glucose	2.1	0.012	1.1
Glutamate	6.1	<.001	(-1.6)
Glutamate, Pyruvate	6.4	<.001	2.6
Isoleucine	1.0	0.036	(-1.1)
Leucine	5.1	<.001	(-2.6)
Leucine, 3-Methyl-2-oxovalerate	1.4	<.001	(-1.2)
Leucine, Isoleucine, Valine, 2-Hydroxy-3-methylvalerate	1.5	0.029	(-1.0)
Malonate, phenylalanine	1.2	0.029	1.3
Methionine, Glutamate	3.2	<.001	1.3
Pyroglutamate, Glutamate, N-Acetylaspartate	3.1	<.001	1.1
Unknown (3 bins)	1.0 - 1.3	0.005 - 0.072	(-1.9) - (-1.1)
Unknown, pyroglutamate	6.8	<.001	(-1.8)
t-Methylhistidine (2 bins)	1.4 - 1.7	0.002 - 0.009	(-2.3) - (-1.3)

* Exact Wilcoxon Rank Sum Test.

** Positive fold change means the peak intensity of the *C. jejuni* 81-176 infected is higher than non-infected Caco-2 cells.