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## **PDMS Flow Cell for Monitoring Bacterial Adhesion Capacity of *Escherichia coli* O157:H7 in Beverages**

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Author AA designed the biological aspects, wrote the protocol, conduct experimental work, performed the statistical analysis and wrote the final draft of the manuscript. Author DMLM designed the engineering aspects for the PDMS fabrication and fluid flow system. Authors AA and DMLM jointly collaborated in editing and reviewing experiments.*

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### **ABSTRACT**

**Aims:** To develop and standardize a polydimethylsiloxane (PDMS) flow cells for monitoring bacterial adhesion capacity of biofilm formation by *Escherichia coli* O157:H7 in Beverages industry.

**Study Design:** PDMS chip was fabricated in house and placed in a metal chamber. The bio-Ferrograph generated different flow rates of bacterial cell suspension in the PDMS cells.

**Methodology:** PDMS flow cells were used to monitor bacteria adhesion capacity of *E. coli* O157:H7 inoculated into some beverages. The Effect of fluid flow rate and temperature on bacteria adhesion capacity was studied in order to standardize the system. Buffer system of adhesion was modified by varying the concentrations of PBS, Saline concentrations and PH value. The impact of elapsing time and initial number of bacterial cells were investigated. Fluorescence imaging of biofilm formation was also captured.

**Results:** Bacterial adhesion capacity reached the maximum at 0.1 ml/min and then dramatically dropped down when fluid flow rate increases. Maximized adhesion capacity occurred with a buffer

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system of 0.01M Phosphate buffer, 1.0% NaCl, pH 7.5 at 30°C. A complete linear relationship ( $R^2$ ; 0.9956 - 0.9815) occurred between adhesion capacity of *E. coli* O157:H7 cells and elapsing time of food beverage. This linear relationship would help to predict and study biofilm formation in fluid and beverage industry. Maximum adhesion capacity occurred with beverages at the following order: skim milk followed by apple juice and then grape juice.

**Conclusion:** PDMS flow cell enables non-destructive, in situ investigation of bacteria adhesion capacity as an initial step for biofilm formation in real time under a wide range of flow rates, nutrient conditions, fluid temperature, and elapsing times. It is inexpensive, simple, disposable, easy-to-use, and can accurately mimic the dynamic flow conditions in beverage industry.

**Keywords:** Bacterial adhesion Capacity; biofilm-forming Bacteria; polydimethylsiloxane (PDMS); flow cell; *E. coli* O157:H7; Beverages; Bio-Ferrograph.

## 1. INTRODUCTION

Biofilm provide good environment to hides and protects pathogens. Bacteria attached and form biofilm are often resistant to disinfection and responsible for the contamination of industrial processes including fluid transport, wastewater management, food industry, and medical implants [1,2]. Poor sanitation of food contact surfaces, equipment, and processing environments has contributed to spread bacterial biofilms, which may contain pathogenic microorganisms. Many types of foodborne disease outbreaks are associated with biofilm especially those involving *Listeria monocytogenes* [3-5], *Salmonella* [6,4], and *E. coli* O157:H7 [7,8]. Produce-associated outbreaks were first reported in 1991 [9] including apple juice, milk, and grape juice. They have remained a prominent food vehicle, accounting for 34% of foodborne outbreak-related cases [9-14]. In 2012, unpasteurized apple juice has caused *E. coli* outbreaks in Michigan

(<https://foodpoisoningbulletin.com/2012/michigan-e-coli-cases-linked-to-unpasteurized-cider/>).

Another outbreak occurred in Canada with *E. coli* infections linked to the unpasteurized beverage (<https://foodpoisoningbulletin.com/2014/e-coli-illnesses-prompt-recall-of-unpasteurized-apple-cider-in-canada/>). Lately, outbreaks associated with unpasteurized milk has significantly increased in the United States during the period; 2007–2012 [15].

Centers for Disease Control and Prevention (CDC) estimate that 65% of human bacterial infections involve biofilms [16]. Small numbers of surviving organisms can regrow, damaging beverage and dairy products. This might destroy a company's reputation in the event of a product recall due to negative health outcomes [17]. In beverages industry, biofilm forms at different

surfaces specially the inner surface of pipelines. Biofilm consists of both microbes and their extracellular polymeric substances (EPS), usually polysaccharides [18]. EPS facilitate the initial attachment of bacterial cells to the surface, maintain biofilm structure, and enhance biofilm resistance to anti-microbial agents [19]. Adhesion of bacterial cells is a time dependent process and divided into two phases: the reversible and the irreversible [19]. Microorganisms first bind reversibly to the organic matter and then irreversible with their flagella and fimbriae. Adhesion of bacterial cells to a surface is influenced by the following factors: pH, temperature, cell hydrophobicity, cell surface charge, motility, cell structure including EPS and flagella, ionic concentration, the growth phase and nutrient content [20].

Biofilm formation in tubes and packaging material used in the food industry and others have become of great interest. Available methods for measuring bacterial adhesion and biofilm formation are important tools in monitoring foodborne pathogens in beverage and medical industry. Measurement of bacterial adhesion and biofilm formation is however, not straightforward. The reason behind that is the difficulty to generate and maintain a fluid flow environment along with controlling growth conditions. Microtiter plates based methods have become the routine assay for biofilm forming bacterial in medical field and food industry [21]. However, they are vulnerable and inaccurate for quantitative evaluation of bacterial adhesion and surface colonization [22]. Microtiter plates based methods do not provide an accurate mimicking of the dynamic flow situation and they lack control over experimental conditions. Therefore, difficult to compare between groups. Biofilm formation under dynamic fluid flow conditions [23] and the rheological behavior of biofilms have become of great interest [24]. There are disadvantages of

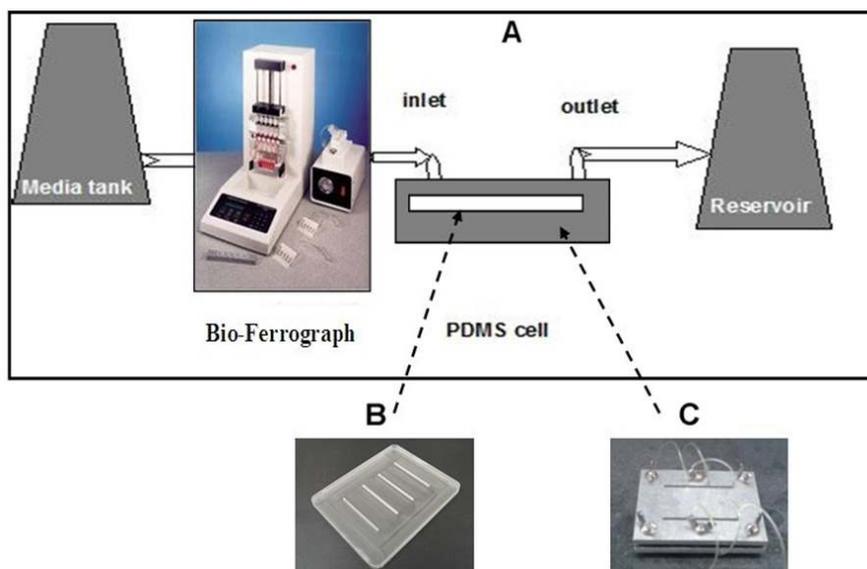
using available fluid flow devices; to list a few: a- materials incorporated into flow cells cannot be autoclaved, b- left over residual debris of previous experiments often causes contamination [25], c- destructive methods such as sonication are often required to remove multicellular structures of biofilm.

In our previous work, quantification of biofilms was successfully measured under static conditions for different foodborne pathogens; *Salmonella enteritidis*, *Listeria monocytogenes*, and *E. coli* O157:H7 using PDMS chip biofilm assay [26]. Abolmaaty and Meyer [26] suggested that the large surface area available and the hydrophobic characteristic of the PDMS internal surfaces allowed the adsorption of hydrophobic targets such as bacterial cells and uniformly distribute them along the larger surface area of the channels. It was observed that the adhesion of microorganisms to PDMS channel was dramatically higher than microliter plates when nutrient in their surroundings become limited [20, 26]. The objective of this research was to standardize a Polydimethylsiloxane (PDMS) mini-fluidic chip assay and to evaluate cell adhesion, in which the device can be easily used as an inexpensive flow cell for quantitative monitoring and studying biofilm stages in beverage industry. *E. coli* O157:H7 was used for this study as a model of biofilm forming bacteria.

## 2. MATERIALS AND METHODS

### 2.1 Fabrication of PDMS Chip

The PDMS flow cell was fabricated from polydimethylsiloxane (PDMS) with the use of reusable master mold as described in our previous work [26]. The mold was fabricated with the aid of photo mask designed at the machine shop, Mechanical Engineering, University of Rhode Island. Polydimethylsiloxane (PDMS) was mixed thoroughly with a silicone elastomer curing agent (10:1 w/w) and poured onto mold. The PDMS was degassed in a vacuum for five min and then the chamber was vented. The PDMS was baked at 90°C for 45 min. After curing, the PDMS chip was peeled off of the mold and then a biopsy punch was used to make inlets and outlets. A metal chamber consisting of an upper and lower plate was custom made at the machine shop. Each plate contained holes for the screws in allowing the PDMS chip to be temporarily sealed with a removable glass plate. The upper plate of the chamber was designed with two openings allowing access to the inlets and outlets of the chip (Fig.1.c). Fig. 2 illustrates the schematic diagram of the PDMS flow cell showing the passing of cell suspension from media tank via bio-Ferrograph ( Fig. 1.a) to the inlet of PDMS flow cell and then to the outlet reservoir tank.



**Fig. 1. PDMS cell flow system**

Schematic diagram of the PDMS flow cell showing the passing of cell suspension from media tank via bio-Ferrograph to the inlet of PDMS flow cell (B) covered with sterile glass plate and then placed in the metal chamber (C) and then to the outlet reservoir tank.

## 2.2 Microorganism and Routine Cultivation

*Escherichia coli* O157:H7 strain C9490 was obtained from the Centers for Disease Control, Atlanta GA 30333 and was routinely grown for 12h in 100 ml of Tryptic Soy Broth plus 0.5% dextrose (TSB+) at 37°C in 250 ml baffled flasks with rotary agitation (200 rpm). Cells were harvested by centrifuging broth cultures at 16,000 g for 10 min at 4°C. The cells were then resuspended in phosphate buffered PBS (PBS; 0.005 M phosphate buffer plus 0.05% NaCl, pH 7.4) and diluted in PBS to achieve required cell densities unless otherwise stated. Exponentially growing cells ( $\sim 1 \times 10^9$  cells/ml) were harvested by centrifuging broth cultures at 13,000 g for 10 min at 4°C. Pellets were resuspended in sterile PBS, pelleted again and the concentration of cells was adjusted as required by re-suspending in either PBS or beverages as needed. Cell densities were determined with the use of Tryptic Soy Agar (TSA) plates (Difco, Detroit, Michigan). Plate counts were correlated with absorbance readings at 600 nm using Spectra Max 340.

## 2.3 Effect of Fluid flow Rate on Bacteria Adhesion Capacity

Bacterial adhesion to a surface, the initial step of biofilm formation is of particular interest. PDMS flow cell was prepared as follows: PDMS chip was aseptically washed with ethanol, air dried, covered with sterile glass plate and then placed in the metal chamber as described above. The effect of fluid flow rate on the adhesion of bacterial cells was studied by passing a cell suspension of  $1 \times 10^4$  cells / ml in PBS through the Mini-fluidic chips via the bio-ferrograph (Guilfoyle, Inc., Belmont, MA) at different flow rates (0.004, 0.008, 0.01, 0.04, 0.08, 0.1, 0.2, 0.4, 0.6 ml/min) for a constant passage time of 100 min at room temperature. The bio-ferrograph permitted extremely accurate control of the solutions flow rate through the minichannel. After each passage time was complete, unattached bacterial cells was washed out by passing PBS through the flow cell using bio-Ferrograph for 1 min. PDMS mini-channels were then filled with 200  $\mu$ l of TSB broth and incubated for 12h at 37°C. PDMS chips were made into two groups: first group for CFU measurement using plate counts and the second one for Crystal Violet (CV) staining. Each group was made in triplicates. To ensure that PDMS channels do not dry up during the incubation, they were placed in boxes, designed for 1 ml automatic pipette tips

that are partly filled with 5 ml distilled water. Control samples were conducted in triplicates as described above by adding just 0.2 ml of sterile TSB to the channels. After the incubation periods were complete, the glass plate was removed to open the PDMS chip. Planktonic bacterial medium was removed and PDMS channels were washed three times with 0.2 ml sterile PBS to remove loosely associated bacteria. The biofilm was physically scrapped off using sterile spatula with the aid of hyaluronidase digestive enzyme and then collected into sterile PBS in 1.5 ml Eppendorf tube. Hyaluronidase (MP Biomedicals, LLC, Solon OH) was prepared in PBS buffer at a different concentration (2, 4, 8 mg/ml) and tested for bacterial survivals. A cell suspension of  $1 \times 10^3$  cells/ml were incubated at 37°C for 30 min with 4 mg/ml hyaluronidase. Survivals were determined with the use of Tryptic Soy Agar (TSA) plates. Serial dilutions of biofilm suspensions were prepared in sterile PBS and then 0.1 ml of the dilution was smeared onto TSA plates. Plates were incubated for 12h at 37°C for the determination of CFU. PDMS channels of the second group were air dried for 45 min and each well was stained with 150  $\mu$ l of 1% crystal violet (CV) solution in water for 45 min at room temperature. After staining, channels were washed with sterile distilled water three times. At this point, biofilms were visible as purple layer formed on the side of each channel. The quantitative analysis of biofilm production was performed by adding 220  $\mu$ l of 95% ethanol to destain the biofilm. Two Hundred microliters from each channel was transferred to a new microtiter plate and absorbance of the crystal violet present was measured at 595 nm using Spectra Max 340. All assays were performed in triplicates, means and standard errors were calculated for all repetitions of the experiment.

## 2.4 Effect of Temperature on Bacterial Adhesion Capacity

The driving forces of cell adhesion to the PDMS channels is based on the hydrophobic interaction between bacterial cell wall and the surface chemistry of the inner surface of the minichannels. Temperature might have an impact on the attachment of bacterial cells to the surface. The influences of the temperature during the passage period (100 min) was studied by passing  $1 \times 10^4$  cells/ml through the PDMS minichannels at different temperature (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50°C) using the bio-Ferrograph at the optimum flow rate which was obtained earlier (0.1 ml/min). After each passage

time was completed, PDMS channels were washed with sterile PBS using bio-Ferrograph to remove bacteria loosely associated bacteria. PDMS mini-channels were then filled with 200  $\mu$ l of TSB broth and incubated for 12 h at 37°C. Biofilm was measured using both plate counts and CV staining as described above.

## **2.5 Effect of Buffer System on Cells Adhesion**

Hence, the buffer system might manipulate motility, cell hydrophobicity, ionic concentration, cell surface charge; the buffer system modified by varying the concentrations of PBS, saline concentrations and pH value. *Experiments were conducted as stated earlier but with changing one variable at a time* and tested for bacterial adhesion capacity.

## **2.6 PDMS Flow Cell Application with Some Food Fluids**

Exponential growth of *E. coli* O157:H7 was harvested, washed as described above and adjusted to  $1 \times 10^4$  cells/ml in sterile PBS. Different beverages (skim milk, apple juice, grape juice) were purchased from retail stores, distributed into 250 ml flasks and then subjected to 15 min autoclaving at 121°C. Beverages were then cooled down in ice and inoculated with  $1 \times 10^4$  cells/ml.

### **2.6.1 Effect of elapsing time on cell adhesion**

The effect of elapsed time of beverage flow on the adhesion of bacterial cells to the inner surface of PDMS mini-channels was studied by passing each inoculated beverage at different times intervals (5, 10, 15, 20, 30, 40, 60, 80, 100, 120, 150, and 180 min) using a Bio-Ferrograph at the optimum flow rate obtained earlier. After each passage was complete, PDMS flow cells were filled with 200  $\mu$ l of TSB broth and allowed for biofilm formation as stated above. The PDMS flow chips were divided into two groups: first group for the CFU determination of the biofilm using plate counts and the second group for Crystal Violet (CV) staining as described above.

### **2.6.2 Effect of cell density in food fluids on bacterial adhesion**

The effect of varying cell density on bacterial adhesion to PDMS surface was studied as follows: different concentrations of *E. coli*

O157:H7 prepared in different beverages as mentioned above, ranging from 5 to  $1 \times 10^7$  cells/ml, was introduced to PDMS flow cell using bio-Ferrograph at a flow rate of 0.1 ml/min for a constant passage time of 100 min. Biofilm formation was then conducted for 12h as mentioned above. The PDMS biofilm assay was performed in triplicate, means and standard errors were calculated for all repetitions of the experiments.

### **2.6.3 Fluorescence imaging of biofilm formation**

Food fluids (skim milk, apple juice, grape juice) inoculated with bacterial cells was subjected to PDMS flow cells for 100 min as mentioned above. After the incubation period was complete, the medium was removed from each PDMS channel. PBS buffer containing 4 mg/ml hyaluronidase was introduced to the mini-channels via bio-Ferrograph for 10 min at a flow rate of 0.1 ml/min. This procedure would facilitate antigen antibody reaction with Anti-*Escherichia coli* O157:H7. BacTrace Anti-*Escherichia coli* O157:H7, FITC labeled, was obtained from KPL, Inc. Gaithersburg, Maryland and prepared in PBS buffer. Antibody was diluted to (1-64) and then added to corresponding channel. Channels were incubated for 60 min at 37°C. After the incubation period was complete, channels were emptied from the antibodies and washed three times with sterile distilled water. Channels were examined under Epifluorescence microscope using 20x lens.

## **3. RESULTS AND DISCUSSION**

### **3.1 Effect of Fluid Flow Rate on Cell Adhesion**

Biofilm is initiated when microorganisms first bind reversibly and then irreversible with their flagella and fimbriae. A cell suspension in PBS was used to study the attachment of bacterial cells to PDMS flow cell. Hyaluronidase found to render the biofilm during the scraping process, make it more detachable, and resulting in higher yield of biofilm. When different concentrations of hyaluronidase were studied with scraping biofilm, 4 mg/ml found to be the optimum concentration resulted in the maximum yield of biofilm when incubated at 37°C for 20 min. *E. coli* O157:H7 cells survived in 4 mg/ml of Hyaluronidase in PBS at 37°C for 30 min (data are not shown). Hyaluronidase found to render

tissue more readily permeable to injected fluids [27] and accelerates the passage of antibiotics from the circulation into the synovial fluid. It improves the systemic bioavailability of proteins [28].

The yield of biofilm measured by both CFU counts and CV indicated that maximum cell adhesion was achieved at low flow rates ranging from (0.004 – 0.1 ml/min) for 100 min via bio-Ferrograph (Fig. 2). The attachment of cells was then gradually reduced to reach the minimum at 0.6 ml/min. It has been confirmed that the motility rates would highly influence the initiation of biofilm formation when cells are attached to the surface. Biofilm formation was dramatically reduced three folds when bio-Ferrograph adjustments elevated up from 0.1 to 0.6 ml/ min (Fig. 2).

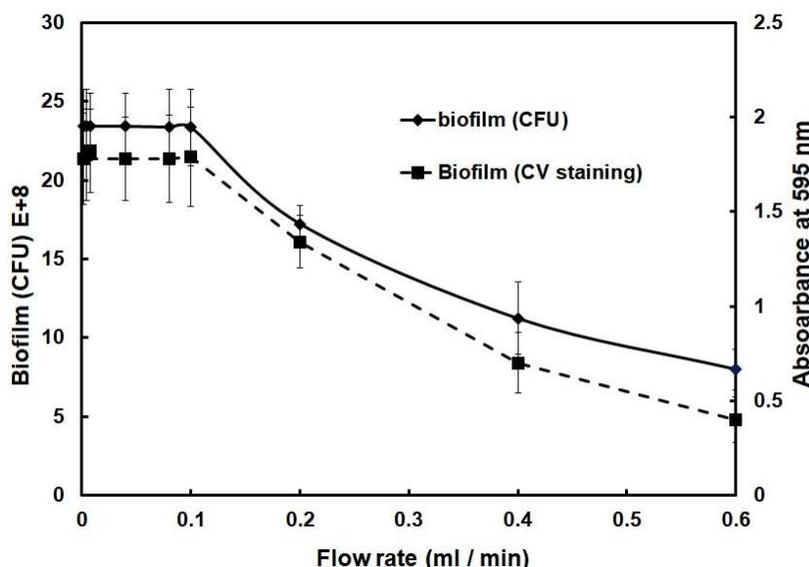
### 3.2 Effect of Temperature on Cells Adhesion

Our data indicated that varying the adhesion temperature had a great impact on adhesion of bacterial cells when passing through PDMS surface at a flow rate of 0.1 ml/min for 100 min via bio-Ferrograph (Fig. 3). A bell shaped curve was generated with the yield of biofilm as a result of different adhesion temperatures of cell

suspension. Both low adhesion temperature (5, 10 and 10°C) and high (45, and 50°C) reported small yield of biofilm. Certainly, high and low adhesion temperature found to notably inhibit adhesion capacity while 25, 30, and 35°C yielded maximum and near maximum rates of adhesion capacity (Fig. 3).

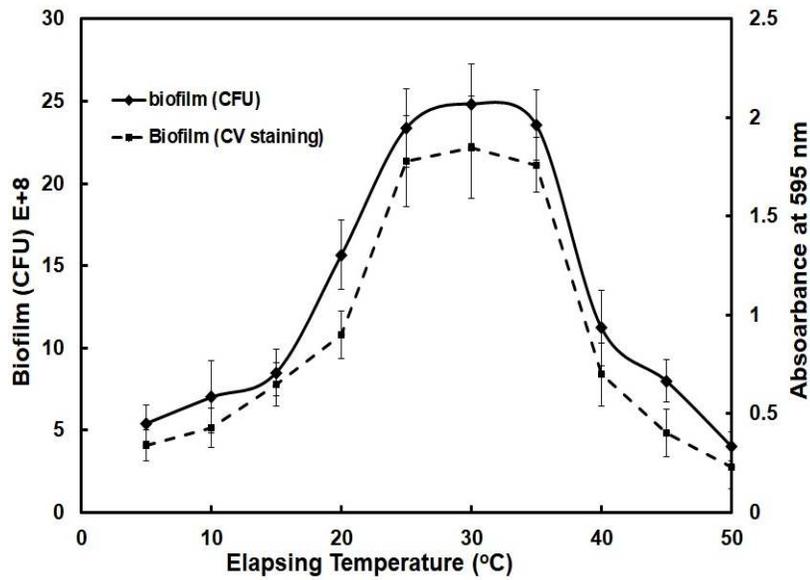
### 3.3 Effect of pH, Phosphate Buffer (PB) and NaCl Concentration on Bacterial Adhesion Capacity

Maximum bacterial adhesion capacity occurred at pH 7.5 when cell suspension was passed through the Mini-fluidic chips via the bio-ferrograph at 0.1 ml/min) for a constant passage time of 100 min at room temperature. The adhesion rates were dramatically dropped down at both sides of the curve reporting no adhesion at pH 4 (Fig. 4). We assessed the optimum PB concentration at pH 7.5 with 0.05% NaCl present with each buffer concentration and found that 0.01M PB elevated adhesion capacity to the maximum (Fig. 5). The concentration of NaCl found to be critical. Concentrations of 0.04 and 5% NaCl found to notably inhibit adhesion capacity while 1.0% yielded maximum and near maximum rates of adhesion capacity (Fig. 6). Our data confirmed that buffer system has such great impact on initiation of biofilm formation.



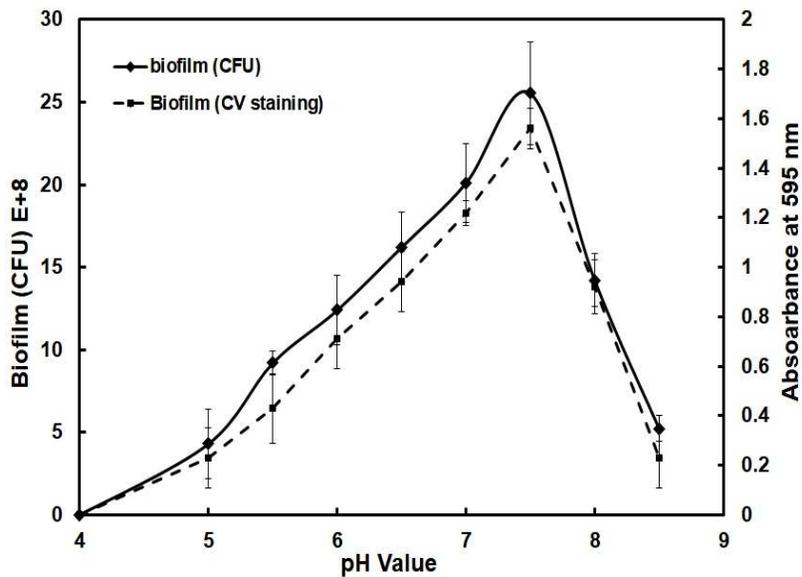
**Fig. 2. Effect of fluid flow rate on bacteria adhesion capacity**

A cell suspension of  $1 \times 10^4$  cells / ml in PBS was passed through the Mini-fluidic chips via the bio-Ferrograph at different flow rates (0.004, 0.008, 0.01, 0.04, 0.08, 0.1, 0.2, 0.4, 0.6 ml/min) for a constant passage time of 100 min at room temperature. PDMS flow cells were filled with 200  $\mu$ l TSB. After 12h of incubation at 37°C, the yield of biofilm were evaluated using plate counts (CFU) and Crystal Violet (CV) staining as mentioned above.



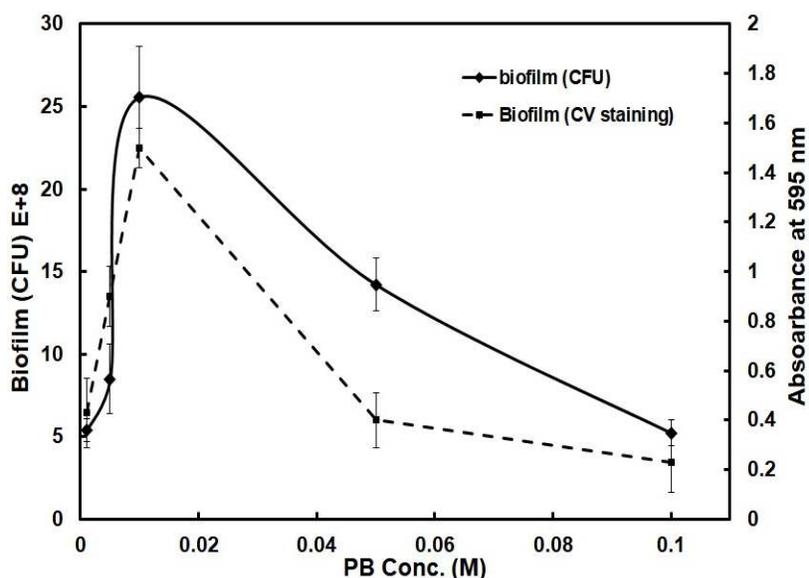
**Fig. 3. Effect of temperature on bacterial adhesion capacity**

A cell suspension of  $1 \times 10^4$  cells / ml in PBS was passed through the Mini-fluidic chips via the bio-Ferrograph at 0.1 ml/min for a constant passage time of 100 min at different temperature ( 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50°C). PDMS flow cells were filled with 200  $\mu$ l TSB. After overnight incubation at 37°C, the yield of biofilm was evaluated using plate counts (CFU) and Crystal Violet (CV) staining as mentioned above.



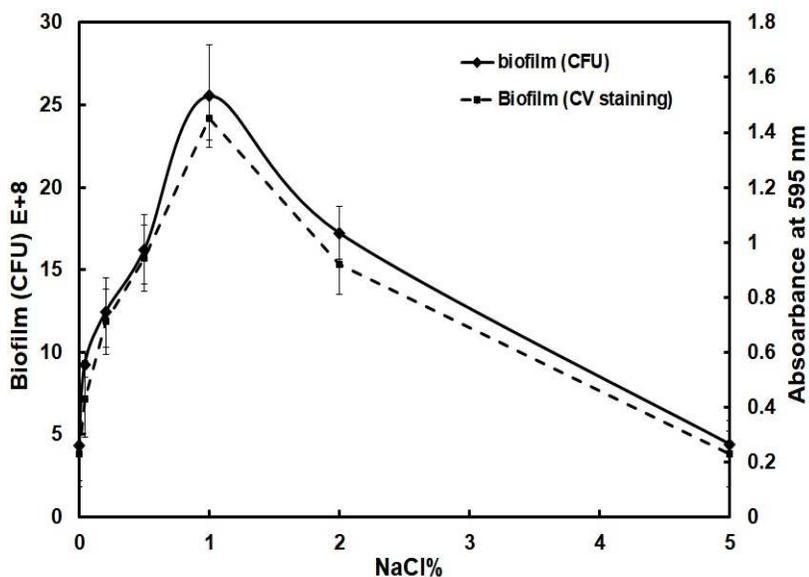
**Fig. 4. Effect of pH on bacterial adhesion capacity**

A cell suspension of  $1 \times 10^4$  cells / ml in various PBS prepared at different pH values (4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5) was passed through the mini-fluidic chips via the bio-Ferrograph at 0.1 ml/min for a constant passage time of 100 min at room temperature. PDMS flow cells were filled with 200  $\mu$ l TSB. After 12 h of incubation at 37°C, the yield of biofilm were evaluated using plate counts (CFU) and Crystal Violet (CV) staining as mentioned above.



**Fig. 5. Effect of phosphate buffer (PB) on bacterial adhesion capacity**

A cell suspension of  $1 \times 10^4$  cells / ml in different concentration of phosphate buffer ( 0.001, 0.005, 0.01, 0.02, 0.05, 0.1M) plus 0.85 NaCl, pH 7.4, was passed through the mini-fluidic chips via the bio-Ferroglyph at 0.1 ml/min for a constant passage time of 100 min at room temperature. PDMS flow cells were filled with 200  $\mu$ l TSB. After 12h of incubation at 37°C, the yield of biofilm were evaluated using plate counts (CFU) and Crystal Violet (CV) staining as mentioned above.



**Fig. 6. Effect of NaCl concentration on bacterial adhesion capacity**

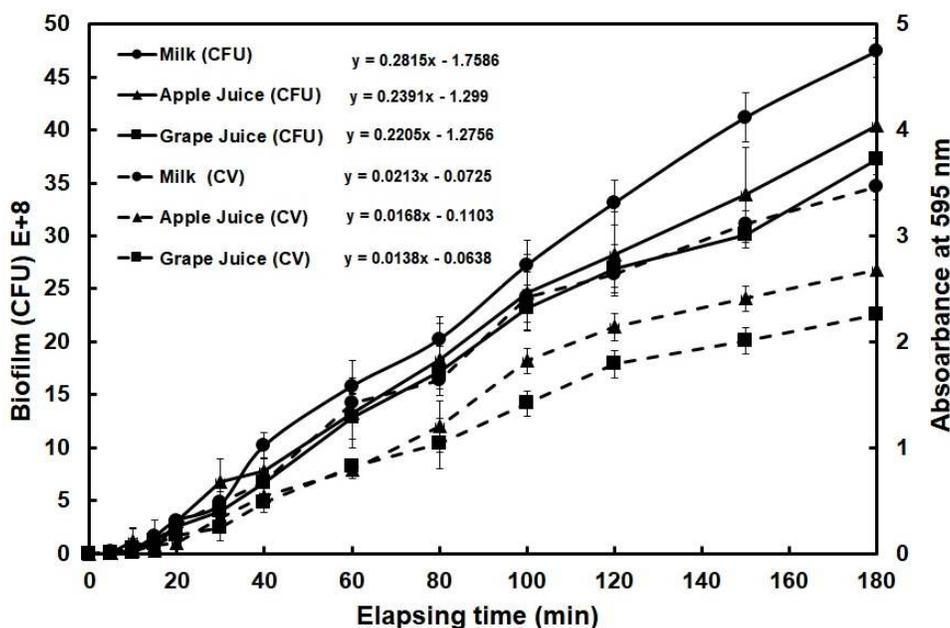
A cell suspension of  $1 \times 10^4$  cells / ml in PBS prepared with different saline concentrations (0.04, 0.2, 0.5, 1.0, 2.0, 5.0) at pH 7.4 was passed through the mini-fluidic chips via the bio-Ferroglyph at 0.1 ml/min for a constant passage time of 100 min at room temperature. PDMS flow cells were filled with 200  $\mu$ l TSB. After 12h of incubation at 37°C, the yield of biofilm were evaluated using plate counts (CFU) and Crystal Violet (CV) staining as mentioned above.

### 3.4 Effect of Different Elapsed Time of Food Fluids on Bacterial Adhesion Capacity

This study was conducted to investigate the influences of nutrient composition and fluid food system on adhesion capacity. Three food fluids were selected (grape juice, milk, and apple juice), inoculated with *E. coli* O157:H7 and pumped via bio-Ferrograph into PDMS flow cell at a flow rate of 0.1 ml/min for different elapsing time (5, 10, 15, 20, 30, 40, 60, 80, 100, 120, 150, and 180 min). Both total counts (CFU) and CV readings generated a wonderful linear pattern (Fig. 7) with a Correlation Coefficient of (0.9956 - 0.9815) between elapsing time and the yield of biofilm that was initiated from cell suspensions in skim milk, orange juice and apple juice. The highest yield biofilm formation was obtained from skim milk followed by apple juice and then grape juice. PDMS flow cell would be very beneficial to predict biofilm formation in beverage industry and other manufacture where pipelines are involved to transfer fluids throughout any process. PDMS flow cell is easy to design to mimic different surface and therefore tackle any biofilm surfaces.

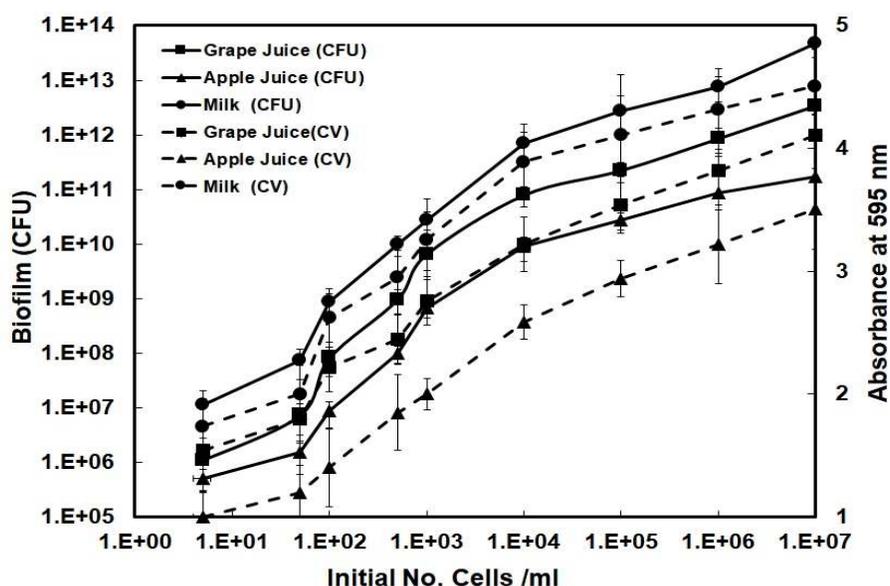
### 3.5 Effect of Various Number of *E. coli* O157:H7 Cells Suspended in Food Fluids on Bacterial Adhesion Capacity

Food fluids including grape juice, skim milk, and apple juice was inoculated with different concentration of bacterial cells. The bio-Ferrograph was used to deliver food fluids through PDMS flow cell at a constant flow rate of 0.1ml/min for a constant passage time of 100 min. A proportional increase in biofilm yield was observed with the increase in cell densities, ranging from 5 to  $1 \times 10^7$  cells/ml (Fig.8) indicating that a PDMS chip can be a useful tool for quantitatively monitoring of biofilm development inside beverage pipelines. The highest yield was obtained from skim milk samples followed by apple juice and then grape juice (Fig. 8). The Power curve found to be a good key concept for understanding the relationship between initial number of cells attached to PDMS inner surface and biofilm formation measured as CFU and crystal violet. These data generated power equations as illustrated in Fig. 8 with Correlation Coefficients between (0.86 – 0.94). Undoubtedly,



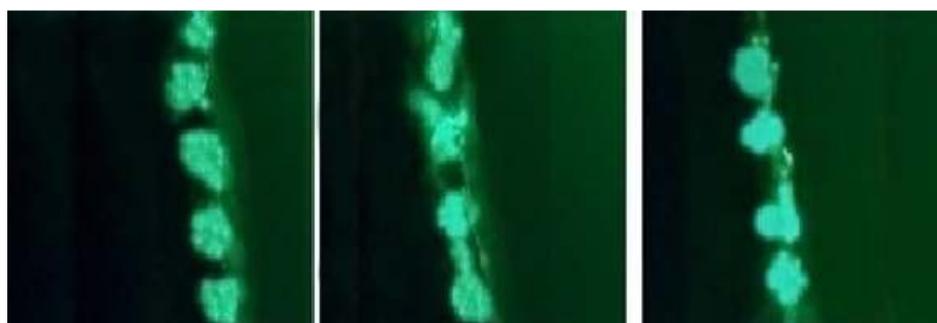
**Fig. 7. bacterial adhesion capacity with different elapsed time of food fluids**

A cell suspension of  $1 \times 10^4$  cells / ml in different sterile food fluids was each passed through the mini-fluidic chips via the bio-Ferrograph at 0.1 ml/min for different passage time (5, 10, 15, 20, 30, 40, 60, 80, 100, 120, 140, 160, 180 min) at room temperature. PDMS flow cells were filled with 200  $\mu$ l TSB. After 12h of incubation at 37°C, the yield of biofilm were evaluated using plate counts (CFU) and Crystal Violet (CV) staining as mentioned above.



**Fig. 8. bacterial adhesion capacity with different number of *E.coli* O157:H7 cells suspended in food fluids**

Different number of *E. coli* O157:H7 cells inoculated into different sterile food fluids was each passed through the mini-fluidic chips via the bio-Ferrograph at 0.1 ml/min for a constant passage time of 100 min at room temperature. PDMS flow cells were filled with 200  $\mu$ l TSB. After 12 h of incubation at 37°C, the yield of biofilm were evaluated using plate counts (CFU) and Crystal Violet (CV) staining as mentioned above.



**Milk      Apple Juice      Grape Juice**

## Fluorescent Imaging of Biofilm

**Fig. 9. Fluorescence imaging of biofilm formation**

Food fluids seeded with  $1 \times 10^4$  cells/ml was subjected to PDMS flow cells for 100 min via the bio-Ferrograph at 0.1 ml/min as mentioned above. After 12 h of incubation at 37°C, PBS buffer containing 4 mg/ml hyaluronidase was pumped through the mini-channels 10 min at a flow rate of 0.1 ml/min. PDMS chip was emptied and washed with PBS. Anti-*Escherichia coli* O157:H7, FITC labeled, (1-64) was added and incubated for 60 min at 37°C. Channels were washed and examined under Epifluorescence microscope using 20x lens.

these power equations would facilitate quantitative prediction of initial number of bacterial contamination in beverage pipeline. Meanwhile, the linear relationship obtained in

Fig. 7 would help to elapsed time required to adhere bacterial to the surface. This chip can be also used to quantitatively detect biofilm forming bacteria in environmental samples with the

assessment of initial number of bacterial cells added to the channels. This PDMS flow cell can be very beneficial to study and evaluate different models for potential risk analysis models beverage industry.

### 3.6 Fluorescence Imaging of Biofilm Formation

Biofilm formation was visually detected using *Escherichia coli* O157:H7 Antibody, FITC labeled. Fig. 8, shows a digital image of fluorescent imaging obtained from Epifluorescence microscope for the detection of biofilm generated from different food fluids inoculated with *E. coli* O157:H7.

### 4. CONCLUSION

This study introduce a novel method, based on flow chamber principle, which enables non-destructive, in situ investigation of bacteria adhesion capacity as an initial step for biofilm formation in real time under a wide range of flow rates and nutrient conditions, fluid temperature, and elapsing times. This flow device is inexpensive, simple, disposable, and easy-to-use for online real-time monitoring of biofilm forming-bacteria in different beverages. This microfluidic chip provides a platform that can accurately mimic the dynamic flow conditions in beverage industry and makes the results easy to compare between groups in a similar manner in real environment.

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### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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