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Hydrodynamic cavitation for the rapid separation and electrochemical detection of *Cryptosporidium parvum* and *Escherichia coli* O157:H7 in ground beef

Ugochukwu C. Nze[a,*], Michael G. Beeman[a], Christopher J. Lambert[a], Ghadhanfer Salih[b], Bruce K. Gale[a,b], Himanshu J. Sant[a,b,**]

[a] Department of Mechanical Engineering, University of Utah, Salt Lake City, UT, 84112, USA
[b] Espira Inc., 825 N 300W Suite N-223, Salt Lake City, UT, 84103, USA

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ABSTRACT:

Foodborne illnesses are a major contributor to misery and health challenges in both rich and poor nations. Illnesses from pathogens such as *Escherichia coli* and *Cryptosporidium parvum* oocysts account for most of the cases of diarrhea in the world. Many standard methods exist for detecting these pathogens in water. However, these standard methods do not readily translate to the detection of the same pathogens in food. Detection techniques for pathogens in food are often inadequate, due to their inability to completely separate pathogens from food matrices. In this paper, we present a technique to separate and detect both *Escherichia coli* cells and *Cryptosporidium parvum* oocysts that have been embedded in ground meat. We achieve this objective by combining enzymatic digestion of the meat, hydrodynamic cavitation to disassemble pathogens from the meat, immunomagnetic separation to purify meat samples and indirect electrochemical detection of the target pathogens. Our use of hydrodynamic cavitation to separate pathogens is compared against an industry standard separation technique. Results indicate that the use of hydrodynamic cavitation amplifies the detection capabilities of our sensing technique and is overall comparable to or better than conventional stomacher sample preparation.

1. Introduction

The presence of pathogens in food and water is a serious concern worldwide and is specifically of interest to the governing bodies that regulate food and water safety. Pathogens such as Shiga toxin-producing *Escherichia coli (E. coli)* and *Cryptosporidium parvum* oocysts (*C. parvum*) are of special concern due to their prevalence in food products and drinks and their highly infectious nature. Cryptosporidiosis, an illness caused by *C. parvum,* is one of the top five most common causes of diarrhea in the world (Boatright and Greenfield, 2005). Cryptosporidiosis is generally considered a waterborne disease (Centers for Disease Control and Prevention (CDC), 1996). However, several foodborne outbreaks of Cryptosporidiosis have been reported due to the consumption of fresh produce and ground beef contaminated with *C. parvum* (Ryan et al., 2018). Likewise, *E. coli* is another bothersome pathogen. In 2015, *E. coli* was found to be responsible for 6% of the 902 foodborne disease outbreaks reported to the Centers for Disease Control and Prevention (CDC), making it the third largest source of foodborne illnesses that year (Centers for Disease Control and Prevention (CDC), 2017). A large number of the outbreaks of *E. coli* in the United States have been traced back to the consumption of undercooked ground beef (Hussein, 2007). While sanitation methods exist for commercial beef, these methods may not provide adequate disinfection of the beef products. A recent study shows that up to 24.3% of commercial ground beef in the U.S. is possibly contaminated with *E. coli* (Bosilevac and Koohmaraie, 2011). Findings such as these necessitate continuous monitoring of food for contamination.

Several standard methods exist that enable the detection of pathogens in food. These methods typically follow a three-step process, including separation of the target pathogen from the food matrix, isolation of the target pathogen from the food matrix, and detection of the isolated pathogen. Of these three steps, inadequate separation of

* Corresponding author.
** Corresponding author. Department of Mechanical Engineering, University of Utah, Salt Lake City, UT, 84112, USA.
E-mail addresses: u.c.nze@utah.edu (U.C. Nze), m.beeman@utah.edu (M.G. Beeman), chris.lambert@utah.edu (C.J. Lambert), ghadhanfer@yahoo.com (G. Salih), bruce.gale@utah.edu (B.K. Gale), himanshu.sant@utah.edu (H.J. Sant).

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pathogens from the food matrix is the most likely step preventing detection of pathogens (Brehm-Stecher et al., 2009). Methods for separating pathogens from food matrices rely on physical principles, chemical degradation, and biological separation (Benoit and Donahue, 2003). Currently, the United States Food and Drug Administration (FDA) recommends the use of physical separation techniques, such as stomaching or homogenization via blending, for separating pathogens from non-leafy green food matrices (Feng et al., 2011). However, stomaching is lacking as a separation method because bacterial detachment stops when cells in suspension reach a certain concentration (Brehm-Stecher et al., 2009). Additionally, the use of a stomacher yields large suspended particles, often requiring further filtration steps downstream to truly separate pathogens from the food matrix (Clime et al., 2015). A possible alternative to stomaching proposed in this work is the use of hydrodynamic cavitation.

Hydrodynamic cavitation occurs in instances where the pressure drops precipitously, for example when fluid flows through a constriction, such as occurs in a Venturi tube or an orifice plate. The change in channel diameter results in an increase in fluid velocity and a reduction of local static pressure. When the pressure in the constricted channel drops below the vaporization pressure of the carrier fluid, vapor cavities will form in the fluid. After exiting the region where the local static pressure is below vapor pressure, these vapor cavities collapse, releasing enough energy to potentially disrupt cells and separate bacteria from food matrices (Jyoti and Pandit, 2001). Hydrodynamic cavitation has previously been reported as an effective disinfection method for media containing pathogens (Arrojo et al., 2008; Cerecedo et al., 2018; Jyoti and Pandit, 2001; Loraine et al., 2012; Mezule et al., 2009). Disinfection of contaminated media via cavitation can occur due to total rupture of the cell membrane (Balasundaram and Harrison, 2006), or by halting a pathogen's ability to replicate while keeping its cell membrane intact (Mezule et al., 2009).

In this work, we demonstrate the ability to finetune hydrodynamic cavitation parameters such that we avoid the total fragmentation of pathogens while still aggressively separating pathogens from food particles. Additionally, we demonstrate the combination of hydrodynamic cavitation and enzymatic digestion for the separation of *Escherichia coli* O157:H7 and *Cryptosporidium parvum* oocysts embedded in ground beef. We couple this method with rapid detection strategies previously developed by our group to demonstrate enhanced pathogen detection with the use of hydrodynamic cavitation when compared against pure chemical degradation of the food matrix. Previous work conducted in our group has indicated our biosensing technique's inability to detect fragmented pathogens (Beeman et al., 2018). This work will also demonstrate that hydrodynamic cavitation is a viable alternative strategy for the separation of pathogens from meat when compared against the use of a stomacher blender.

2. Materials and apparatus

2.1. Dual conjugation of anti-pathogen antibody and polyguanine to polystyrene beads

One-micrometer CP01F Streptavidin coated polystyrene microspheres were purchased from Bangs Labs (Fishers, IN). Biotinylated Anti- *C. parvum* antibody at 10 μg per mL (Catalog# A400BIOT-1X) and Dilution/Blocking Buffer (Catalog# B100-20) were purchased from Waterborne, Inc. (New Orleans, LA). Dynabeads™ anti-*Cryptosporidium* magnetic beads (Catalog# 73001) were purchased from Idexx Laboratories (Westbrook, ME). *C. parvum* oocysts (oocysts) in 10% Formalin and 1x Phosphate-buffered saline were purchased from the Cryptosporidium Production Laboratory located at The University of Arizona (Tucson, AZ). Biotinylated Anti-*E. coli* antibody at 4–5 mg per mL (Catalog# B65109B) was purchased from Meridian life science Inc. (Memphis, TN). Dynabeads™ anti-*E. coli* O157:H7 antibody-coated magnetic beads (Catalog# 71993) were purchased from ThermoFisher Scientific (Carlsbad, CA). A non-pathogenic strain of *Escherichia coli*, (ATCC® 700728™) was purchased from the American Type Culture Collection (Manassas, VA). 5′ Biotinylated with 3' FAM 20-mer poly-Guanine oligonucleotides (PolyG) were synthesized by the DNA/Peptide Facility, part of the Health Sciences Center Cores at the University of Utah (Salt Lake City, UT).

2.2. Elution of polyguanine oligonucleotides from polystyrene beads

Sodium Acetate in crystal form was obtained from Cayman Chemical (Ann Arbor, MI). 99.5% Formamide (Catalog# 17899) was purchased from ThermoFisher Scientific (Rockford, IL). Phosphate Buffered Saline Tablets, Biotechnology Grade (Catalog# 97062–730), were purchased from VWR Life sciences Amresco (Solon, OH).

2.3. Meat sample to represent solid food

Kroger Unseasoned Meat Tenderizer (Papain), Kroger Unbleached Cone Coffee Filters #4, and ground beef were purchased from a local grocery store.

2.4. Apparatus for cavitation and stomaching

A peristaltic pump (Item# EW-07553-70), Masterflex L/S Easy-Load II Pump Heads (Item# HV-77200-60), and Masterflex C-Flex tubing L/S 17 (Item# HV-06424-17) were purchased from Cole-Parmer (Vernon Hills, IL). A Seward Stomacher 400 Circulator blender was used for stomaching.

2.5. Apparatus for electrochemical detection

Electrochemical measurements were made using a Palmsens EmStat3+ potentiostat (Order code: ES3P-USB) with accompanying Pstrace 4.8 control software (Houten, The Netherlands). Screen printed carbon electrodes (Catalog# DRP-96X110) were purchased from Dropsens in a 96 well plate format (Llanera, Asturias Spain). The electrochemical cells consisted of a carbon counter electrode, a 3 mm in diameter carbon working electrode, and a silver pseudo-reference electrode. A custom 3D printed magnetic separation rack was used to concentrate the magnetic beads into a pellet. A Labnet Mini LabRoller Dual Format Rotator was used to re-suspend all solutions (Edison, NJ). An Autolab Faraday cage was purchased from Metrohm Autolab B.V. (Utrecht, Netherlands) and used to prevent electrical interference from surrounding electronics during electrochemical measurements.

3. Methods

3.1. Working principle of the pathogenic sensor

There are seven steps to this biosensing technique: 1) Pretreatment of meat samples; 2) Cavitation of meat samples; 3) Immunomagnetic capture of pathogens in meat samples; 4) Removal of non-target particles from the system; 5) Amplification of the pathogens with the use of polyG coated secondary beads; 6) Elution of polyG from the secondary beads; and 7) Electrochemical detection of polyG. Fig. 1 illustrates this method as applied to *C. parvum*.

3.2. Culturing of E. coli and C. parvum

To grow *E. coli*, non-pathogenic *E. coli* O157:H7 was purchased from the American Type Culture Collection (ATCC) in a freeze-dried format and was propagated according to ATCC's instructions. Briefly, 1 mL of Difco Nutrient broth (Catalog # 234000, Becton Dickinson, Sparks, MD, USA) was used to rehydrate the freeze-dried pellet, and this solution was mixed well. Following rehydration, the solution was transferred to a tube containing an additional 5 mL of nutrient broth. A 200 μL aliquot

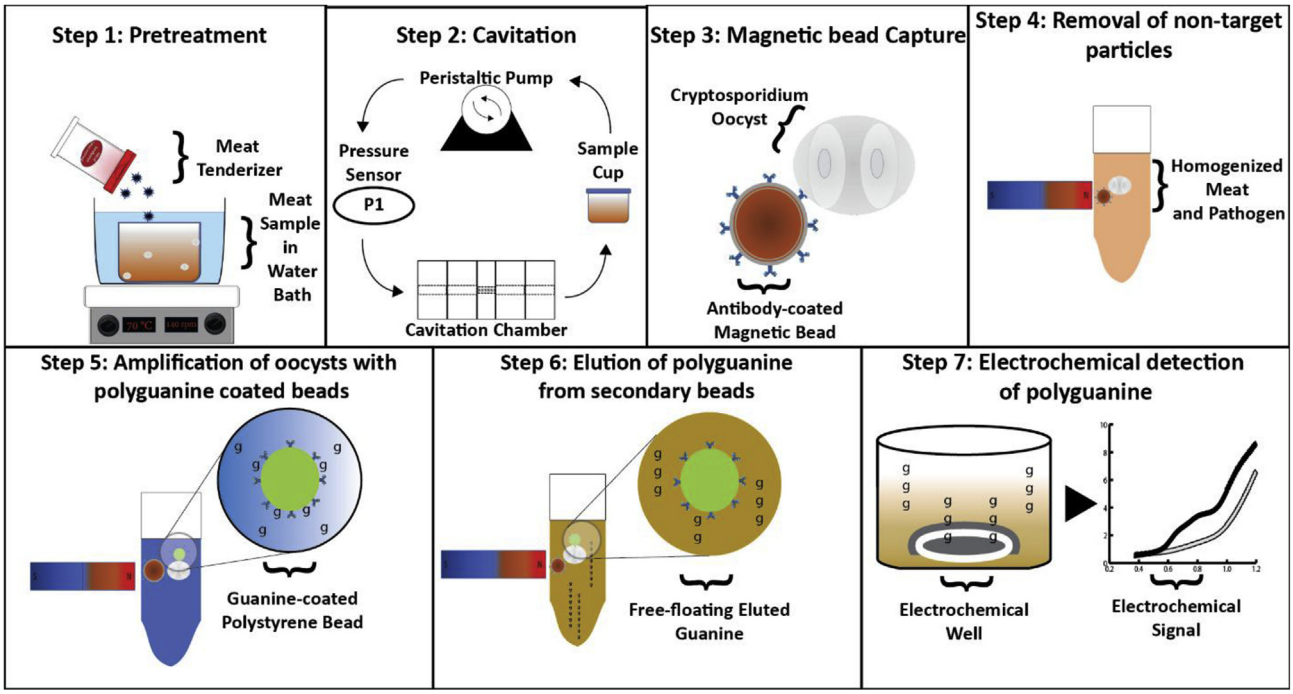

Fig. 1. Process overview for the electrochemical sensing of Cryptosporidium oocysts.

of this solution was spread on an agar plate containing Difco Nutrient Agar (Catalog # 213000, Becton Dickinson, Sparks, MD, USA). Both the broth solution and the agar plate were incubated at 37 °C for 24 h. After propagation, the cultured broth was centrifuged at 1000 g for 10 min to concentrate the bacterial cells into a pellet. The supernatant from the broth was removed, and the bacteria were resuspended in 3 mL of Difco Nutrient Broth mixed with 20% (vol/vol) sterilized glycerol. The culture was aliquoted in Nalgene Cryogenic vials (Thermo Scientific, Waltham, MA, USA) and stored at −135 °C until ready for use.

To prepare *E. coli* samples for inoculation 100 µL of aliquoted *E. coli* solution was spread on a Difco Nutrient Agar plate and incubated for 24 h at 36 °C. A sterile pipette tip was used to scrape a portion of the bacterial colony and transfer the colony into a solution of 1x PBS.

C. parvum oocysts were diluted using 1x PBS to achieve the desired concentration.

3.3. Inoculation of meat

Fresh ground beef samples were purchased from a local grocery store and prepared for use in our experiments. Ground beef samples containing 85% lean content 15% fat content were partitioned into 50 g samples, flattened into thin sheets to aid with thawing, and stored at −20 °C for further use. Samples meant for testing were rapidly thawed in accordance with the methods described by the U.S. FDA's Bacteriological Analytical Manual (H Andrews and S Hammack, 2003). After thawing, inoculation was conducted by immersing 10 g of ground beef in 10 mL of 1x PBS. 100 µL of the desired pathogen is then added to each sample. Samples were then incubated for a further 90 min to allow pathogens to grow on meat.

3.4. Dual conjugation of anti-pathogen antibody and polyguanine to polystyrene beads

A protocol to create polystyrene beads that were conjugated with both anti-pathogen antibodies and polyguanine oligonucleotides was developed based on similar work by Bangs Laboratories (2014). First, stock 1.0 µm CP01F Streptavidin coated polystyrene microspheres (PSB) were resuspended by rotating the solution on a Labnet Mini LabRoller Dual Format Rotator end over end for 30 min. Then 20 µL of stock PSBs were aliquoted in a 1.7 mL centrifuge tube. 1 mL of Waterborne PBS containing phosphate buffered saline, pH 7.4, with 1% w/v bovine serum albumin, EDTA, and sodium azide was added to the solution. The solution was centrifuged for 5 min at 6000 rcf to form a pellet. The supernatant was removed, making sure not to disturb the pellet that was formed. The solution was then resuspended with an addition 1 mL of waterborne PBS. The centrifugation, removal of supernatant and resuspension steps were repeated twice more. After the third centrifugation, the supernatant was removed. To create anti-*C. parvum* polystyrene beads, 184 µL of Waterborne PBS and 816 µL of 10 µg per mL Waterborne Anti-*C. parvum* antibody was added to the solution. To create anti- *E. coli* polystyrene beads, 998 µL of PBS and 2 µL of 4–5 mg per mL Anti-*E. coli* antibody was added to the solution. The solutions were then covered in aluminum foil to prevent exposure to light and allowed to rotate end over end for 2 h. The solutions were centrifuged again for 5 min at 6000 rcf to form a pellet. The supernatant was removed, making sure not to disturb the pellet that was formed. Six micrograms of 5′ biotinylated 20-mer poly Guanine oligonucleotides with 3' FAM was added into the solution for 3x excess. Finally, the solution was resuspended in 1600 µL of PBS.

3.5. Fabrication of hydrodynamic cavitation device

The cavitation chamber used in this work is fabricated in less than 15 mins using cost-effective, rapid prototyping techniques. Fig. 2A illustrates a schematic of the fabricated cavitation device. To assemble the device, five 25-mm acrylic disks were cut out using a ULS Versalaser CO_2 laser engraving system. These disks were designated for use as either inlet fittings, expansion chambers, or the cavitation orifice plate. The inlet fitting disks and expansion chambers were manufactured using a ¼ inch (6.35 mm) sheet of acrylic. In each of these disks, a 1.2 mm-diameter-through hole was also engraved. Tapped holes were bored through the inlet fitting disks and used to connect the cavitation device to the rest of our system via female luer locks. Cavitation was induced in our system using an orifice plate with one 0.8 mm-diameter-through hole. The pressure difference between the expansion chambers and the cavitation orifice plate enabled the creation and destruction of

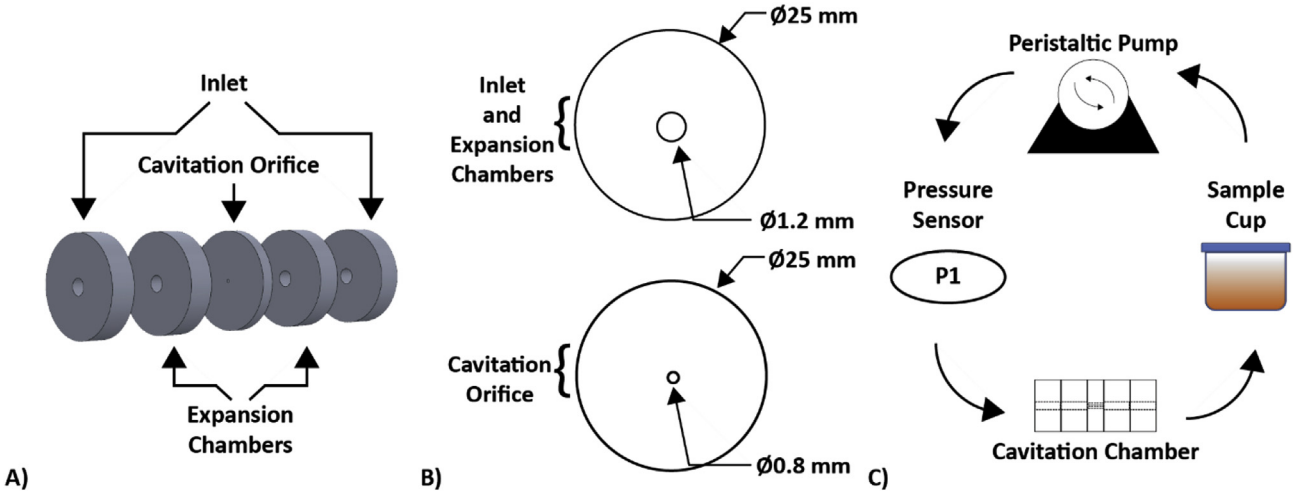

Fig. 2. A) Exploded view of the cavitation chamber. B) Dimension of Inlet, Expansion chambers and Cavitation orifice plate. C) Experimental setup used in the study. It consisted of the cavitation device, a peristaltic pump, a pressure sensor, and the sample cup. The sample was circulated in and out of the cavitation chamber using a peristaltic pump. Input pressure was measured using the pressure sensor P1.

cavitation bubbles.

3.6. Optimization of cavitation parameters

C. parvum has been chosen as the model pathogen for optimization of cavitation parameters due to its size. Since *C. parvum* is large, whole parvum oocysts can easily be differentiated from disintegrated oocyst particulates. *E. coli* was not chosen for this study because its smallest dimension neared the detection limits of our cytometer. Therefore, it may have been difficult to distinguish several closely located fragments of *E. coli* from a whole *E.* coli cell.

Four samples containing 40 mL of DI water spiked with *C. parvum* at 20,000 oocysts/mL were created. *C. Parvum* samples were stained with Hoechst 33342 dye and incubated at 37 °C for 10 min so that whole oocysts could be distinguished from other components of our sample based on size and fluorescence of particles. Three of these samples underwent a cavitation treatment for 7.5 min at input pressures of 8, 14, and 20 PSI (55.2, 96.5, 137.9 kPa) respectively. The fourth sample did not undergo a cavitation treatment as a control. After each treatment was applied, anti-*C. parvum* PSBs were attached to the oocysts using methods described above to observe the binding capabilities of *C. parvum* post-treatment. Post-treatment, 300 µL aliquots of each sample were obtained, and samples were characterized with a BD FACSCANTO II flow cytometer.

Using flow cytometry, population side-scatter and forward-scatter intensities of each sample are recorded concurrently. The gates of the cytometer were set so that only events that registered positive for Hoechst 33342 but negative for all other interferences were counted. We also subtract the background scattering intensity of the sample due to buffer effects. This data was used to quantify the generation of sample debris due to *C. parvum* destruction.

3.7. Cavitation of meat

To facilitate the maximum recovery of pathogenic material from the beef sample, a complete homogenization of our sample is needed. One major hindrance to the homogenization of complex samples like ground beef is the existence of proteins and connective tissues, such as collagen and myofibrillar protein, which are difficult to break down. The presence of these proteins in our samples would lead to immediate clogging of the cavitation device resulting in inadequate separation of pathogenic material from the meat sample. Therefore, a pretreatment step is required to soften these proteins before passage through the cavitation chamber. For this work, Papain facilitated the degradation of both myofibrillar proteins and collagen (Calkins, Ph.D. and Sullivan, 2001). Papain is an aggressive enzyme, found commonly in papaya fruit and meat tenderizer, which can be employed to break down connective tissues. To tenderize the beef, inoculated beef samples were mixed with a solution containing 90 mL of deionized water and 6.6 g of Papain. These samples were placed in a water bath at 70 °C for one and a half hours with constant stirring to allow for the optimal enzymatic activity of Papain (Calkins, Ph.D. and Sullivan, 2001). This pretreatment step created a mixture of liquefied beef, fat, and collagen. Samples were pre-filtered by passing them through a coffee filter with 0.8 mm-diameter-holes cut throughout it at a roughly 30% fill density to collect any solids. The filtrate recovered from this process typically contained less than 10% of the initial solid sample. The liquid beef mixture filtrate was then passed through the hydrodynamic cavitation device for seven and a half minutes at an input pressure of 11 PSI. Fig. 2C illustrates the cavitation schema.

3.8. Cavitation of inoculated meat

Eight ground beef samples were inoculated with either *E. coli* (1.5×10^5 CFU/mL), *C. parvum* (2×10^4 oocysts/mL) or just DI water as a control using the methods described above. After inoculation, all sample received a tenderization pretreatment for one and a half hours and were pre-filtered to collect any solids. Samples then either underwent cavitation at 11 PSI for 7 and a half minutes or were left as is. Four 1 mL aliquots of each sample were then collected. Our electrochemical stratagem was applied to each aliquot. Square wave voltammetric scans were performed twice on each aliquot.

3.9. Cavitation vs. stomaching

A comparison between our method and stomaching was made to determine which method was more effective for electrochemical detection. To perform this test, three ground beef samples, referred to as samples A, B, and C were inoculated with *E. coli* (9.8×10^4 CFU/mL) using the methods described above. After inoculation, all samples were mixed with a solution containing 90 mL of DI water and 6.6 g of meat tenderizer (Papain). Samples A and B were placed in a water bath at 70 °C for one and a half hours to allow for the tenderization and breakdown of the meat. Sample C was allowed to rest at room temperature for the same amount of time. Sample A was pre-filtered to collect any solids and then underwent cavitation at 11 PSI for 7.5 min.

Sample B was filtered and did not receive either the cavitation or stomacher treatment. Sample C was processed using a Seward stomacher 400 Circulator blender at 230 RPM for 2 min and filtered to collect any solids. Four 1 mL aliquots of each sample were then collected. Each aliquot underwent the pathogen detection method developed by our group (described below). Finally, square wave voltammetry (SWV) was applied to each aliquot to measure the electrochemical response of a sample.

3.10. Electrochemical detection of polyguanine

Electrochemical detection of the polyG labels was used to indirectly measure the amount of pathogen separated from the meat, with a higher electrochemical response corresponding to the increasing separation of the pathogen. Electrochemical detection of the polyG labels was performed using methods similar to what has been previously described by our group (Beeman et al., 2018; Gordon, 2017). Briefly, a solution containing 1 mL of sample and 10 μL of magnetic beads was added to a 1.7 mL microcentrifuge tube. This solution was then allowed to mix end over end at 32 rpm for 40 min. After mixing, the microcentrifuge tube was placed in a custom-made magnetic rack with N42SH grade neodymium magnets to carry out the immunomagnetic separation process. The tube remained on the magnetic rack for 3 min, being inverted once per minute. After 3 min the supernatant from each tube was discarded, and 1 mL of PBS wash buffer was added. This process was repeated twice to ensure the washing of any non-target particles in the system.

Following immunomagnetic separation, 15 μL of dual conjugated PSBs were added to the solution. This solution was mixed end over end for another 40 min. After 40 min, each tube was once again placed on the custom magnetic rack for 3 min, being inverted once per minute, and then the supernatant was discarded, and 1 mL of PBS wash buffer was added. This process was repeated two times to wash any unbound PSBs out of the system.

To elute the PolyG from the polystyrene beads, the supernatant was removed from the solution and 250 μL of an elution buffer containing equal parts of a 95% formamide, diluted with DNase/RNase free water, and an 80 mM sodium acetate, diluted in DNase/RNase free water, was added. The tubes were put into a water bath held at 90 °C for 10 min. Each sample was transferred to a well on the Dropsens 96 well screen-printed carbon electrode plate. The supernatant was allowed to adsorb on the electrode plate for 10 min. After 10 min, a Palmsens EmStat3+ potentiostat with accompanying Pstrace v4.8 software is used to record the current density of the guanine redox reaction using SWV. SWV is conducted twice per well, with 8 s of equilibrium time between each scan. Each scan is conducted using the following parameters: a starting potential of 0.34 V, an ending potential of 1.2 V, a step potential of 0.005 V, an amplitude of 0.02 V, and a frequency of 100 Hz. The first SWV scan (scan 1) was used to measure the oxidation of polyG. The second SWV scan (scan 2) measured the baseline current exhibited by the detector in the absence of oxidation of guanine. Scan 2 was subtracted from scan 1 to observe the baseline subtracted current exhibited by each sample. For each sample, the baseline subtracted peak current occurring at 1 V (vs. Ag pseudo-reference electrode) was taken as the quantitative measure of the amount of polyG in the sample.

4. Results and discussion

In this work, we aim to show that hydrodynamic cavitation is a viable alternative to stomaching for separating pathogens embedded in meat particles.

4.1. Optimization of cavitation parameters

In this work, we adjusted our cavitation parameters so as not to disintegrate our pathogens of interest. Finetuning in such a way allows our hydrodynamic cavitation technique to be compatible with several downstream analytic methods, such as the electrochemical detection method developed by our group. Flow cytometry reveals no great increase in debris due to the disintegration of oocysts between samples which had received no cavitation treatment (983 counts), samples which had received cavitation conducted at an input pressure of 8 PSI (55.2 kPa, 1427 counts), and samples which received cavitation conducted at an input pressure of 14 PSI (96.5 kPa, 1250 counts). However, samples which receive a cavitation treatment at an input pressure of 20 PSI (137.9 kPa) display an almost three-fold increase in debris observed (3321 counts). A one-way ANOVA was conducted to compare the effects of cavitation on the generation of *C. parvum* debris. It is observed that there is no significant difference in the amount of *C. parvum* debris generated when cavitation is performed at 14 PSI or below compared to when there is no cavitation performed at all. For input pressures of 14 PSI and below the increase in debris due to cavitation is not significant within a 99% confidence interval with $p > 0.7024$ for 8 PSI and $p > 0.9103$ for 14 PSI. However, when the pressure is increased above 14 PSI, a significant difference in found in the amount of *C. parvum* debris observed within a 99% confidence interval with $p < 0.0019$.

Flow cytometry was also used to measure the incidence of PSBs bound to oocysts in each aliquot as a percentage of total presence of oocysts in the aliquot. The antibody conjugated to our PSBs was raised for the detection of epitopes on the outer wall of *C. parvum* oocysts. This antibody does not efficiently attach to oocysts walls that have been fragmented and damaged. Therefore, the number of polystyrene beads bounded to cryptosporidium was used as a secondary indicator of oocyst fragmentation. It is observed that increasing the cavitation input pressure reduces the occurrence of oocysts bonded to polystyrene beads. Experiments conducted without cavitation have the highest success of bonding with 72% of oocysts bonding to PSB. The addition of a cavitation treatment results in a minor decrease in the occurrence of oocysts bonded to PSBs with 61% and 60% of oocysts bound in samples that receive cavitation at input pressures of 8 PSI and 14 PSI respectively. However, a drastic reduction in binding is observed for samples which receive a cavitation treatment with an input pressure of 20 PSI. Only 40% of oocysts present in samples that receive cavitation at an input pressure of 20 PSI are observed to be bound to polystyrene beads, which suggests that cavitation pressure above 14 PSI results in the fragmentation of oocysts. Due to these results, a median input pressure of 11 PSI was chosen for use in future experiments. Fig. 3 summarizes the results received from flow cytometry.

4.2. Cavitation of inoculated meat

One of the main objectives of this work is to demonstrate the combination of hydrodynamic cavitation and enzymatic digestion for the separation of pathogens from meat. The first step of this process is the pretreatment of meat using enzymatic digestion. While the pretreatment steps performed resulted in a semi-liquid solution of beef, fat, and collagen, it was unclear whether this solution alone would facilitate maximum recovery of pathogens or whether further processing was needed. A comparison was made between meat samples which had only received enzymatic digestion and meat samples that underwent both cavitation and enzymatic digestion. Square wave voltammetry results indicate that the inclusion of hydrodynamic cavitation as a sample preparation step results in an increase in the electrochemical current response for all samples. The electrochemical sensograms observed for this work are similar to the sensorgrams to what has been previously reported by our group (Beeman et al., 2018). A representative sensorgram for electrochemical detection can be found in Fig. 4. A summary of the results can be found in Table 1.

Square wave voltammetry results suggested that cavitation resulted in an increase in the baseline electrochemical response for uninoculated samples. As expected, samples which were neither inoculated with a pathogen nor underwent any cavitation treatment exhibit the lowest

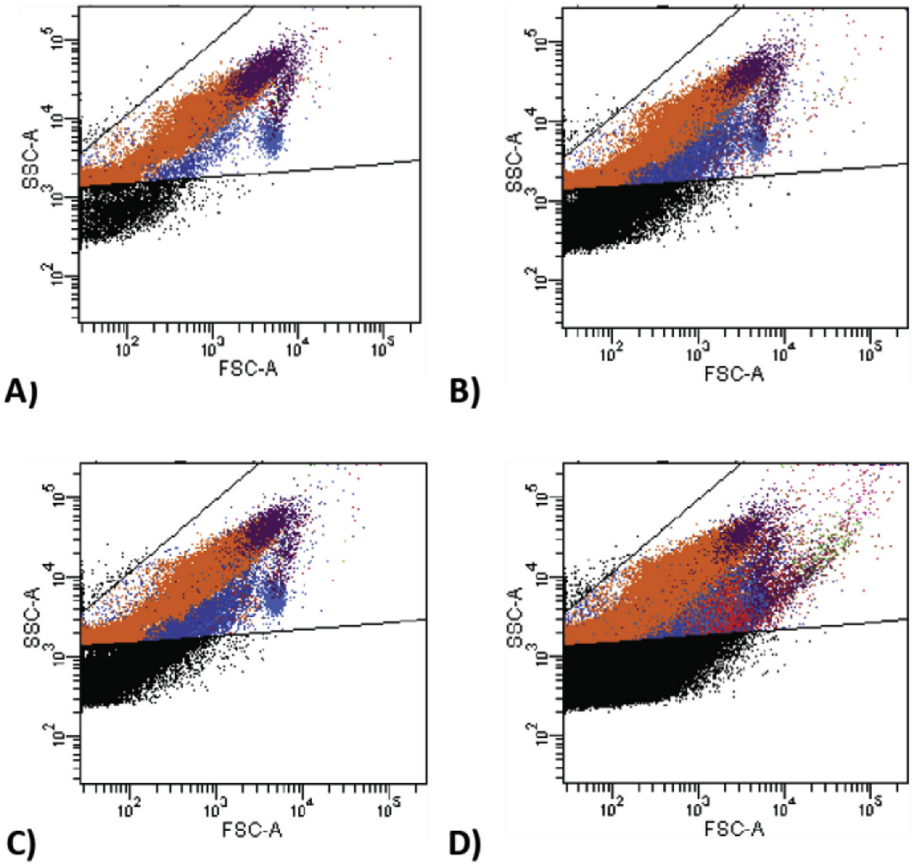

Fig. 3. Population forward scatter vs. side scatter of samples containing oocysts (blue), polystyrene beads (orange), anti-Cryptosporidium magnetic beads (purple), oocyst debris (red), and background debris (black) as observed via flow cytometry. A) Sample which received no cavitation; B) Sample which received cavitation at an input pressure of 8 PSI; C) Sample which received cavitation at an input pressure of 14 PSI; D) sample which received cavitation at an input pressure of 20 PSI. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

current response, 2.89 ± 0.11 μA for *E. coli* tests and 2.34 ± 0.18 μA for *C. parvum* tests, respectively. An at least 3% increase in current response is observed between uninoculated samples which undergo cavitation and uninoculated samples which do not receive the cavitation treatment, 3.18 ± 0.23 μA for *E. coli* tests and 2.41 ± 0.33 μA for *C. parvum* tests, respectively. This increase is likely due to the increased presence of small particulates after the cavitation treatment as shown in the flow cytometry results. These particulates can increase the incidence of PSBs non-specifically bound in our system, which in turn increases the electrochemical signal received from a sample. However, all inoculated samples still display higher electrochemical signals than the uninoculated samples.

Samples that were inoculated by pathogens also generated higher electrochemical responses after receiving the cavitation treatment. The cavitation treatment resulted in a 17.6% increase in the current response observed for samples containing *E. coli* and a 12.5% increase in signal strength is observed for samples containing *C. parvum* when compared against inoculated samples which did not receive cavitation. A one-way ANOVA was conducted to compare the effects of cavitation on the electrochemical signal. For both pathogens, the increase in electrochemical signal due to cavitation is significant within a 90% confidence interval with $p < 0.0733$ for *C. parvum* and $p < 0.0465$ for *E. coli*. Additionally, a significant difference is also observed between inoculated meat samples which only receive tenderization pretreatment and meat which receive both tenderization and cavitation, $p < 0.0999$ for *E. coli*. This indicates that the combination of tenderization pretreatment and cavitation disembeds pathogens from meat particles better than meat tenderization alone.

4.3. Cavitation vs. stomaching

A comparison between pathogenic separation via hydrodynamic cavitation and pathogenic separation via stomaching is also made. Square wave voltammetry results indicate that the inclusion of hydrodynamic cavitation as a sample preparation step results in the increase of electrochemical current response when compared with stomacher samples. A summary of the results can be found in Fig. 5. Samples which only receive a tenderization treatment display a current response of 2.336 ± 0.203 μA. Samples which receive only the stomacher treatment generated a current response of 3.201 ± 0.071 μA. Finally, samples which received both the tenderization treatment and hydrodynamic cavitation treatment display a current response of 3.415 ± 0.221 μA. A one-way ANOVA was conducted to compare the effects of stomaching and cavitation on the electrochemical signal. Both cavitation and stomaching significantly increase the electrochemical response of our sensor within a 99% confidence interval, with $p < 3.20 \times 10^{-5}$ for cavitation and $p < 1.80 \times 10^{-4}$ for stomaching. A comparison between stomaching and cavitation shows that they elicit nearly similar current responses for our electrochemical sensor, with $p = 0.25$. These results illustrate that the cavitation technique we have developed is at least equivalent to the stomacher approach and potentially slightly better and can be used as an alternative method for detaching pathogens embedded in ground beef.

Pathogenic separation via stomaching displays several disadvantages when compared to cavitation. One disadvantage is that the use of a stomacher is less cost-effective than the proposed cavitation system. The complete assembly of our cavitation system, as proposed, simply requires a peristaltic pump, a digital pressure transducer, a cavitation chamber, and peristaltic pump tubing. Other materials used in this work such as the meat tenderizer and coffee filter can easily be found in a local supermarket. All these materials can be procured and assembled at less than 1/3rd the cost of a stomacher with similar capabilities. Thus, the proposed pathogenic separation system is more cost-effective than other established methods. Another disadvantage is that samples which received both the pretreatment and cavitation were almost completely homogenized. Greater than 90% of the samples that

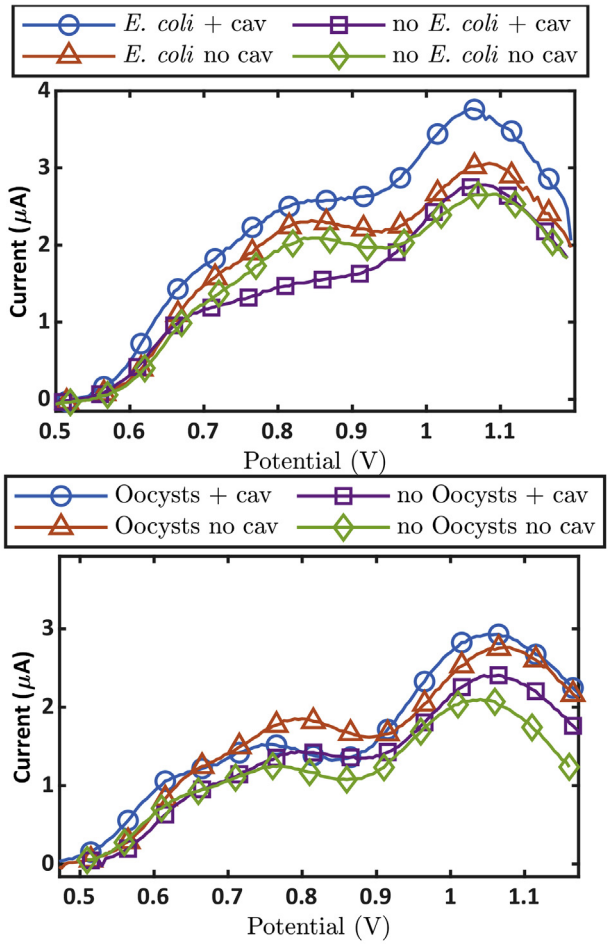

Fig. 4. Representative square wave voltammetric sensorgrams of samples that underwent different treatments. Treatments include: samples that were not inoculated with *E. coli* (top) or *C. parvum* (bottom) and did not undergo cavitation (Green line), samples that were not inoculated with *E. coli*/*C. parvum* and did undergo cavitation (Purple line). samples that were inoculated with *E. coli*/*C. parvum* but did not undergo cavitation (orange line), and samples that were inoculated with *E. coli*/*C. parvum* and did undergo cavitation (Blue line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Effect of cavitation on the electrochemical signal.

Inoculant	Pathogen Inoculation	Cavitation Treatment	Mean current (μA)	SD	CV (%)
E. coli	–	–	2.89	± 0.11	4%
	–	+	3.18	± 0.23	7%
	+	–	3.29	± 0.54	16%
	+	+	3.87	± 0.08	2%
C. parvum	–	–	2.34	± 0.18	8%
	–	+	2.41	± 0.33	14%
	+	–	2.56	± 0.28	11%
	+	+	2.88	± 0.07	2%

receive this treatment can easily flow through our downstream sample filter. However, stomaching is unable to completely homogenize the sample, with 42.9% of the stomached sample unable to pass through our sample filter. Additionally, stomaching of ground meat samples generates a considerable amount of debris. This makes stomaching incompatible with techniques such as PCR due to an efflux of PCR inhibitors such as fat and several proteins from the meat (Kanki et al., 2009). Cavitation has the potential to degrade such PCR inhibitors,

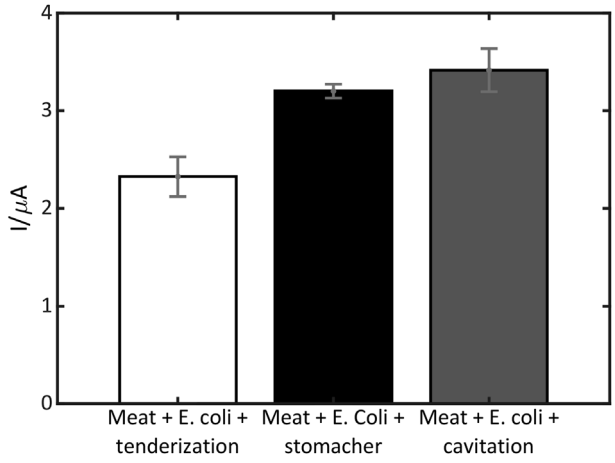

Fig. 5. Comparison of electrochemical response generated by inoculated samples which received the cavitation treatment vs. inoculated samples which solely received a stomaching treatment.

negating this need for further sample preparation steps before PCR is performed on ground meat samples. Finally, stomaching is a batch process and can thereby not be combined with any inline detection or sample processing units. The hydrodynamic cavitation system used in this work is a semi-batch process, and it allows for the automated introduction of new samples and removal of processed samples using simple three-way valves. These advantages make hydrodynamic cavitation a superior choice for semi-continuous monitoring of commercial foodstuffs.

4.4. Cavitation of various complex samples

This hydrodynamic cavitation procedure can also be fine-tuned for processing other complex sample matrices. To apply this technique to other complex samples, one should optimize parameters such as the operating pressures of the device, the flow rate of the sample, and the design of the cavitation chamber (Arrojo and Benito, 2008). The differences between the inlet pressure, the minimum pressure, and the outlet pressure in a cavitation device are critical parameters in determining the number of vapor cavities generated within the device. Depending on the sample of interest, these three pressures can be varied such that enough vapor cavities form to disintegrate a sample. These pressures can be controlled through the additions of a stagnation plate or throttling device to the cavitation system. The operating pressure of a cavitation system can also be controlled by varying the volumetric flow rate of a sample. Depending on the geometric design of the cavitation orifice and the properties of the fluid in which the sample is contained, a minimum fluid velocity must be established before any cavitation can be observed (Braeutigam et al., 2010; Carpenter et al., 2017). The design of the cavitation orifice can be varied to produce the desired vapor cavity collapse. Orifice plates with a single large orifice hole or multiple smaller orifice holes can be considered. Single orifice cavitation devices produce less violent vapor cavity collapses than their multiple hole counterparts (Carpenter et al., 2017). However, multiple hole devices may be more susceptible to device clogging due to their small hole diameter size.

5. Conclusions

This work demonstrates the electrochemical detection of pathogens embedded in ground beef prepared using hydrodynamic cavitation as a pathogen release technique. Cavitation parameters were selected such that pathogens in the system were not disintegrated, enabling 60% binding between the detection conduit in our system and *Cryptosporidium parvum* oocysts. The developed cavitation technique

improved the electrochemical detection of both *Escherichia coli* O157:H7 and *Cryptosporidium parvum* oocysts embedded in ground meat when compared against samples that simply underwent enzymatic digestion. An increase in the electrochemical response of 17.6% and 12.5% for *E. coli* and *C. parvum*, respectively, was observed after applying the cavitation treatment. This suggests that the application of hydrodynamic cavitation released more pathogens from the food matrix than enzymatic digestion alone. The use of hydrodynamic cavitation was shown to be a viable alternative for separation of embedded pathogens in meat when compared to a Seward stomacher blender. The electrochemical response from meat which received the stomacher treatment was comparable to samples which received the tenderization and hydrodynamic cavitation treatment, with samples displaying a current response of 3.201 ± 0.071 μA and 3.415 ± 0.221 μA respectively. The cavitation technique used in this work offers several advantages when compared to stomaching, including its lower cost, increased sample recovery, and potential automation. Furthermore, the complexity of a beef food matrix indicates the possibility of applying this method to detect pathogens in other complex food matrices. Work is underway to streamline the sample preparation and detection process to build an integrated and fully automated system for identification and sensitive quantification of multiple pathogens from food and drinking water samples.

Declaration of interests

Bruce K. Gale and Himanshu J. Sant have a financial interest in Espira Inc. (Salt Lake City, UT, USA). All conflicts are managed by the University of Utah Conflict of Interest Committee.

CRediT authorship contribution statement

Ugochukwu C. Nze: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing - original draft. **Michael G. Beeman:** Conceptualization, Formal analysis, Investigation, Writing - review & editing. **Christopher J. Lambert:** Conceptualization, Investigation, Writing - review & editing. **Ghadhanfer Salih:** Writing - original draft. **Bruce K. Gale:** Conceptualization, Supervision, Writing - review & editing. **Himanshu J. Sant:** Conceptualization, Supervision, Writing - review & editing.

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