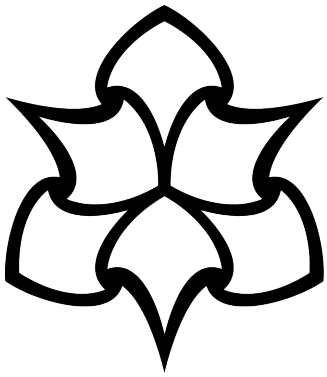

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Non-thermal plasma-based inactivation of bacteria in water using a microfluidic reactor

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ABSTRACT

Failure of conventional water treatment systems may lead to the contamination of water sources, which can cause outbreaks of waterborne healthcare associated infections. Advanced oxidation processing by non-thermal plasma has the potential to treat water without the addition of chemicals. Antibiotic resistant *Pseudomonas aeruginosa* and *Escherichia coli* were chosen to investigate the use of non-thermal plasma generated in a microfluidic reactor to disinfect bacteria contaminated water. The microfluidic reactor used in this study utilized a dielectric barrier discharge, in a gas-liquid phase annular flow regime. Microbiological analysis of water inoculated with *P. aeruginosa* and *E. coli* was carried out before and after plasma treatment. Using air as the carrier gas, effective disinfection of water was achieved. At the lowest flow rate (35 μL/min), *P. aeruginosa* and *E. coli* viability were drastically reduced, with an approximate 8 log maximum decrease in viability following an estimated residence time of 5 s of plasma treatment. Scanning electron microscopy indicated changes in cell morphology due to the plasma treatment. Live/Dead assays revealed that the membranes of the cells had been damaged after plasma treatment. This work demonstrated that non-thermal plasma has the potential to disinfect against microbial contamination in water.

1. Introduction

Consumption of potable water contaminated with microorganisms that pass untreated through chemical and physical water treatment processes can be hugely detrimental to human health (Shi et al., 2012; Naidoo and Olaniran, 2014). Waterborne healthcare associated infections (HCAIs), caused by opportunistic waterborne microorganisms such as *Pseudomonas aeruginosa* and *Escherichia coli*, are one of the most prevalent causes of mortality and morbidity incidences in settings such as healthcare (Ferranti et al., 2014). During several outbreaks of HCAIs in the UK, pathogen contamination in treated water was verified as the source (Walker and Moore, 2015; Kinsey et al., 2017). More than 5500 patients were killed by *E. coli* infections in 2015 in the UK, which lead to high aggregate costs of £2.3 billion to the NHS (Andrew et al. (2018). Furthermore, concerns over multi-drug resistant bacteria in waterborne HCAIs outbreaks have increased due to the reduction of therapeutic effectiveness of antibiotics in treating infected patients (Hayward et al., 2020). According to the O'Neill commission in 2016, approximately 700,000 patients die worldwide annually due to infections caused by antibiotic resistant microorganisms, with the rate predicted to grow substantially (O'Neill, 2016). Thus, before such pathogens reach patients, efforts have focused on controlling the misuse of antibiotics, implementing safe practices of better hygiene and improving sources of safe water.

Typically, disinfectants are added to remove pathogenic microorganisms and to control microbial growth in treated water. Chlorination is an established method of disinfection, but this practice is limited by the production of toxic by-products in cases of hyperchlorination and inefficient disinfection of some pathogens of clinical concern such as *P. aeruginosa* (Bridier et al., 2011; Mao et al., 2018; Hou et al., 2019).

Abbreviations: CFU, Colony forming unit; DBD, Dielectric barrier discharge; EPS, Extracellular polymeric substance; HCAIs, Healthcare associated infections; NHS, National health service; OD, Optical density; PBS, phosphate buffered saline; PI, propidium iodide; SEM, scanning electron microscopy; SCV, small colony variant; UV, ultra-violet; VBNC, viable but nonculturable.

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Installation of point-of-use filters (0.2 μm filtration units) at the exit of water outlets have been reported as effective in terms of treating contaminated water without the drawbacks of chemical based disinfection to subdue HCAI outbreaks (Cervia et al., 2008; Loveday et al., 2014; Falkinham et al., 2015). Over time, however, deposition on the filter increases, resulting in reduced permeate flux, efficiency and reusability of the filter due to biofouling and biofilm formation (Jepsen et al., 2018; Lin et al., 2020). Furthermore, the use of point-of-use filters is costly in terms of hospital wide application and replacement (Hopman et al., 2017; Mathers et al., 2018; Shaw et al., 2018a; Livingston et al., 2018).

There are a range of advanced oxidation processes that are increasingly suggested as a disinfection technology (Miklos et al., 2018), and those processes using non-thermal plasma have gained significant interest as an effective treatment technology for economical and safe chemical-free disinfection of water (Foster, 2017). Plasma-liquid interactions lead to the formation of several reactive species such as hydrogen peroxide, ozone and hydroxyl radicals, in addition to producing ultra-violet (UV) radiation, which may inactivate bacteria (Foster, 2017). Several plasma reactors have adopted various types of plasma discharge and show promising effectiveness against antimicrobial resistance strains of various bacteria inoculated in liquid (Bai et al., 2011; Kim et al., 2013; Mai-Prochnow et al., 2014; Tian et al., 2015; Johnson et al., 2016; Rashmei et al., 2016; Zhang et al., 2017; Shaw et al., 2018b; Pai et al., 2018; Kondeti et al., 2018). Previous macroscale studies have seen dielectric barrier discharge (DBD) based systems cause a 7 log reduction of static samples of *E. coli* after 20 s of treatment, while treatment of static samples of *P. aeruginosa* saw a 4.8 log reduction after 4 min (Ziuzina et al., 2013; Choudhury et al., 2018).

The application of microfluidic reactors has been increasingly investigated for the treatment of chemical contaminants in water over conventional macroscale reactors which have mass transport and fluid control limitations (Dapeng and Jiuhui, 2009; Silva, 2015; Da Silva et al., 2016; Azzouz et al., 2018; Patinglag et al., 2019). Microfluidics refers to devices and methods that allow the control of the spatial and temporal behaviour of fluids within dimensions less than a millimetre (Luo and Duan, 2012). Using a microfluidic reactor can be advantageous due to the large surface area to volume ratio, shorter diffusion distances and high mass transfer efficiency compared to traditional macroscale reactors. Recently, DBD systems within microfluidic platforms have been employed for continuous single and dual phase flow for synthesis applications at atmospheric pressure (Ishi et al., 2015; Wengler et al., 2018).

The aim of this work was to demonstrate the proof of principle that a non-thermal plasma generated along a dual phase liquid-gas flow in a microfluidic reactor was effective for the rapid disinfection of water. Such a system has the possibility to be 'scaled out' and operated as arrays of multiple devices to produce an effective continuous flow water disinfection system at point of use.

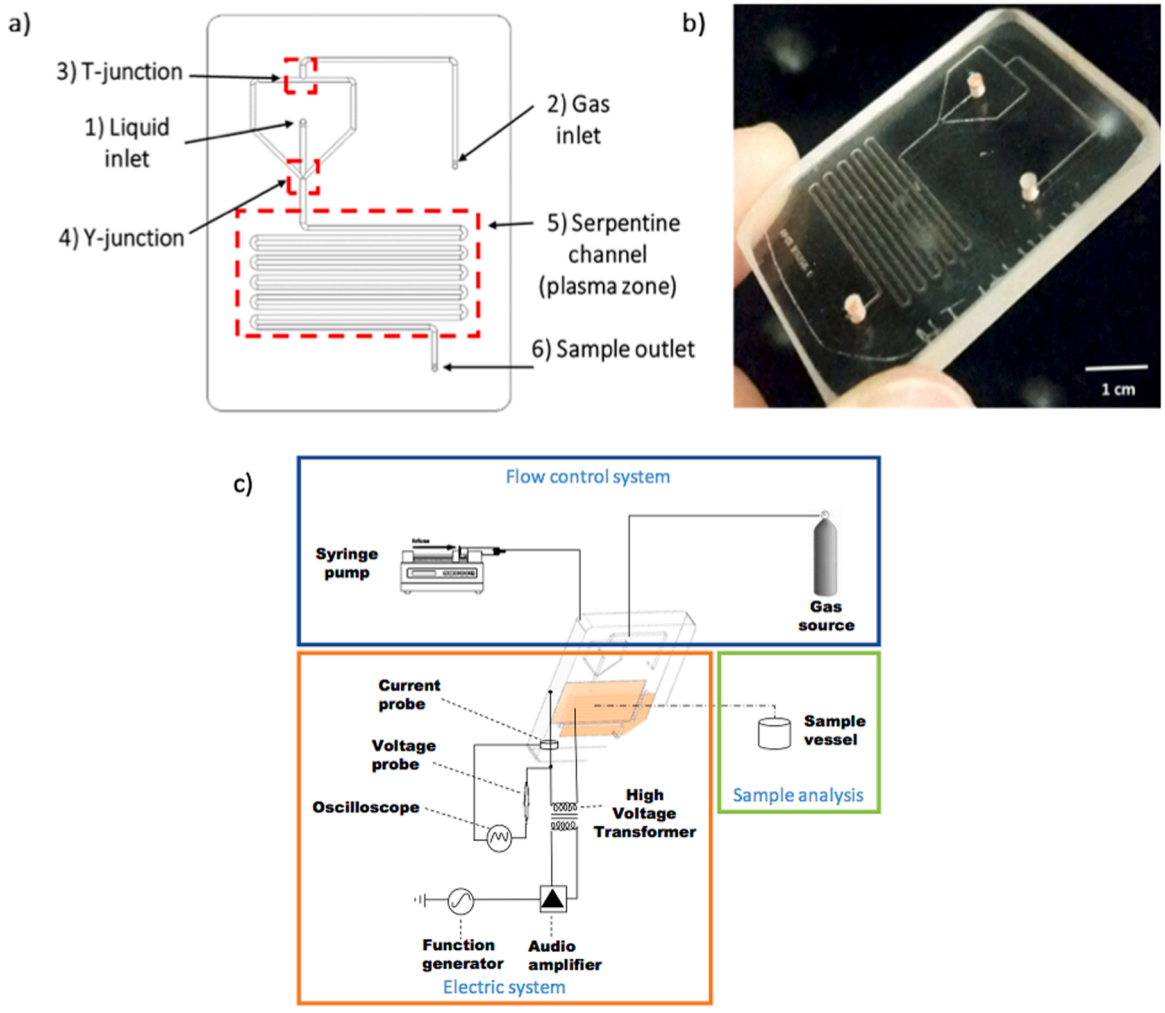

Fig. 1.. (a) Diagram of the microfluidic plasma device. (b) Photograph of the glass microfluidic device used in this study. (c) Diagram of the experimental setup used for all the investigations carried out in this study.

2. Materials and methods

2.1. Microfluidic plasma reactor

The design, manufacture and experimental set-up of the glass microfluidic plasma reactor employed for these experiments have previously been described in detail (Patinglag et al., 2019). Fig. 1 shows a simplified schematic of the microfluidic plasma reactor operated for the present study. Microchannels were etched on a 1 mm glass bottom plate, with a depth of 50 μm and width of 330 μm. Both inlet and outlet holes [1/16″ outer diameter] were drilled through a 1 mm thick glass top plate, aligned with the etched wafer and thermally bonded by heating in a furnace. Fluorinated ethylene propylene tubing [1/16″ OD × 0.030″ inner diameter] was glued, using Araldite two-part epoxy adhesive (Huntsman Advance Materials, UK), to the glass microreactor.

2.2. Microbial samples

E. coli (NCIMB 10,244) and *P. aeruginosa* (ATCC 47,085) were obtained from the National Collection of Industrial, Food and Marine Bacteria, UK and the American Type Culture Collection, US, respectively. Stocks were stored at −80 °C in a freezing mix containing dipotassium hydrogen phosphate (12.6 g/L), potassium dihydrogen phosphate (3.6 g/L), tri-sodium citrate (0.9 g/L), ammonium sulphate (1.8 g/L), glycerol (300 g/L), magnesium sulphate (1.8 g/L) and deionised water. All components used to prepare the freezing mix were from Thermo Fisher Scientific, UK. Frozen stocks were thawed and streaked on nutrient agar media (Oxoid, UK) under sterile conditions and incubated at 37 °C for 24 h. The inoculated agar plates were kept at 4 °C and replaced every 4 weeks. All media such as nutrient broths, phosphate buffered saline (PBS) and agar plates were autoclaved under pressure of 15 psi at 120 °C for 20 min.

A PBS tablet (Oxoid, UK) was dissolved in 100 mL of deionised water, autoclaved and filtered through a 0.22 μm membrane (Pall Acrodisc). The sterile PBS used contained sodium chloride (8.0 g/L), potassium chloride (0.2 g/L), di-sodium hydrogen phosphate (1.15 g/L) and potassium dihydrogen phosphate (0.2 g/L) at a pH of 7.3 ± 0.2 at 25 °C. The resulting culture was centrifuged at 1721 xg for 5 min. Cells were diluted in 10 mL sterile PBS solution, prepared using sterile membrane filtered water (MilliporeElix, USA), to an optical density (OD) of 0.4 at 600 nm using a UV–vis spectrophotometer (Jenway 6305, UK), calibrated against sterile PBS. The pellet was washed by suspending the pellet in filtered PBS solution, re-centrifuged at 1721 xg for 5 min and re-suspended in filtered PBS. The microbial suspension was diluted in filtered PBS to obtain an optical density (OD_{600nm}) of 0.4 for *E. coli* and *P. aeruginosa* using a spectrophotometer, which corresponded to 5.4×10^8 CFU/mL for *E. coli* and 7.0×10^8 CFU/mL for *P. aeruginosa*. Cell counts were determined in colony forming unit (CFU)/mL using serial dilutions and the spread plate method, before and after plasma treatment. A sample of the effluent was also collected to determine the pH after plasma treatment. Using universal indicator paper, due to the small volumes, no change was observed from the original pH of the PBS solution.

2.3. Operation of the device

A gas–liquid two-phase annular flow was generated by parallel injection of compressed air and inoculated liquid from separate inlets of the microfluidic plasma reactor. An in-house regulated gas line was employed to control the gas flow pressure at 2 bars in a channel depth of 50 μm and the total feed liquid flow rate was controlled using the syringe pump at 35 to 100 μL/min. Both air and the inoculated liquid sample were introduced into separate inlets of the glass microfluidic reactor (Fig. 1A, inlets 1 and 2). The electrode voltage from the AC power supply was controlled at a peak-to-peak applied voltage of approximately 10 kV, frequency was set to 17 kHz and average power consumption of 13.0 ± 0.7 W.

Bacterial colonies from planktonic and mixed culture samples were counted before entering the inlet and after exiting the outlet. The collection of samples was carried out after 3 to 5 min without plasma to stabilize the liquid flow and 5 min after initiating plasma generation in the reactor. The experiments were repeated at least three times using independently grown cultures. To test for bacterial adhesion and/or blockage over time, samples were left to flow continuously for 24 h using liquid flow rates of 35, 40, 60, 80 and 100 μL/min liquid flow rates.

2.4. Determination of viable cells

The concentration of viable cells was evaluated using a standard spread plate method. Agar was prepared according to the manufacturer's instructions. The agar solution was sterilized and cooled to 50 °C before addition of the desired antibiotic. Selective Petri dishes were prepared with streptomycin (50 μg/mL) for *E. coli* and ampicillin (100 μg/mL) for *P. aeruginosa*. For mixed bacterial cultures, MacConkey agar (Oxoid, UK) was used for selective differentiation of both bacteria, i.e. *E. coli* colonies appeared as pink in colour while *P. aeruginosa* colonies exhibited no colour. Serial dilutions of the bacterial suspension were prepared, with dilutions up to $1{:}10^8$. Diluted samples of 100 μL were spread onto the surface of the agar plate using a sterile spreader. The inoculated Petri dishes were incubated at 37 °C for 24 h before colony counting. The limit of detection for spread plating 100 μL of the diluted sample was 1 CFU for $1{:}\ 10^8$ dilution and the limit of quantification from a countable range of 30 – 300 CFU.

2.5. Microbial adhesion test

The microfluidic reactor was assessed for blockages and bacterial adherence to the microchannel walls. Bacteria suspended in PBS at a concentration of 10^8 CFU/mL were continuously infused for 24 h into the microfluidic reactor using liquid flow rates of 35, 40, 60 80, 90 and 100 μL/min and 2 bar gas pressure. Excess bacterial suspension in the microchannel was removed and the channel dried with filtered air driven by the syringe pump for 1 h. One percent w/v fluorescein salt/PBS mixture (excitation wavelength of 485 nm and emission filter of 520 nm) (Sigma-Aldrich, UK) was pumped into the microfluidic reactor and excess fluid removed with pumped filtered air. The microchannel was visualised using epifluorescent microscopy (Nikon Eclipse E600 epifluorescence microscope, Japan) mounted with an F-View II black and white digital camera (Soft Imaging System Ltd., UK, supplied by Olympus, UK). This system was operated using Cell-F image visualisation software (Olympus, UK) for image capture and analysis. The microchannel was observed using 1000x magnification to image the fluorescence intensity of the microchannel. The presence of fluorescence at an increased intensity indicated the adherence of bacteria.

2.6. Live/Dead assay

To examine cell viability before and after plasma treatment, samples were treated using fluorescent stains propidium iodide and SYTO 9 (LIVE/DEAD™ BacLight™ bacteria viability kit, Invitrogen, Scotland). Fluorescent stains were diluted in dimethyl sulfoxide (Sigma-Aldrich, UK) according to the manufacturer's instructions and individually diluted in a 1:10 ratio in sterile distilled water. Diluted propidium iodide and SYTO 9 were mixed together in a 1:1 ratio.

Bacterial samples (10 μL), before and after plasma treatment, were loaded separately on the surface of clean stainless-steel coupons (L x W x H: 1.5 mm x 1.5 mm x 1 mm) (SS316 with 2B finish) and dried in a microbiological Class II safety hood. Before bacterial samples were loaded on stainless steel coupons, the surfaces were cleaned with 50% nitric acid, followed by sterile deionised water, ethanol and finally rinsed with sterile deionised water for 15 min each, blown with compressed air and left to dry in a microbiological Class II safety hood.

Populations of live and/or dead bacteria without plasma treatment were investigated as controls to validate that viable bacteria were SYTO9 positive (green) and damaged or nonviable bacteria were propidium iodide positive (red). A control for dead bacterial cells was prepared by centrifuging, at 1721 xg, 5 mL of the microbial culture (OD_{600}: 0.4 for both *E. coli* and *P. aeruginosa*) and washing the pellet with PBS. The cells were re-centrifuged and resuspended in 5 mL of 70% ethanol and left for 1 h. For live bacterial controls, microbial cultures were centrifuged as above and suspended in 5 mL of PBS. For mixed controls, equal volumes of live and dead microbial cultures were combined and vortexed in sterilised Eppendorf tubes.

Prepared mixed solution of bacterial cells and fluorescent stain (10 μL) was spread on steel coupons with samples and left to air dry in the dark. Samples were stored in the dark at 4 °C and analysed for potentially viable or damaged cells using an epifluorescent microscope. The percentage bacterial coverage of live and dead bacteria was measured using separate selective filters for propidium iodide (excitation wavelength of 535 nm and emission filter of 617 nm) and SYTO 9 (excitation wavelength of 485 nm and emission filter of 498 nm) across the same field of view. A minimum of 10 fields of view using each filter were taken per sample.

2.7. Scanning electron microscopy (SEM)

Stainless steel coupons loaded with bacterial samples and unloaded coupons used as a control were immersed in 4% glutaraldehyde (Sigma-Aldrich, UK) overnight at 4 °C to fix the bacterial cells. Autoclaved and filtered deionised water (10 mL) was used at a 45° angle to wash the coupons. Sample coupons were left to dry for 1 h followed by sequential immersion in an ethanol (Sigma-Aldrich)/water mixture (30%, 50%, 70%, 90% and 100%) for 10 min at each concentration and left to dry for a further hour. Prior to SEM analysis, samples were stored at room temperature in a desiccator with silica gel. Samples were attached to aluminium pin stubs with adhesive carbon tabs before sputter coating with gold/palladium coating (Model: SC7640, Polaron, Au target, coating time: 30 s, 5 mA current. 800 V). The SEM was carried out using a Supra 40VP with SmartSEM software (Carl Zeiss Ltd. UK) and images were taken using an acceleration voltage of 2 kV and a working distance of approximately 6 mm.

2.8. Statistical analysis

IBM SPSS Statistic (Version 24) was used to perform statistical calculations. The standard error of the mean is shown on graphs and tables using error bars, and lower/upper limits, respectively. ρ values were determined at the 95% confidence level using two-way ANOVA. Post hoc analysis with Tukey's test was performed for all data. The level of statistical significance was set at $\rho < 0.05$ for the ANOVA and t-test.

3. Results and discussion

3.1. Microbial adhesion

Attachment and colonization of bacteria on the surface of the microchannel introduces the risk of blockage from attached bacteria that initiates aggregation and promotes the formation and growth of biofilms over time (Kragh et al., 2016). This can lead to recontamination of treated water during water distribution. For every treatment, a control experiment with the inoculated sample flowing into the reactor treated only with the flow of air as the carrier gas but without plasma ignition was carried out. No adhesion of large bacterial aggregates was observed on the channel walls but a cloudy appearance was observed in the fluorinated ethylene propylene tubing using *E. coli* inoculated water (Fig. S1). Similar results were observed with *P. aeruginosa*. Bacteria tend to adhere to surfaces, forming microcolonies and developing biofilms (Tuson and Weibel, 2013). In continuous flowing systems, fluid flow can enhance transfer of substrates such as nutrients in the bulk liquid to the adhered bacterial colonies or biofilms (Taherzadeh et al., 2012). This allows the development of colonies and established biofilms on the surface of the channel, which may significantly affect the number of bacteria leaving the reactor via the outlet and cause blockages in the microchannel over time.

The number of bacteria introduced into and removed from the device were counted. No blockage was observed after running the inoculated water with both bacteria for 24 h at various liquid flow rates. No significant reduction in bacterial counts was observed for both *E. coli* and *P. aeruginosa* ($\rho > 0.05$) as the solution containing these bacteria flowed through the reactor (Fig. S2). This could be due to the enhanced fluid velocity and pressure from both air and liquid in the microchannel with small channel diameter on the order of micrometre exerting shear forces on the attaching cells, inhibiting cell attachment and thus resulting in detachment of these cells (Rijnaarts et al., 1993; Donlan, 2002; Taherzadeh et al., 2012). No fluorescing bacteria were observed on channel walls in areas where the plasma was formed (Fig. S1 (d)). This could be due to multiple processes and reactive species formed during plasma ignition. Bacteria are killed by the physical and chemical reactions induced by the plasma species such as chemical etching of the cellular surface which disintegrate their bodies and detach them from the surface (Burts et al., 2009; Scholtz et al., 2015).

3.2. Plasma treatment of bacteria in monoculture samples

Planktonic bacterial samples were treated at various residence times by non-thermal plasma generated in the microchannel. The residence time of the inoculated liquid in the plasma zone was regulated through changing the liquid flow rate entering the microfluidic plasma reactor.

The surviving CFU/mL of the *E. coli* and *P. aeruginosa* as a function of residence time showed that the viability of *E. coli* and *P. aeruginosa* decreased with increasing residence time. There was significant difference ($\rho < 0.05$) between the untreated and plasma treated bacterial samples at various residence times (Fig. 2). Both bacterial strains were 100% inactivated (no viable colonies were observed), using 35 μL/min liquid flow rate with residence time of 5.3 s in the plasma zone. However, compared to *P. aeruginosa, E. coli* was inactivated more rapidly; no colonies were observed at 4.6 s residence time following 24 h of incubation of collected samples after treatment. A statistically significant

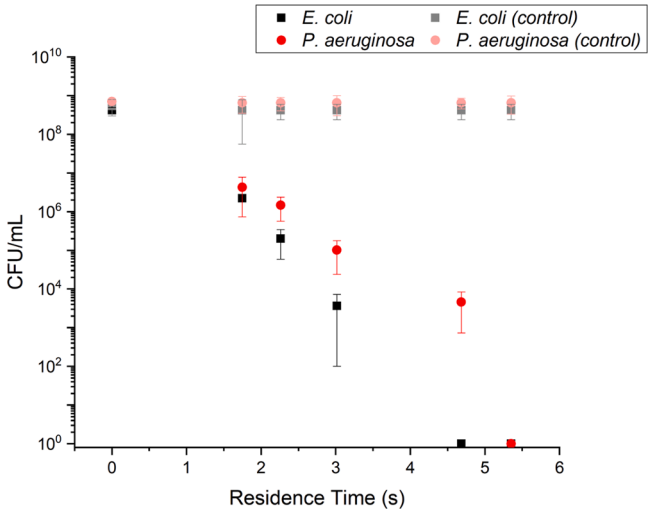

Fig. 2.. Surviving CFU/mL of monoculture *E. coli* and *P. aeruginosa* in water before and after plasma treatment at various residence times. CFU/mL at 0 s indicates the starting CFU of *E. coli* and *P. aeruginosa* in water introduced into the inlet while the subsequent results refer to samples collected from the outlet. Error bars represent standard error of the mean ($n = 3$). Statistical significance, $\rho < 0.05$.

difference ($\rho < 0.05$) was observed between the plasma treated *E. coli* and *P. aeruginosa* as monoculture samples at various residence times. This result indicated that *P. aeruginosa* may require a higher residence time for further interaction between the bacteria and reactive species formed in the gas and liquid phase during plasma ignition or increased plasma density for a higher rate of inactivation (Lu et al., 2014; Ma et al., 2017; Pai et al., 2018; Chandana et al., 2018).

Though both *E. coli* and *P. aeruginosa* are Gram-negative bacteria, the outer membrane of *P. aeruginosa* is 10 to 100-fold less permeable than *E. coli* to reactive molecules or species (Breidenstein et al., 2011; Gellatly and Hancock, 2013) and thus may lead to slow transport of reactive species formed in the gas and liquid phase during plasma ignition into the bacteria (Ziuzina et al., 2015; Šimončicová et al., 2019).

After 72 h of incubation post plasma treatment, colonies of *E. coli* and *P. aeruginosa* were observed on agar plates with samples treated at residence times of between 1 and 3 s. The number of new colonies was low, less than 100 CFU/mL. No new colonies were detected with samples treated with residence times greater than 4 s.

The differences in the residence time of the sample in plasma between this microfluidic reactor system and plasma reactors reported in literature, demonstrate the significance of mass transfer for disinfection by non-thermal plasma in shorter periods, i.e. reducing treatment times from minutes and hours to just seconds. Malik (2010) reported that the surface-area-to-volume ratio of water under treatment affected mass transfer, indicating that high treatment efficiency observed using water spray or film was due to their large surface-area-to-volume ratio . Compared to conventional reactors, with liquid depths of cm or mm, reducing the channel size down to those found in microfluidic devices in the order of microns increases the surface-area-to-volume ratio as the thickness of the water film is reduced to micrometres. This exposes the solution inside the microfluidic reactor to high concentrations of short-lived reactive species. Thus, greater mass transfer and penetration of reactive chemicals from gas into liquid was obtained in this continuous flow configuration using the microfluidic reactor compared to bulk reactors in static configuration where the plasma interacts at the interface of the bulk liquid requiring longer distance and diffusion times.

Complete inactivation of bacteria was achieved using air as the carrier gas for non-thermal plasma generation. Previous studies have shown that the antimicrobial effect of different carrier gases is associated with the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Higher concentrations of ROS with pure O_2, where long-lived species such as ozone can be generated, have been associated to a higher degree of sterilization (Zhang et al., 2017; Zhou et al., 2020). Various studies have shown however, that air, where both ROS and RNS are formed, can achieve comparative results to those using with pure gases, achieving 100% inactivation of bacteria (Cubas et al., 2019; Du et al., 2012). The use of air is advantageous since it is abundant and a cheap source for plasma generation and is hence a cost-effective gas for plasma-based water disinfection system. As the main components of air are oxygen and nitrogen, a combination of ROS and RNS are formed in plasmas. Short-lived species (NO, OH and superoxide) and long-lived species (nitrates, nitrites, ammonium) induce an anti-microbial effect but the full mechanism of the direct oxidative effect induced by each species individually or the synergy of these chemicals (combined with the effects of energetic UV radiation and intense electric fields) has yet to be understood (Zhang et al., 2017; Zhou et al., 2020). In the liquid or gas phase, ROS and RNS can induce depolarization as they enter the bacterial cell which leads to membrane permeation and adsorption of short- and long-lived oxidative species (Lunov et al., 2017). Accumulation in the bacterial cell results in oxidative stress and nitration of cell components. As a result, different types of protein essential for cell functions are damaged and in response, signalling pathways get activated which can lead to DNA damage, cell cycle arrest and triggering of cell death pathways. Several long-living species, such as O_3 and H_2O_2 can induce post-plasma reactions, through the generation of subsequent •OH products via dissociation (Liao et al., 2017; López et al., 2019).

3.3. Plasma treatment of mixed culture samples

A mixture of both *E. coli* and *P. aeruginosa* was used to investigate the efficiency of the microfluidic plasma reactor in treating mixed microbial bacterial cultures. Reduction in viable bacteria with increasing residence time was observed in the mixed culture sample of *E. coli* and *P. aeruginosa* with initial total concentrations of 7.40×10^5 CFU/mL and 5.40×10^5 CFU/mL respectively. No colonies of either bacteria were observed after a residence time of 5 s in the devices with the plasma activated (Fig. 3).

The results of plasma treated mixed culture samples showed no statistical difference ($\rho > 0.05$) between the amount of surviving *E. coli* and *P. aeruginosa*. However, compared to the results with monoculture *E. coli* from the previous section, several colonies of *E. coli* were observed on agar plates with samples treated to less than 4 s of residence time, but all were eliminated after >5 s residence time. Several papers described inactivation of bacteria after plasma treatment, yet few had further examined possible regrowth of colonies after more than 24 h incubation (Yingguang et al., 2011; Sanaei and Ayan, 2015; Ercan et al., 2018). After 72 h of incubation, emerging colonies of *E. coli* and *P. aeruginosa* were observed from samples treated to less than 4 s of residence time in plasma but colony counts of less than 500 CFU/mL were recorded. No new colonies were detected with residence times of more than 4 s. This could be due to the increasing number of bacteria killed with increasing residence time until eventually all bacteria were deactivated.

The regrowth of small colonies from samples treated with shorter residence times may be indicative of small colony variant (SCV) bacteria or viable but nonculturable (VBNC) bacteria. SCV bacteria refers an aberrant form of bacteria generated in response to environmental stress, characterized with a slow growth rate, reduced metabolic activity and increased resistance to antibiotics, which are able to recover over time when provided with essential factors such as nutrients (Proctor et al., 2006). Similar to SCV bacteria, VBNC bacteria (which refers to live bacteria in a state of low metabolic activity and cannot be cultured), remain a concern for potential risks upon exposure. Shorter residence times in plasma may not be sufficient to induce the same capacity of oxidative stress compared to higher residence time to effectively kill the bacteria but could potentially reduce their metabolic activity. The ability of SCV and VBNC bacteria to be resuscitated in favourable conditions, such as nutrient availability and temperature, increases the risk of colonisation and health problems (Proctor et al., 2006; Kriegeskorte et al., 2014; Ramamurthy et al., 2014).

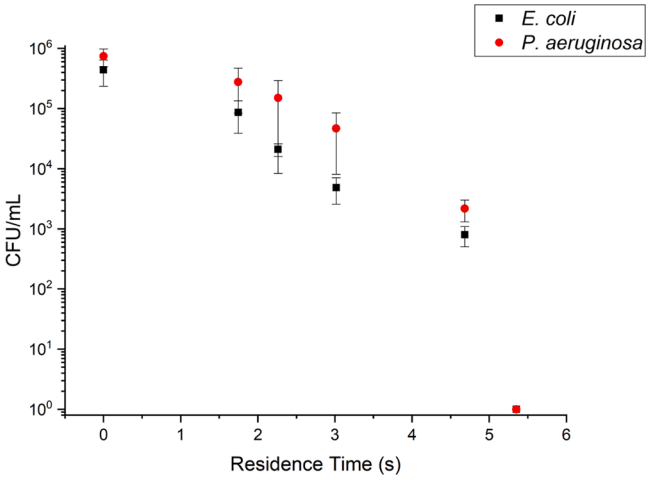

Fig. 3.. Surviving populations of *E. coli* and *P. aeruginosa* in mixed samples after plasma treatment at various residence times. Error bars represent standard error of the mean ($n = 3$). Statistical significance, $\rho < 0.05$.

3.4. Visualisation of bacteria using scanning electron microscopy (SEM)

SEM analysis was used to visualise the effect of non-thermal plasma treatment on the bacteria. Images of untreated *E. coli* and *P. aeruginosa* confirmed the presence of viable cells with the cell membrane appearing smooth and intact (Fig. 4a/d). After plasma treatment, both bacterial species showed distinct signs of damage such as cell membranes with a damaged integrity (white arrows in Fig. 4b), irregular shape and size (white arrows in Fig. 4c/f) and dimples on the membrane surface (white arrows in Fig. 4e).

The appearance of distinct damage on cells after plasma treatment has been previously reported for *E. coli* and *P. aeruginosa*. Plasma treatment compromises cellular viability that results in rupture of the cell membrane, leakage of contents and exposure of genetic material to the surrounding environment, affecting metabolic functions and ability to replicate and grow, leading to death of the bacteria (Ziuzina et al., 2015; Sun et al., 2018). The morphology change observed was associated with the electrostatic disruption of the cell membrane by non-thermal plasma, specifically electrostatic stress caused by charge collection on the outer membrane exceeding the tensile strength of the cell membrane (Mendis et al., 2000). Irregular shape and elongation of cell shape were associated with bacterial stress response and prolonged inhibition of division, which can lead to cell lysis and death (Mojsoska et al., 2017; Jia et al., 2019).

SEM results for shorter residence times showed a mixture of bacteria with intact membranes, damaged membranes and subtle morphology changes. In line with previous plate assay data, regrowth of small colonies observed could be due to treated bacteria with intact membranes that had been damaged by the plasma treatment process, but not killed (Fig. 4b/e). Studies have shown that membrane damage does not guarantee cell death and cells can retain their viability in a VBNC or SCV state (Yost and Joshi, 2015; Mojsoska et al., 2017; Wei and Zhao, 2018; Ye et al., 2020). SEM images of the bacteria treated for higher residence times showed a mixture of damaged membranes and subtle morphological changes (Fig. 4c/f). Compared to shorter residence times, longer residence times showed the absence of bacteria with intact membranes which could be due to more prolonged interaction between the bacteria and reactive species formed in plasma and hence, the number of bacteria killed. In relation to the antimicrobial activity of non-thermal plasma, subtle morphology changes such as variation in shapes, elongation and dimpling observed after plasma treatment were associated with the progressive damage of the cell architecture that leads to ruptured walls

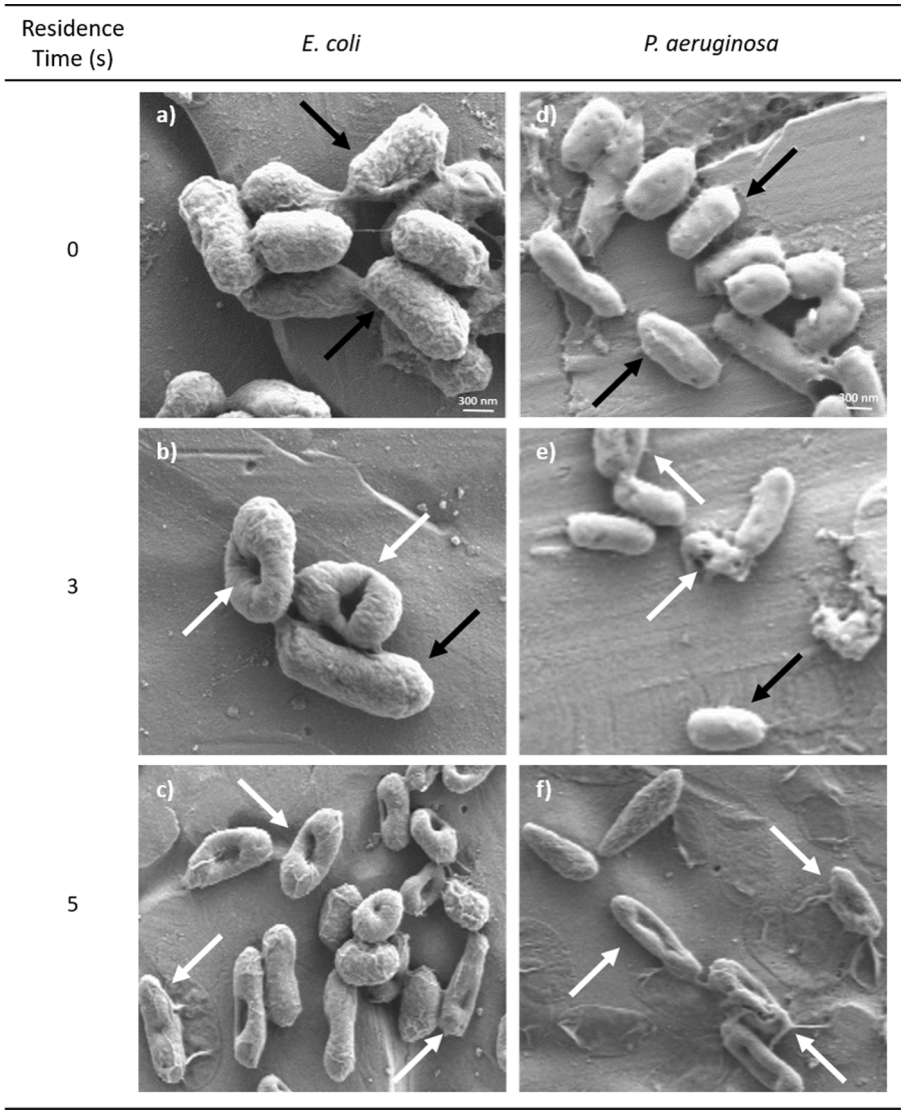

Fig. 4.. SEM images of E. coli (a, b, c) and P. aeruginosa (d, e, f) before and after plasma treatment. Images (a) and (d) correspond to control samples, images (b) and (e) to samples treated with a 3 second residence time and images (c) and (f) to samples treated with a 5 second residence time. Black arrows indicate intact cells while white arrows indicate irregular shape, dimples or damaged cells.

and the release of cellular contents (Joshi et al., 2011; Dobrynin et al., 2011; Yost and Joshi, 2015).

3.5. Cell viability

A Live/Dead assay was conducted on cells treated with non-thermal plasma since SEM was limited in determining viability of treated cells. Plasma treated *E. coli* and *P. aeruginosa* were double stained with DNA intercalating dyes SYTO9/propidium iodide (PI) and observed using an epifluorescence microscope. Viable control *E. coli* and *P. aeruginosa* showed green fluorescence (Fig. 5a/d) while non-viable plasma treated *E. coli* and *P. aeruginosa* showed red fluorescence (Fig. 5b/c/e/f).

Results showed that plasma affected the permeability of the cell membrane, with PI emitting red fluorescence upon binding to the DNA. The results correlated with the SEM results, which showed distinct damage of the membrane caused by non-thermal plasma treatment such as damage to the membrane, dimples or holes, which allowed the DNA intercalating PI dye to enter the bacteria. These morphological changes were related to the decreased viability of bacteria observed after plasma treatment for shorter and higher residence times using the plate assay. Compared to longer residence time, shorter residence time of the bacteria with plasma treatment may not always lead to complete cell death and could potentially induce bacteria into a VBNC or SCV state. Results showed a small fraction of <1% of green fluorescence detected which indicated live cells (Fig. 5b/e) in samples obtained using shorter residence times. This may correspond to some of the bacteria observed to have intact membranes in the SEM images, which may be in a VBNC or SCV state (Fig. 4b/e). However, PI stains nucleic acids in bacteria with damaged membranes but even with damaged membranes, some cells are able to maintain their viability.

SEM observation of cell membrane damage is not conclusive evidence of cell death, since similar subtle morphological changes were observed for both shorter and longer residence times. No green fluorescence was detected in samples treated with higher residence times and after 72 h incubation (Fig. 5c/f). This correlated with the plate assay where no regrowth of colonies was observed in samples treated with higher residence times and after 72 h incubation. The overall effect of non-thermal plasma was to progressively induce substantial damage in the cell structure, which was residence time dependant. A significant decrease in growth, viability and culturability was observed with higher residence times compared to shorter residence times.

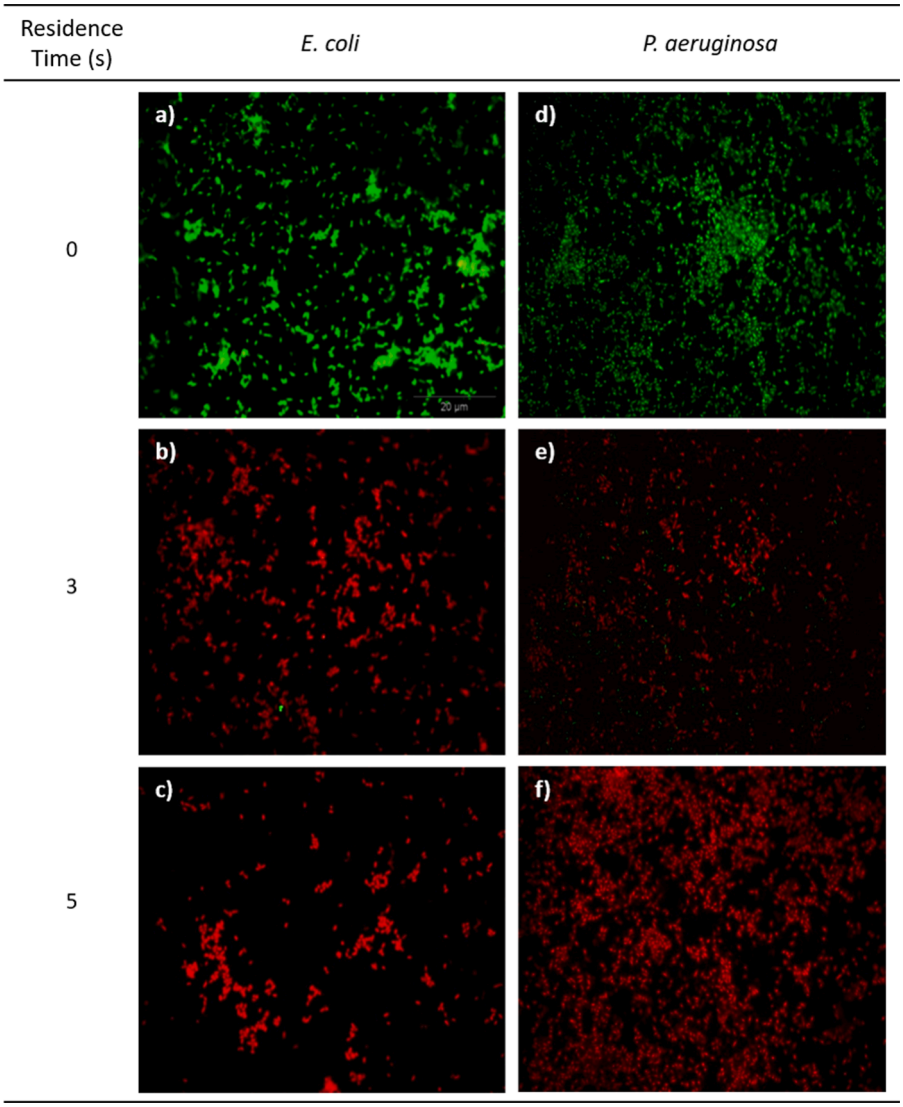

Fig. 5.. E. coli (a, b, c) and P. aeruginosa (d, e, f) viability according to Live/Dead assay results before and after plasma treatment of 3 and 5 s residence time. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

4. Conclusion

Antimicrobial efficacy of a microfluidic plasma reactor was investigated. This microfluidic plasma reactor was able to achieve bacterial inactivation following just seconds of treatment. Both *E. coli* and *P. aeruginosa* showed the same trend, with decreasing bacterial viability as residence time in plasma increased. Using air as the carrier gas, effective disinfection of water was achieved. Full inactivation of both bacteria (10^8 CFU/mL maximum number of each bacteria treated) as monoculture and mixed culture was achieved after 5 s of residence time in the plasma region of the microfluidic plasma reactor. Mixed bacterial culture samples may induce some protection to plasma compared to the monoculture samples. Compared to *E. coli, P. aeruginosa* was less susceptible to the plasma treatment. The ability of plasma to penetrate the bacteria was confirmed with SEM and Live/Dead assays, and post treatment culturing up to 72 h. SEM analysis revealed changes in cell morphology, with ruptured cell membranes, while Live/Dead assays and post treatment culturing revealed viable and non-viable cells after plasma treatment. This work demonstrated the potential for using microfluidic non-thermal plasma systems for the anti-microbial decontamination of water. The microfluidic plasma reactor allowed the control of fluid flow dynamics and utilized its inherent advantages such as a large surface-area-to-volume ratio to facilitate improved mass transfer and plasma penetration of water, leading to cell damage and reducing the viable bacterial counts by 8 orders of magnitude in seconds. The operation of multiple devices in parallel would enable the treatment of substantial volumes of water on demand and this would be ideal for healthcare settings.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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References

Andrew, M., Carl, B., Alex, B., 2018. Raising Standards of Infection Prevention and Control in the NHS. House of Commons.
Azzouz, I., Habba, Y.G., Capochichi-Gnambodoe, M., Marty, F., Vial, J., Leprince-Wang, Y., Bourouina, T., 2018. Zinc oxide nano-enabled microfluidic reactor for water purification and its applicability to volatile organic compounds. Microsyst. Nanoeng. 4, 17093.
Bai, N., Sun, P., Zhou, H., Wu, H., Wang, R., Liu, F., Zhu, W., Lopez, J.L., Zhang, J., Fang, J., 2011. Inactivation of Staphylococcus aureus in water by a cold, He/O2 atmospheric pressure plasma microjet. Plasma Process. Polym. 8 (5), 424–431.
Breidenstein, E.B., de la Fuente-Núñez, C., Hancock, R.E., 2011. Pseudomonas aeruginosa: all roads lead to resistance. Trends Microbiol. 19 (8), 419–426.
Bridier, A., Briandet, R., Thomas, V., Dubois-Brissonnet, F., 2011. Resistance of bacterial biofilms to disinfectants: a review. Biofouling 27 (9), 1017–1032.
Burts, M.L., Alexeff, I., Meek, E.T., McCullers, J.A., 2009. Use of atmospheric non-thermal plasma as a disinfectant for objects contaminated with methicillin-resistant Staphylococcus aureus. Am. J. Infect. Control 37 (9), 729–733.
Cervia, J.S., Ortolano, G.A., Canonica, F.P., 2008. Hospital tap water: a reservoir of risk for health care-associated infection. Infect. Dis. Clin. Pract. 16 (6), 349–353.
Chandana, L., Sangeetha, C.J., Shashidhar, T., Subrahmanyam, C., 2018. Non-thermal atmospheric pressure plasma jet for the bacterial inactivation in an aqueous medium. Sci. Total Environ. 640, 493–500.
Choudhury, B., Portugal, S., Mastanaiah, N., Johnson, J.A., Roy, S., 2018. Inactivation of Pseudomonas aeruginosa and Methicillin-resistant Staphylococcus aureus in an open water system with ozone generated by a compact, atmospheric DBD plasma reactor. Sci. Rep. 8 (1), 17573.
Cubas, A.L.V., de Medeiros Machado, M., dos Santos, J.R., Zanco, J.J., Ribeiro, D.H.B., André, A.S., Debacher, N.A., Moecke, E.H.S., 2019. Effect of chemical species generated by different geometries of air and argon non-thermal plasma reactors on bacteria inactivation in water. Sep. Purif. Technol. 222, 68–74.
Da Silva, B.T., 2015. Development of catalytic microreactors by plasma processes: application to wastewater treatment. Educ. Chem. Eng. 8 (4), e119–e123.
Da Silva, B., Habibi, M., Ognier, S., Schelcher, G., Mostafavi-Amjad, J., Khalesifard, H., Tatoulian, M., Bonn, D., 2016. Silver nanocluster catalytic microreactors for water purification. Eur. Phys. J. Spec. Top. 225 (4), 707–714.
Dapeng, L., Jiuhui, Q., 2009. The progress of catalytic technologies in water purification: a review. J. Environ. Sci. 21 (6), 713–719.
Dobrynin, D., Friedman, G., Fridman, A., Starikovskiy, A., 2011. Inactivation of bacteria using dc corona discharge: role of ions and humidity. New J. Phys. 13 (10), 103033.
Donlan, R.M., 2002. Biofilms: microbial life on surfaces. Emerg. Infect. Dis. 8 (9), 881.
Du, C.M., Wang, J., Zhang, L., Li, H.X., Liu, H., Xiong, Y., 2012. The application of a non-thermal plasma generated by gas–liquid gliding arc discharge in sterilization. New J. Phys. 14 (1), 013010.
Ercan, U.K., İbiş, F., Dikyol, C., Horzum, N., Karaman, O., Yıldırım, Ç., Çukur, E., Demirci, E.A., 2018. Prevention of bacterial colonization on non-thermal atmospheric plasma treated surgical sutures for control and prevention of surgical site infections. PLoS ONE 13 (9), e0202703.
Falkinham, J.O., Hilborn, E.D., Arduino, M.J., Pruden, A., Edwards, M.A., 2015. Epidemiology and ecology of opportunistic premise plumbing pathogens: legionella pneumophila, Mycobacterium avium, and Pseudomonas aeruginosa. Environ. Health Perspect. 123 (8), 749–758.
Ferranti, G., Marchesi, I., Favale, M., Borella, P., Bargellini, A., 2014. Aetiology, source and prevention of waterborne healthcare-associated infections: a review. J. Med. Microbiol. 63 (10), 1247–1259.
Foster, J.E., 2017. Plasma-based water purification: challenges and prospects for the future. Phys. Plasmas 24 (5), 055501.
Gellatly, S.L., Hancock, R.E., 2013. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathog. Dis. 67 (3), 159–173.
Hayward, C., Ross, K.E., Brown, M.H., Whiley, H., 2020. Water as a source of antimicrobial resistance and healthcare-associated infections. Pathogens 9 (8), 667.
Hopman, J., Tostmann, A., Wertheim, H., Bos, M., Kolwijck, E., Akkermans, R., Sturm, P., Voss, A., Pickkers, P., Hoeven, H., 2017. Reduced rate of intensive care unit acquired gram-negative bacilli after removal of sinks and introduction of 'water-free'patient care. Antimicrob. Resist. Infect. Control 6 (1), 59.
Hou, A.M., Yang, D., Miao, J., Shi, D.Y., Yin, J., Yang, Z.W., Shen, Z.Q., Wang, H.R., Qiu, Z.G., Liu, W.L., Li, J.W., 2019. Chlorine injury enhances antibiotic resistance in Pseudomonas aeruginosa through over expression of drug efflux pumps. Water Res. 156, 366–371.
Ishii, C., Stauss, S., Kuribara, K., Urabe, K., Sasaki, T., Terashima, K., 2015. Atmospheric pressure synthesis of diamondoids by plasmas generated inside a microfluidic reactor. Diam. Relat. Mater. 59, 40–46.
Jepsen, K.L., Bram, M.V., Pedersen, S., Yang, Z., 2018. Membrane fouling for produced water treatment: a review study from a process control perspective. Water 10 (7), 847 (Basel).
Jia, M., Liu, Z., Wu, C., Zhang, Z., Ma, L., Lu, X., Mao, Y., Zhang, H., 2019. Detection of Escherichia coli O157: H7 and Salmonella enterica serotype Typhimurium based on cell elongation induced by beta-lactam antibiotics. Analyst 144 (15), 4505–4512.
Johnson, D.C., Bzdek, J.P., Fahrenbruck, C.R., Chandler, J.C., Bisha, B., Goodridge, L.D., Hybertson, B.M., 2016. An innovative non-thermal plasma reactor to eliminate microorganisms in water. Desalin. Water Treat. 57 (18), 8097–8108.
Joshi, S.G., Cooper, M., Yost, A., Paff, M., Ercan, U.K., Fridman, G., Friedman, G., Fridman, A., Brooks, A.D., 2011. Nonthermal dielectric-barrier discharge plasma-induced inactivation involves oxidative DNA damage and membrane lipid peroxidation in Escherichia coli. Antimicrob. Agents Chemother. 55 (3), 1053–1062.
Kim, H.S., Wright, K.C., Hwang, I.W., Lee, D.H., Rabinovich, A., Fridman, A., Cho, Y., 2013. Concentration of hydrogen peroxide generated by gliding arc discharge and inactivation of E. coli in water. Int. Commun. Heat Mass Transf. 42, 5–10.
Kinsey, C.B., Koirala, S., Solomon, B., Rosenberg, J., Robinson, B.F., Neri, A., Halpin, A. L., Arduino, M.J., Moulton-Meissner, H., Noble-Wang, J., Chea, N., 2017. Pseudomonas aeruginosa outbreak in a neonatal intensive care unit attributed to hospital tap water. Infect. Control Hosp. Epidemiol. 38 (7), 801–808.
Kondeti, V.S.K., Phan, C.Q., Wende, K., Jablonowski, H., Gangal, U., Granick, J.L., Hunter, R.C., Bruggeman, P.J., 2018. Long-lived and short-lived reactive species produced by a cold atmospheric pressure plasma jet for the inactivation of Pseudomonas aeruginosa and Staphylococcus aureus. Free Radic. Biol. Med. 124, 275–287.
Kragh, K.N., Hutchison, J.B., Melaugh, G., Rodesney, C., Roberts, A.E., Irie, Y., Jensen, P. Ø., Diggle, S.P., Allen, R.J., Gordon, V., Bjarnsholt, T., 2016. Role of multicellular aggregates in biofilm formation. mBio 7 (2).
Kriegeskorte, A., Grubmüller, S., Huber, C., Kahl, B.C., von Eiff, C., Proctor, R.A., Peters, G., Eisenreich, W., Becker, K., 2014. Staphylococcus aureus small colony variants show common metabolic features in central metabolism irrespective of the underlying auxotrophism. Front. Cell Infect. Microbiol. 4, 141.
Liao, X., Liu, D., Xiang, Q., Ahn, J., Chen, S., Ye, X., Ding, T., 2017. Inactivation mechanisms of non-thermal plasma on microbes: a review. Food Control 75, 83–91.
Lin, W., Ye, C., Guo, L., Hu, D., Yu, X., 2020. Analysis of microbial contamination of household water purifiers. Appl. Microbiol. Biotechnol. 1–13.

Livingston, S.H., Cadnum, J.L., Gestrich, S., Jencson, A.L., Donskey, C.J., 2018. A novel sink drain cover prevents dispersal of microorganisms from contaminated sink drains. Infect. Control Hosp. Epidemiol. 39 (10), 1254–1256.
López, M., Calvo, T., Prieto, M., Múgica-Vidal, R., Muro-Fraguas, I., Alba-Elías, F., Alvarez-Ordóñez, A., 2019. A review on non-thermal atmospheric plasma for food preservation: mode of action, determinants of effectiveness, and applications. Front. Microbiol. 10 article 622.
Loveday, H., Wilson, J., Kerr, K., Pitchers, R., Walker, J., Browne, J., 2014. Association between healthcare water systems and Pseudomonas aeruginosa infections: a rapid systematic review. J. Hosp. Infect. 86 (1), 7–15.
Lu, H., Patil, S., Keener, K.M., Cullen, P.J., Bourke, P., 2014. Bacterial inactivation by high-voltage atmospheric cold plasma: influence of process parameters and effects on cell leakage and DNA. J. Appl. Microbiol. 116 (4), 784–794.
Lunov, O., Zablotskii, V., Churpita, O., Lunova, M., Jirsa, M., Dejneka, A., Kubinová, Š., 2017. Chemically different non-thermal plasmas target distinct cell death pathways. Sci. Rep. 7 (1), 1–16.
Luo, D., Duan, Y., 2012. Microplasmas for analytical applications of lab-on-a-chip. TrAC Trends Anal. Chem. 39, 254–266.
Ma, S., Kim, K., Huh, J., Hong, Y., 2017. Characteristics of microdischarge plasma jet in water and its application to water purification by bacterial inactivation. Sep. Purif. Technol. 188, 147–154.
Mai-Prochnow, A., Murphy, A.B., McLean, K.M., Kong, M.G., Ostrikov, K.K., 2014. Atmospheric pressure plasmas: infection control and bacterial responses. Int. J. Antimicrob. Agents 43 (6), 508–517.
Malik, M.A., 2010. Water purification by plasmas: which reactors are most energy efficient? Plasma Chem. Plasma Process. 30 (1), 21–31.
Mao, G., Song, Y., Bartlam, M.G., Gao, G., Wang, Y., 2018. Long-term effects of residual chlorine on Pseudomonas aeruginosa in simulated drinking water fed with low AOC medium. Front. Microbiol. 9, 879.
Mathers, A.J., Vegesana, K., German Mesner, I., Barry, K.E., Pannone, A., Baumann, J., Crook, D.W., Stoesser, N., Kotay, S., Carroll, J., Sifri, C.D., 2018. Intensive care unit wastewater interventions to prevent transmission of multispecies Klebsiella pneumoniae carbapenemase–producing organisms. Clin. Infect. Dis. 67 (2), 171–178.
Mendis, D., Rosenberg, M., Azam, F., 2000. A note on the possible electrostatic disruption of bacteria. IEEE Trans. Plasma Sci. 28 (4), 1304–1306.
Miklos, D.B., Remy, C., Jekel, M., Linden, K.G., Drewes, J.E., Hübner, U., 2018. Evaluation of advanced oxidation processes for water and wastewater treatment – A critical review. Water Res. 139, 118–131.
Mojsoska, B., Carretero, G., Larsen, S., Mateiu, R.V., Jenssen, H., 2017. Peptoids successfully inhibit the growth of gram negative E. coli causing substantial membrane damage. Sci. Rep. 7, 42332.
Naidoo, S., Olaniran, A.O., 2014. Treated wastewater effluent as a source of microbial pollution of surface water resources. Int. J. Environ. Res. Public Health 11 (1), 249–270.
O'Neill, J., 2016. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations, 2016. HM Government and Wellcome Trust, UK.
Pai, K., Timmons, C., Roehm, K.D., Ngo, A., Narayanan, S.S., Ramachandran, A., Jacob, J.D., Ma, L.M., Madihally, S.V., 2018. Investigation of the roles of plasma species generated by surface dielectric barrier discharge. Sci. Rep. 8 (1), 16674.
Patinglag, L., Sawtell, D., Iles, A., Melling, L.M., Shaw, K.J., 2019. A microfluidic atmospheric-pressure plasma reactor for water treatment. Plasma Chem. Plasma Process. 39 (3), 561–575.
Proctor, R.A., Von Eiff, C., Kahl, B.C., Becker, K., McNamara, P., Herrmann, M., Peters, G., 2006. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat. Rev. Microbiol. 4 (4), 295–305.
Ramamurthy, T., Ghosh, A., Pazhani, G.P., Shinoda, S., 2014. Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. Front. Public Health 2, 103.
Rashmei, Z., Bornasi, H., Ghoranneviss, M., 2016. Evaluation of treatment and disinfection of water using cold atmospheric plasma. J. Water Health 14 (4), 609–616.
Rijnaarts, H.H., Norde, W., Bouwer, E.J., Lyklema, J., Zehnder, A.J., 1993. Bacterial adhesion under static and dynamic conditions. Appl. Environ. Microbiol. 59 (10), 3255–3265.
Sanaei, N., Ayan, H., 2015. Bactericidal efficacy of dielectric barrier discharge plasma on methicillin-resistant Staphylococcus aureus and Escherichia coli in planktonic phase and colonies in vitro. Plasma Med. 5 (1).
Scholtz, V., Pazlarova, J., Souskova, H., Khun, J., Julak, J., 2015. Nonthermal plasma-a tool for decontamination and disinfection. Biotechnol. Adv. 33 (6), 1108–1119.
Shaw, E., Gavaldà, L., Càmara, J., Gasull, R., Gallego, S., Tubau, F., Granada, R., Ciercoles, P., Dominguez, M.A., Manez, R., Carratalà, J., 2018a. Control of endemic multidrug-resistant Gram-negative bacteria after removal of sinks and implementing a new water-safe policy in an intensive care unit. J. Hosp. Infect. 98 (3), 275–281.
Shaw, P., Kumar, N., Kwak, H.S., Park, J.H., Uhm, H.S., Bogaerts, A., Choi, E.H., Attri, P., 2018b. Bacterial inactivation by plasma treated water enhanced by reactive nitrogen species. Sci. Rep. 8 (1), 11268.
Shi, H., Cheng, X., Wu, Q., Mu, R., Ma, Y., 2012. Assessment and removal of emerging water contaminants. J. Environ. Anal. Toxicol. 2, 2161-0525, S2: 003.
Šimončicová, J., Kryštofová, S., Medvecká, V., Ďurišová, K., Kaliňáková, B., 2019. Technical applications of plasma treatments: current state and perspectives. Appl. Microbiol. Biotechnol. 103 (13), 5117–5129.
Sun, Y., Zhang, Z., Wang, S., 2018. Study on the bactericidal mechanism of atmospheric-pressure low-temperature plasma against Escherichia coli and its application in fresh-cut cucumbers. Molecules 23 (4), 975.
Taherzadeh, D., Picioreanu, C., Horn, H., 2012. Mass transfer enhancement in moving biofilm structures. Biophys. J. 102 (7), 1483–1492.
Tian, Y., Ma, R., Zhang, Q., Feng, H., Liang, Y., Zhang, J., Fang, J., 2015. Assessment of the physicochemical properties and biological effects of water activated by non-thermal plasma above and beneath the water surface. Plasma Process. Polym. 12 (5), 439–449.
Tuson, H.H., Weibel, D.B., 2013. Bacteria-surface interactions. Soft Matter 9 (17), 4368–4380.
Walker, J., Moore, G., 2015. Pseudomonas aeruginosa in hospital water systems: biofilms, guidelines, and practicalities. J. Hosp. Infect. 89 (4), 324–327.
Wei, C., Zhao, X., 2018. Induction of viable but nonculturable Escherichia coli O157: H7 by low temperature and its resuscitation. Front. Microbiol. 9, 2728.
Wengler, J., Ognier, S., Zhang, M., Levernier, E., Guyon, C., Ollivier, C., Fensterbank, L., Tatoulian, M., 2018. Microfluidic chips for plasma flow chemistry: application to controlled oxidative processes. React. Chem. Eng. 3 (6), 930–941.
Ye, C., Lin, H., Zhang, M., Chen, S., Yu, X., 2020. Characterization and potential mechanisms of highly antibiotic tolerant VBNC E. coli induced by low level chlorination. Sci. Rep. 10 (1), 1–11.
Yingguang, C., Ping, Y., Xinpei, L., Zilan, X., Tao, Y., Qing, X., Ziyong, S., 2011. Efficacy of atmospheric pressure plasma as an antibacterial agent against Enterococcus faecalis in vitro. Plasma Sci. Technol. 13 (1), 93.
Yost, A.D., Joshi, S.G., 2015. Atmospheric nonthermal plasma-treated PBS inactivates Escherichia coli by oxidative DNA damage. PLoS ONE 10 (10), e0139903.
Zhang, Z., Xu, Z., Cheng, C., Wei, J., Lan, Y., Ni, G., Sun, Q., Qian, S., Zhang, H., Xia, W., Shen, J., 2017. Bactericidal effects of plasma induced reactive species in dielectric barrier gas–liquid discharge. Plasma Chem. Plasma Process. 37 (2), 415–431.
Zhou, R., Zhou, R., Wang, P., Xian, Y., Mai-Prochnow, A., Lu, X., Cullen, P.J., Ostrikov, K. K., Bazaka, K., 2020. Plasma-activated water: generation, origin of reactive species and biological applications. J. Phys. D Appl. Phys. 53 (30), 303001.
Ziuzina, D., Patil, S., Cullen, P., Keener, K., Bourke, P., 2013. Atmospheric cold plasma inactivation of Escherichia coli in liquid median inside a sealed package. J. Appl. Microbiol. 114 (3), 778–787.
Ziuzina, D., Boehm, D., Patil, S., Cullen, P., Bourke, P., 2015. Cold plasma inactivation of bacterial biofilms and reduction of quorum sensing regulated virulence factors. PLoS ONE 10 (9), e0138209.