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Treatment of *Helicobacter pylori* with dielectric barrier discharge plasma causes UV induced damage to genomic DNA leading to cell death

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HIGHLIGHTS

- A dielectric barrier discharge (DBD) plasma torch was used for sterilization.
- *H. pylori* was used as a model organism to test efficiency of the DBD plasma torch.
- Treatment of *H. pylori* with DBD plasma induced DNA damage.
- DBD plasma induced a small temperature rise, UV generation, and H_2O_2 production.
- UV from the DBD plasma device caused DNA injury and inactivation of *H. pylori*.

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ABSTRACT

Gastrointestinal endoscopy is an important tool for the indentification and treatment of disorders of the gastrointestinal tract. However, nosocomial infections of *Helicobacter pylori* have been linked to the use of contaminated endoscopes. Disinfectants such as glutaraldehyde, ortho-phthalaldehyde and peracetic acid are generally used in the reprocesssing of endoscopes, but these chemicals are hazardous to human health. Thus, safer reprocessing and disinfecion methods are needed. In this study, we applied a dielectric barrier discharge (DBD) plasma torch for inactivation of *H. pylori* to investigate a potential new methodology to disinfect endoscopes. Suspensions of *H. pylori* in 10% glycerol were subjected to the DBD plasma torch, which reduced the viable cell count to undetectable levels after 2 min of treatment. Furthermore, urease activity of *H. pylori* was eliminated after 2 min-plasma treatment, while plasma-treatment reduced the intact DNA of *H. pylori* in a time-dependent manner. Next, we examined several potential bactericidal factors produced by the DBD plasma torch. Two min-plasma treatment resulted in a small temperature rise (4 °C), ultraviolet radiation (UV) generation, and the production of hydrogen peroxide. *H. pylori* samples were then exposed to equivalent levels of each of these factors in turn. Our results showed that treatment with heat and hydrogen peroxide at the levels produced after 2-min of plasma treatment did not efficiently inactivate *H. pylori*, whereas exposure to UV had a significant bactericidal effect. Taken together, UV generated by the plasma torch may be crucial for efficient inactivation of *H. pylori* by damaging the bacterial DNA.

1. Introduction

Helicobacter pylori is a major causative agent of gastritis, and a risk factor for duodenal ulcer disease and gastric ulcer disease as well as gastric lymphoma (Graham, 1994; Cave, 1997). Etiological data support a person-to-person transmission of *H. pylori* (Brown, 2000), although nosocomial infection is the only proven mode by which the bacterium can be acquired (Fantry et al., 1995). *H. pylori* is a Gram-negative spiral bacterium that is usually found in the stomach. The bacterium produces urease that decomposes urea in the gastric mucus into carbon dioxide and ammonia (Kusters et al.,

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2006). The formation of ammonia locally neutralizes gastric acid enabling *H. pylori* to fix to the stomach.

Gastrointestinal endoscopy is a useful tool for the identification and treatment of disorders of the gastrointestinal tract. To prevent nosocomial infections, endoscopes should be appropriately reprocessed after disinfection in accordance with reprocessing and infection control guidelines (Nelson, 2003). However, this expensive piece of equipment is fragile and is not resistant to heat or pressure (Reichert and Schultz, 2000). Thus, only a limited number of methods are available for disinfection of gastrointestinal endoscopes. Disinfectants such as glutaraldehyde, ortho-phthalaldehyde and peracetic acid are generally used to disinfect this equipment. A list of liquid chemical sterilants can be found on the U.S. Food and Drug Administration (FDA) Internet homepage (U.S. Food and Drug Administration, 2015). Although glutaradehyde is both cost-effective and efficient at disinfecting endoscopes, its vapors can irritate the respiratory tract after prolonged exposure. Furthermore, an adequate concentration and contact time of solution to all surfaces at an effective temperature is required for complete disinfection. Thus, variance in the conditions can alter the outcome of the disinfection process. Ortho-phthalaldehyde and peracetic acid are listed as possible alternatives to glutaraldehyde, but none of these agents have completely satisfied the ideal properties of displaying rapid bactericidal effects at levels that are non-toxic and non-irritant to humans (British Society of Gastroenterology Endoscopy Committee, 1998; Ayliffe, 2000).

Ethylene oxide gas sterilization can be an effective method for disinfecting medical devices that are not resistant to heat (Reichert and Schultz, 2000). However, there remain problems associated with the potential harmful effects of ethylene oxide to the employee, patient and environment (Shintani, 2017). Increased awareness of safety issues together with environmental concerns may limit the future use of ethylene oxide sterilization.

Hydrogen peroxide gas plasma sterilizers, such as STERRAD®, have been used to sterilize bronchoscopes and other instruments with narrow lumens and channels (Okpara-Hofmann et al., 2005). Similarly, sterilizers using hydrogen peroxide gas and formaldehyde gas have been developed, although it remains unclear whether these procedures are compatible with the materials that make up an endoscope (Reichert and Schultz, 2000; Kanemitsu et al., 2005). Furthermore, this methodology poses a potential hazard to members of staff and requires prolonged aeration of up to 24 h, which considerably increases the overall processing time. Taken together, although current reprocessing methods are effective and meet the requirements of infection-control, more effective techniques with enhanced safety and higher throughput are needed that will not damage the endoscope.

With this goal in mind, we aimed to establish a disinfection method for *H. pylori* using gas plasma. We recently studied innovative disinfection methods for various bacteria, viruses, and fungi (Sakudo and Shintani, 2011; Laroussi et al., 2012; Shintani and Sakudo, 2016). During the course of this research we developed a dielectric barrier discharge (DBD) plasma torch composed of a ceramic tube, stainless steel mesh/wire, and copper tape that delivers a high-voltage and high frequency pulse to air using a power supply to generate gas plasma. Here, we have examined whether the DBD plasma torch can efficiently disinfect a suspension of *H. pylori*. Furthermore, to determine the disinfection mechanism of *H. pylori* mediated by the DBD plasma torch, we investigated potential changes to the bacterial components after DBD plasma treatment using biochemical and morphological analyses. Disinfection factors that may be responsible for the observed bactericidal activity of the DBD plasma torch were analyzed, including heat, ultraviolet radiation (UV) and hydrogen peroxide. In order to identify which of these factors were particularly important for disinfection, we subjected *H. pylori* to each factor in turn to assess their individual contribution. Finally, the proposed disinfection mechanisms are discussed in detail.

2. Materials and methods

2.1. DBD plasma torch

We designed a torch used for plasma generation. The torch was constructed from a ceramic tube (Al_2O_3) with an inner diameter of 4 mm, outer diameter of 6 mm and overall length of 100 mm. Copper tape (0.08 mm thickness and 60 mm length) was wound around the tube as an earth electrode (Fig. 1). A stainless steel mesh (SUS304) with stainless steel wires was located inside the tube to act as a high voltage electrode. The two electrodes were conneced to a high-voltage power supply unit with low frequency (10 kV peak-to-peak, 10 kHz). These power supply settings were successfully used for DBD plasma generation from air in a previous study (Toyokawa et al., 2017). An advantage of generating plasma derived from air is that it does not require a regular supply of purified gas,

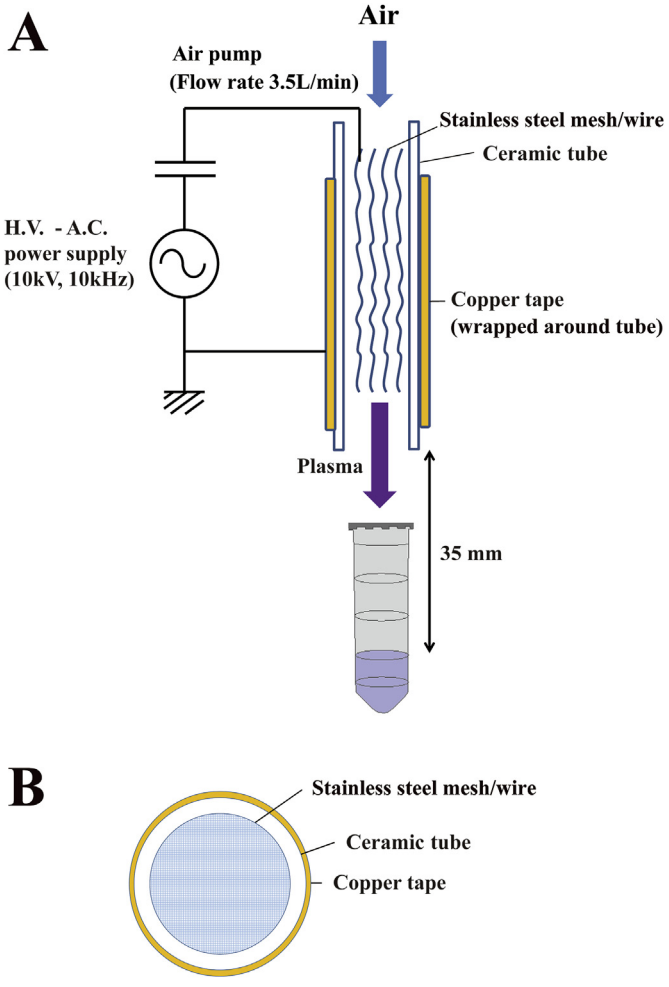

Fig. 1. Schematic representation of the dielectric barrier discharge (DBD) plasma torch. (A) The DBD plasma device comprised a ceramic tube (Al_2O_3), containing a stainless-steel mesh, which was covered with copper tape. The copper tape and stainless steel mesh/wire were connected to a power supply (10 kV, 10 kHz). During gas plasma generation, air flow was maintained at 3.5 L/min using an air pump. The distance from the tip of the plasma torch to the liquid surface was set at 35 mm. (B) Cross-sectional view of the torch, which is composed of a ceramic tube (4 mm inner diameter; 6 mm outer diameter) containing a stainless-steel mesh and covered with copper tape (80 μm thickness) on the outside.

thereby reducing the operating cost. The flow rate of air was maintained at 3.5 L/min using an air pump (Suishin SSPP-2S; Suisaku Co., Sakai, Japan). Aliquots (200 μl) of *H. pylori* suspension in 10% glycerol in a 1.5 mL Eppendorf tube was subjected to treatment using the DBD plasma torch at a distance of 35 mm from the torch tip to the liquid surface.

2.2. Bacterial culture and colony count assay

Cultures of *H. pylori* NCTC11639 (American Type Culture Collection: ATCC43629) on horse blood agar medium (Columbia HP agar medium; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was used for plasma treatment. Culture conditions of *H. pylori* were microaerophilic at 37 °C using a diamond micro-aerobic pack pouch (LSI Medience Corporation, Tokyo, Japan). Plasma-treated *H. pylori* was spread onto the horse blood agar medium and incubated for 3 d at 37 °C. The number of colonies was then counted to determine the colony forming units per mL (CFU/mL).

2.3. Immunochromatography

H. pylori antigens were detected using the Testmate Rapid Pylori Antigen (Wakamoto Pharmaceutical Co. Ltd., Kanagawa, Japan) and ImmunoCard STAT! HpSA (Catalogue number 750720, Meridian Bioscience Inc., Cincinnati, OH, USA), according to the manufacturer's instructions. Testmate Rapid Pylori Antigen kit, which uses labelled monoclonal antibody 21G2, specifically recognizes native catalase *H. pylori* antigen (Suzuki et al., 2002; Sato et al., 2012).

ImmunoCard STAT! HpSA recognizes unknown *H. pylori* antigens (Wu et al., 2006). Briefly, the assay procedure was as follows. A 40 μL aliquot of sample from the 200 μL of plasma-treated *H. pylori* suspension was solublized with diluent buffer. The sample suspension (One drop for Testmate Rapid Pylori Antigen and 4 drops for ImmunoCard STAT! HpSA) was placed on the specimen application region of the test strip. After incubation at room temperature (10 min for Testmate Rapid Pylori Antigen; 5 min for ImmunoCard STAT! HpSA), the labelled antibody-antigen immune complex or free antibody migrate and are further captured by solid-phase anti-IgG antibodies forming a control line. The results were considered positive for *H. pylori* if both the control and test lines were formed and the results were considered negative if only the control line was formed. The band intensity of test lines were then compared between samples before and after DBD plasma torch treatment.

2.4. Urease assay

Urease assays were performed using MR Urea® in accordance with the standard protocol (Institute of Immunology Co., Ltd., Tokyo, Japan). This assay kit relies on *H. pylori* urease converting urea to ammonia. The reaction scheme is shown below.

$$(NH_2)_2CO + 2\ H_2O + H^+ \rightarrow 2NH_4^+ + HCO_3^-$$

The experimental procedure is as following. Briefly, 40 μL of plasma-treated *H. pylori* was added to the substrate reagent incuding 0.5 mL of solvent. After incubation at 25 °C for 2 h, the color change of the solution was compared between untreated and treated samples. The hydrolyzing activity of *H. pylori* urease was determined by monitoring the color change in the solution (yellow to red) containing a pH indicator, phenol red.

2.5. DNA-polymerase chain reaction (PCR)

Bacterial 16S rDNA PCR kit (Takara Bio Inc., Shiga, Japan) was used for amplification of intact genomic DNA from *H. pylori*. Samples (plasma-treated or untreated *H. pylori*) were diluted with distilled water (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) and centrifuged at 15,000 rpm for 3 min. The supernatants were then boiled for 10 min to extract the genomic DNA. The resultant DNA samples were subjected to 30 cycles of PCR comprising of the following steps: 94 °C for 0.5 min, 55 °C for 0.5 min and 72 °C for 1 min using a PC320 thermal cycler (Astec Co. Ltd., Fukuoka, Japan). PCR products were subsequently subjected to agarose gel electrophoresis using a 1% agarose gel (Agarose-RE; Nacalai Tesque, Inc., Kyoto, Japan) and visualized using WSE-5200 Printgraph 2 M (ATTO Corporation, Tokyo, Japan). The amplified products were directly subjected to DNA sequencing using primers F1 and R1 (*bacterial*) in the above kit. Sequencing was performed using a ABI373OXL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

2.6. Thermography

The temperature of each sample was measured using an infrared thermography instrument (FLIR i5; FLIR systems, Wilsonville, OR, USA) as described previously (Toyokawa et al., 2017).

2.7. Measurement of optical emission spectra

A multichannel spectrophotometer (S-2431, Soma Optics Ltd., Tokyo, Japan) attached to a fiber optic probe was used for measuring the emission of 200–800 nm during DBD plasma torch operation. Spectra were collected for 400 msec with 20 times integration time.

2.8. Treatment of H. pylori with ultraviolet radiation (UV) using a transilluminator

Aliquots (200 μL) of *H. pylori* suspension in 10% glycerol were spotted onto cover glasses (Matsunami Glass Industries Ltd., Osaka, Japan) and subjected to UV as follows. The dried spots were irradiated with long-wave ultraviolet radiation (UV-A) using a UV transilluminator UVGL-58 (UVP, Upland, CA, USA) for 0–30 min. The distance between the spots and the UV transilluminator was maintained at 35 mm. The energy (mJ/cm^2) of UV were estimated on the basis of color changes to UV indicators (UV label-H) (NiGK Corporation, Tokyo, Japan). Treated *H. pylori* samples were used for the colony count assay as described above.

2.9. Statistical analysis

The results are the mean ± standard deviation of experiments conducted at least in triplicate. The statistical analysis of significant difference was performed by non-repeated analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The analysis was carried out using GraphPad Prism 5 software (GraphPad Prism Software Inc., La Jolla, CA).

3. Results

Cultures of *H. pylori* suspended in a 10% glycerol solution were treated for 0–5 min with the DBD plasma torch for 0–5 min. The resultant samples were then plated and colony-counted after 3 d of culture. The results showed that the viable cell count of *H. pylori* was 612 ± 76 CFU/mL at 0 min, 396 ± 98 CFU/mL at 0.5 min, 352 ± 13 CFU/mL at 1 min after the plasma treatment (Fig. 2). Samples treated with plasma for 2 min had a viable cell count below the detection limit. From the data, we calculated a decimal reduction time (*D* value, decimal reduction value), which is the time required to achieve 90% (or log unit) inactivation of a population of a given test microorganism. The *D* value calculated from

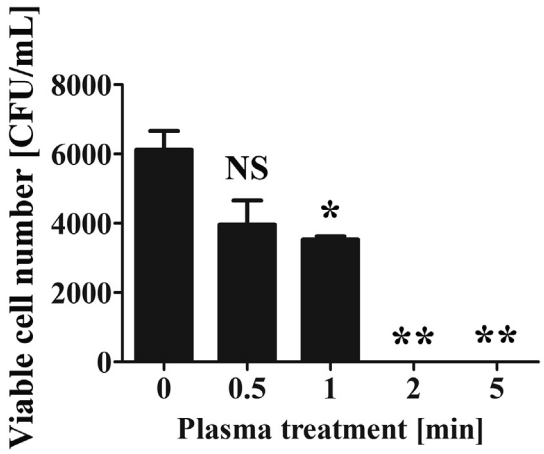

Fig. 2. Treatment with the DBD plasma torch reduced viable cell number of *Helicobacter pylori*. A suspension of *H. pylori* was exposed to the DBD plasma torch for the indicated time (min). Viable cell count [colony forming units (CFU)/mL] was then determined before and after treatment. Differences where $p < 0.05$(*) and $p < 0.01$(**) versus control (0 min) were considered significant, while NS means no significance.

the initial slope within 1-min of DBD plasma treatment was 1.91 min, while that calculated from the single-slope approximation within 2-min of DBD plasma treatment was 1.69 min.

Next, to further examine the effect of the plasma torch on *H. pylori*, plasma-treated *H. pylori* was subjected to Scanning electron microscopy (SEM) (Fig. S1). No major morphological changes were observed after the plasma torch treatment for 5 min or less. These findings suggest that the DBD plasma treatment did not introduce any gross modifications to the bacterial surface of *H. pylori*.

Next, we examined the effect of plasma treatment on components of *H. pylori* using various biochemical analyses. Immunochromatography was performed using a Testmate Rapid Pylori Antigen® kit, which detects *H. pylori* catalase, and a ImmunoCard STAT! HpSA kit, which uses antibodies that recognize unidentified *H. pylori* antigens. Both these approaches revealed no change of band intensity in the test line (catalase or unidentified antigen) before and after plasma treatment (0–5 min) (Fig. 3).

In addition, we also analyzed any change in *H. pylori* urease activity using MR Urea® (Fig. 4). In these experiments, the amount

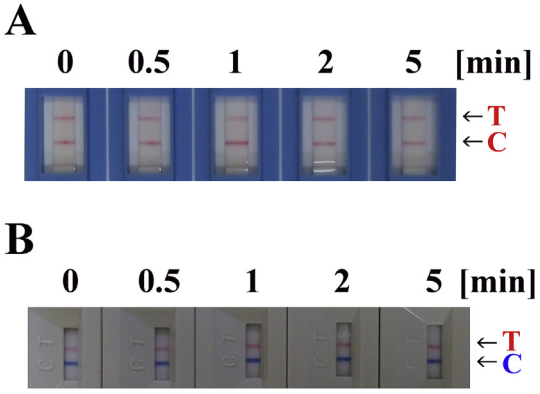

Fig. 3. DBD plasma treatment causes no detectable change in *H. pylori* proteins. A suspension of *H. pyroli* was treated with the DBD plasma torch for the indicated time (min) and then subjected to immunochromatography to detect (A) native *H. pylori* catalase using ImmunoCard STAT! HpSA (Meridian Bioscience Inc.) and (B) unknown *H. pylori* antigen using Testmate Rapid Pylori Antigen (Wakamoto Pharmaceutical Co. Ltd.). The bands corresponding to *H. pylori* proteins are indicated by arrows with the test line (T). The control lines (C) are also highlighted with arrows.

of ammonia produced by the decomposition of urea was used as an index. Urease activity, secreted from the bacterial cells, was detected at 0, 0.5, and 1 min after DBD plasma treatment, but not after 2 or 5 min of treatment. These observations suggest complete inactivation of urease activity brought about by plasma treatment for more than 2 min.

To further investigate the effect of DBD plasma-treatment on *H. pylori*, biochemical changes to the genomic DNA were also analyzed. DNA-PCR was carried out by using sequence-specific primers for bacterial 16S rDNA (Fig. 5). Analysis of the PCR mix ($N = 4$) by agarose gel electrophoresis revealed a band in the untreated *H. pylori* sample (0 min), which was verified by DNA sequencing to correspond to an amplified product corresponding to *H. pylori* 16S rDNA (98–99% identical to Genbank accession number U00679). A band of the same size was also detected in *H. pylori* samples treated with the DBD plasma torch for 0.5, 2, 5, 10, and 15 min, although the intensity of the amplified DNA was slightly reduced in the 2 and 5 min samples compared to 0 min (untreated *H. pylori*) and considerably reduced in the 10 and 15 min samples by comparison with 0 min.

Next, we analyzed the potential disinfection factors generated during operation of the DBD plasma torch in turn. Analysis of the emission spectrum (200–800 nm) of the DBD plasma torch indicated that long wavelength UV radiation UV-A (320–400 nm) mainly generated during plasma production showed strong peaks around 300–500 nm (Fig. 6). The peaks may be due to N_2 emission of the second positive system ($C^3\Pi u - B^3\Pi g$). This region covered not only UV-A but also medium wavelength UV radiation UV-B (280 nm–320 nm) but did not include short wavelength UV radiation UV-C (<280 nm). In addition, very weak peaks around 650–800 nm may arise due to the N_2 first positive system ($B^3\Pi g - A^3\Sigma u^+$) (Moisan et al., 2001).

Next, chemical indicator strips were employed to semi-quantitatively measure chemicals and UV produced during operation of the plasma torch. Specifically, chemical indicator strips of Quantofix peroxide 25 (Macherey-Nagel GmbH&Co.KG) for hydrogen peroxide and UV label-H for UV were used. Plasma torch treatment was performed on the indicator strips for 0, 0.5, 1, 2, or 5 min. Upon exposure to the DBD torch, UV radiation energy detected by the strips increased in a time-dependent manner (Fig. S2). The energy value was estimated as 19.89 ± 3.01 mJ/cm^2 at 0 min (below detectable limit), 119.33 ± 2.08 mJ/cm^2 at 0.5 min, 218.76 ± 21.24 mJ/cm^2 at 1 min, 417.63 ± 19.59 mJ/cm^2 at 2 min, and 1014.25 ± 10.81 mJ/cm^2 at 5 min. The amount of hydrogen peroxide detected by the strips was 2.70 ± 0.27 mg/L (below detectable limit) at 0 min, 3.03 ± 1.37 mg/L at 0.5 min, 3.36 ± 0.68 mg/L at 1 min, 4.01 ± 1.34 mg/L at 2 min, and 5.98 ± 0.61 mg/L at 5 min (Fig. S3). The temperature on the surface of the sample liquid was measured during exposure to the DBD torch using infrared thermography. The surface temperature of the *H. pylori* suspension was 26.0 °C at 0 min, 29.5 °C at 0.5 min, 30 °C at 1 min, 29.8 °C at 2 min, and

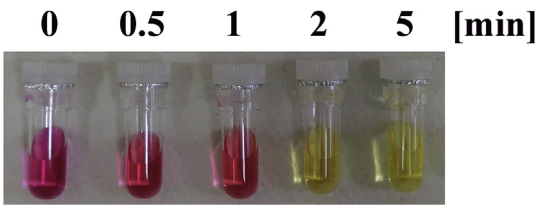

Fig. 4. Urease secreted from *H. pylori* was inactivated by DBD plasma treatment. Urease activity was measured by a standard urease assay using MR Urea® in accordance with the manufacturer's instructions (Institute of Immunology Co. Ltd.) employing the index of colorimetric change after DBD plasma treatment of *H. pylori* for the indicated time (min).

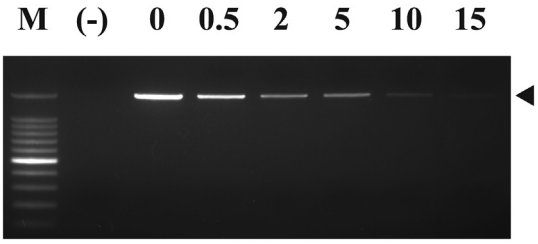

Fig. 5. Changes to the genomic DNA (16S rDNA) of *H. pylori* following DBD plasma treatment. M, marker (100 bp DNA ladder). (−), negative control (water). The bands for *H. pylori* 16S rDNA are indicated by an arrowhead. Numbers refer to the DBD plasma treatment time (min).

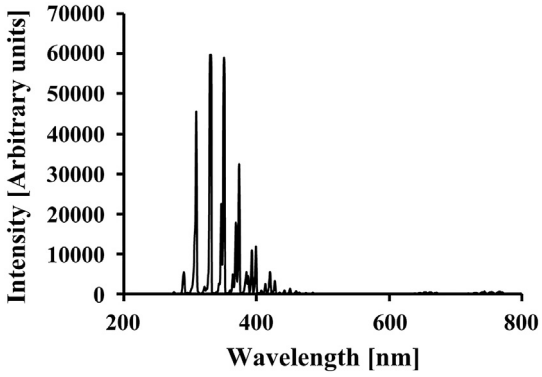

Fig. 6. Spectra of emission during operation of the DBD plasma torch. The optical emission spectrum was analyzed by a S-2431 spectrophotometer during operation of the DBD plasma torch.

30.3 °C at 5 min (data not shown).

Finally, we analyzed which of these bactericidal factors primarily contributes to the disinfection of *H. pylori* by separately analyzing the effect of heat, UV radiation, and hydrogen peroxide on viability. A *H. pylori* suspension was heat-treated for 2 min and cell viability was then determined. No significant changes in viable cell number were observed after treatment at 40 °C (680 ± 37 CFU/mL) or 50 °C (616 ± 12 CFU/mL) compared to the control (35 °C, 701 ± 58 CFU/mL). By contrast, a significant reduction in viable cell number was observed after heat-treatment at 52 °C (445 ± 46 CFU/mL) and 54 °C (200 ± 44 CFU/mL) (Fig. S4). No viable cells were detected after heat-treatment at 56 °C or above. These observations suggest that *H. pylori* cells are inactivated by exposure to temperatures above 52 °C. Next, *H. pylori* suspensions were treated with UV and then subjected to a colony count assay. Treatment with UV-C, but not UV-A, for 2 min using a UVGL-58 device reduced the viable cell number to levels below the detection limit (Fig. S5). Furthermore, a reduction in the number of viable bacteria was observed after 2–10 min treatment with UV-A, with a marked reduction after 15 min or 30 min treatment (Fig. 7). A suspension of *H. pylori* subjected to hydrogen peroxide treatment for 2 min showed some reduction but no significant difference in viable cell number (i.e., 633 ± 275 CFU/mL in untreated control (0%) compared with 295 ± 132 CFU/mL in 0.03% hydrogen peroxide, 221 ± 28 CFU/mL in 0.3% hydrogen peroxide, and 128 ± 14 CFU/mL in 3% hydrogen peroxide) (Fig. S6).

4. Discussion

In this study, we analyzed the potential application of a DBD plasma torch for the disinfection of endoscopes using *H. pylori* as a test organism. Our results suggest the plasma treatment induces

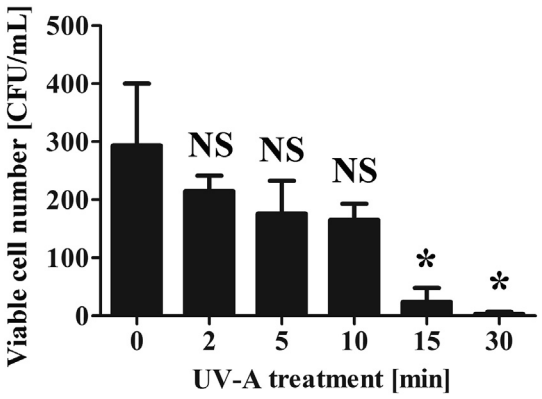

Fig. 7. Stability of *H. pylori* following exposure to long-wave ultraviolet radiation (UV-A) treatment. After UV-A treatment for the indicated time (0–30 min) using a UVGL-58 device (UVP), *H. pylori* samples were then subjected to cell culture assays to determine viable cell number (CFU/mL). Differences where $p < 0.05$(*) versus control (0 min) were considered significant, while NS means no significance.

biochemical changes in *H. pylori* by reducing urease activity and causing DNA damage. Viability and urease activity of *H. pylori* was completely reduced by DBD plasma treatment for 2 min. The results suggest that *H. pylori* was completely inactivated after 2 min-treatment. Thus, the levels of the essential inactivation factors required for complete inactivation of *H. pylori* were reached after this treatment time.

During operation of the DBD plasma torch samples are exposed to heat, UV, and hydrogen peroxide. Heat treatment at temperatures greater than 52 °C for 2 min resulted in almost complete inactivation of *H. pylori*. However, the highest temperature of samples after being subjected to DBD gas plasma treatment for 2 min was 29.8 °C, which was insufficient to inactivate *H. pylori*. It should be noted that viable cell number of *H. pylori* decreased to undetectable levels following DBD gas plasma treatment for 2 min. Measurement of optical emission spectra showed that UV-A was generated during operation of the DBD plasma torch, whereas the level of UV-C was below the detectable limit although UV-C has disinfection effect on *H. pylori*. In terms of the energy level of UV, samples subjected to the DBD plasma torch for 1 min and 2 min were exposed to 218.76 mJ/cm^2 and 417.63 mJ/cm^2, respectively. We replicated a similar exposure of *H. pylori* to UV radiation using a transilluminator (UVGL-58) for 15 and 30 min, which significantly reduced cell viability. The estimated energy of UV-A generated by the UVGL-58 device was 27.26 mJ/cm^2 at 15 min and 41.64 mJ/cm^2 at 30 min. We therefore conclude that exposure to UV energy generated by 1 or 2 min-plasma treatment was sufficient to inactivate *H. pylori*.

By contrast, production of hydrogen peroxide at 2 min-plasma treatment was 4.01 mg/L, equivalent to approximately 0.0004%, which did not have a significant effect on *H. pylori* viability. Thus, the principle means by which the DBD plasma torch reduces cell viability of *H. pylori* is by exposure to UV-A.

Previous studies showed that the main inactivation factors for cells exposed to gas plasma were UV radiation, the presence of reactive chemicals such as hydrogen peroxide, and/or localized heating depending on the process gas used (Moisan et al., 2002; Bol'shakov et al., 2004; Laroussi and Leipold, 2004;Laroussi, 2005; Toyokawa et al., 2017). The present study analyzed the use of a DBD plasma torch, which generates plasma from air, to inactivate *H. pylori*. In this case, our results highlight the importance of UV-A as the principle mechanism for the bactericidal effect of the plasma. However, UV-B was also detected in the emission spectra during operation of the DBD plasma torch. Further studies are

required to analyze the disinfection effect of UV-B on *H. pylori* and its disinfection contribution. Moreover, generation of reactive chemicals other than hydrogen peroxide is possible during the operation of the DBD plasma torch. A potential advantage of the gas plasma torch is that the reactive chemicals may act synergistically with UV to enhance the observed disinfection by treating regions of the 3D endoscope that are not exposed to radiation.

Analysis of the *H. pylori* genome before and after plasma treatment showed that there was a marked reduction in the amount of intact genomic DNA upon exposure to the DBD device, which correlated with treatment time. Previous studies have analyzed gas plasma generated from various gases, including air, helium, oxygen and nitrogen (Kim and Kim, 2006; Maeda et al., 2015; Modic et al., 2017), which lead to DNA oxidation/modification as well as degradation. These modifications include single- and/or double-strand breaks in the genomic DNA. Furthermore, gas plasma induces the formation of 8-OHdG (8-hydroxy-2′- deoxyguanosine) in DNA (Sakudo et al., 2017), while exposure to UV radiation produces thymidine dimers and strand breaks (Moreau et al., 2008). Thus, additional analyses using sensitive techniques for detecting changes in the DNA are needed to reveal the precise mechanism(s) by which the plasma inactivates *H. pylori*. In particular, because low level photochemical damage induced by UV can be self-repaired (Bucher et al., 2016), detailed analysis of DNA damage, such as dose/time dependence, will need to be assessed. Elucidation of the inactivation mechanism may assist in improving the efficiency of the system by allowing us to fine-tune plasma generation to maximize disinfection. Moreover, the precise spatial pattern of plasma produced at the outlet of the torch is likely to have an effect on the efficiency of disinfection treatment. Additional studies to investigate the impact of these factors on disinfection are required.

Gas plasma is particularly suited to surface disinfection, because the plasma only penetrates to a depth of about 100–1000 nm (Shintani et al., 2010). This low penetration character of plasma reduces any damage to the material being disinfected and helps to maintain its structural integrity. Thus, gas plasma is a promising tool for maintaining the hygiene standards of medical devices in the hospital setting. However, to date, there has been a paucity of studies analyzing the effect of gas plasma on surface materials commonly used in medical devices. Thus, further examination of the interaction between gas plasma and material surfaces are necessary before the methodology can be used as a disinfection/sterilization procedure for endoscopes.

It should be noted that the present research has limitations because only a laboratory strain of *H. pylori* in a suspension of glycerol was used. In the environment, *H. pylori* may be present in different forms from our experimental model. In particular, *H. pylori* can form biofilms under nutrient rich conditions (Sasaki et al., 1999) and exist in a viable but non-culturable (VNC) form (Linke et al., 2010). *H. pylori* associated with biofilms, VBNC, or as a result of clumping may display increased resistance to disinfection by plasma treatment. Therefore, additional studies using *H. pylori*-contaminated endoscopes from the hospital and clinic are required before this sterilization technique can be fully evaluated.

Finally, we discuss our results in terms of the following definitions of sterilization and disinfection. Sterilization is the reduction of initially present microorganisms. As the likelihood of survival of an individual microorganism is never zero, for critical applications such as medical devices (endoscopes), a sterility assurance level of at least 10^{-6} is required. High level disinfection means inactivation of microorganisms to a level of 10^{-6}, which is required for medical devices. Treatment must be active against bacterial spores, Cryptosporidiidae, mycobacteria, non-enveloped viruses, and fungi as well as enveloped viruses and vegetative bacteria. Thus, high-level disinfection treatments can be used for endoscopes and need to inactivate vegetative bacteria, enveloped viruses, fungi, non-enveloped viruses, *Mycobacterium tuberculosis*, and bacterial spores. So far, the DBD plasma torch has been tested on a single species of vegetative bacteria (*H. pylori*). Thus, on the basis of current knowledge, DBD plasma treatment is only a low-level disinfecting treatment. However, our preliminary experiments showed that non-enveloped virus feline calcivirus (FCV), which is a relatively resistant microorganism (Cannon et al., 2006), is also efficiently inactivated by treatment with the DBD plasma torch (Fig. S7). Therefore, the plasma method may be effective against a broad range of microorganisms. Further investigation using various microorganisms would be required to ascertain whether the DBD plasma torch can achieve high-level disinfection or sterilization. On the basis of these criteria, adequate dose and exposure time as well as variance in other conditions, which influence the outcome of disinfection process by the DBD plasma torch, should be determined.

5. Conclusions

The present study showed that short exposure to a DBD plasma torch efficiently inactivated *H. pylori*. Further development of this DBD plasma system may form the basis of an innovative method to reprocess and sterilize endoscopes. The present study also suggests that UV generated in the gas plasma may induce damage to *H. pylori* DNA. Future optimization of the gas plasma system may facilitate increased inactivation efficiency.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.02.115.

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