Title: Effect of Low Temperature Atmospheric Argon Plasma On Metabolic Activity and Viability of Periodontal Pathogens

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Running Title: LTAP Deactivates Periodontal Pathogens

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ABSTRACT: Current treatment options for periodontal disease, although effective, have limitations, and the profession is continually searching for new ways to treat this disease. An emerging area of clinical research is the use of low-temperature atmospheric pressure plasma technology for the deactivation of oral bacteria and bacterial biofilms. This study tested the hypothesis that periodontal pathogens can be rapidly inhibited or killed by low-temperature atmospheric argon plasma (LTAP) in vitro. Our objectives were to determine the effect of LTAP on bacterial metabolic activity and viability of Aggregatibacter actinomycetemcomitans (Aa) and Porphyromonas gingivalis (Pg). LTAP treated bacteria were tested with the WST metabolic activity and proliferation, and Live/Dead BacLight viability assays. Additionally, Aa survival was tested by colony forming units (CFU) on agar plates after treatment with LTAP. Un-ignited argon gas alone, and exposure to oxygen from ambient air were also tested as controls. Our findings show that the LTAP plasma brush was effective in inhibiting metabolic activity and reducing viability of the periodontal pathogens Aa and Pg (up to 99.78% and 84.65% respectively) after 2 min treatment. The effects of LTAP were independent of gas blowing effect, or transient exposure to ambient air.

Keywords: argon plasma, plasma treatment, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis
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INTRODUCTION

Periodontal disease is a chronic inflammatory disease that results in destruction of the tooth-supporting structures and can lead to tooth loss. Approximately 38% of adults 30 years and older, and 64% of adults 65 years and older in the US afflicted with moderate to severe forms of periodontitis (1), with serious esthetic, social, and functional implications. Periodontal disease has been implicated as a risk factor for many systemic conditions including diabetes, atherosclerosis, heart disease, and chronic obstructive pulmonary disease (COPD), most likely through the systemic increase of pro-inflammatory cytokines in the blood stream. In addition, a keystone periodontal pathogen has been implicated in triggering auto-immune response and rheumatoid arthritis in susceptible patients (2, 3).

The bacterial etiology of periodontal disease is well established, and therefore the reduction of the pathogenic bacterial load is key to treatment. Unfortunately, the complete disinfection of periodontal sites is not feasible (4-6). Lasers have been proposed as an adjunct treatment but have shown only modest, if any, improvements in clinical outcomes (7-9). Locally delivered antimicrobials and systemic antibiotics have also been proposed, however both may have side effects and are ineffective against biofilms. Systemic antibiotics carry the risks of adverse drug reactions, interaction with other medications, and development of antibiotic resistance (10-13).

A promising and innovative area of clinical research is low-temperature atmospheric pressure plasma technology, made up of charged particles, and contains plasma-species from electrical discharge including ionized gas and high-energy, chemically reactive neutral particles (atoms and/or molecules with unpaired electrons – also known as free radicals). Plasma has been shown to kill bacteria by way of UV irradiation of genetic material, erosion as UV photons break
chemical bonds, and erosion through etching as the reactive plasma-species chemically react with bacteria (14). Currently plasma has many industrial and medical applications, including decontamination of food (15, 16), air ventilation systems, and water (17). Prospective medical uses include sterilization of medical instruments and surfaces, decontamination of medical waste (17), selective ablation of cancer cells (18), and disinfection of ocular cells and tissues (19). The use of plasma has also been proposed in dentistry, as it can increase the bond strength of restorations by altering dentin surfaces (20). Plasma can also initiate chemical bonding of hydroxyethylmethacrylate (HEMA), a monomer used in bonding agents, to dentin collagen fibrils (21), and modify the adhesive-composite interface to minimize the effects of excess water (22, 23).

The decontamination property of plasma technology renders it ideal for testing in treatment of periodontal disease. Yang et al showed that their low-temperature argon plasma brush could remove 99.9999% of *S. mutans* and *L. acidophilus* from different surfaces (24). They observed massive cell structural damage with SEM, and leakage of nucleic acids and proteins through spectrometry. Yu et al, in a similar study with *E. coli* and *M. luteus*, found that the bacteria could be significantly reduced in 2-3 min for *M. luteus*, and 3-4 min for *E. coli* (25). In addition, low-temperature atmospheric nitrogen plasma inactivated *G. stearothermophilus* spores, commonly used to test the effectiveness of autoclaves, as well as endotoxin (26). Deactivation of biofilms can also be achieved with this technology, as previously shown with gram positive and negative films in 4 and 10 min respectively (27). There have been many other studies showing evidence of the bactericidal effects of low temperature atmospheric plasma (28-34).

The aim of this study is to investigate the effect of low-temperature atmospheric argon plasma (LTAP) on the metabolic activity and viability of the periodontal pathogens:
Aggregatibacter actinomycetemcomitans (Aa), and Porphyromonas gingivalis (Pg). Aa is a Gram-negative, facultative rod aslo associated with aggressive periodontitis (35). Aa releases leukotoxin, among other virulence factors (36, 37), and can cause alveolar bone destruction in as little as 2 to 3 months (35). Pg is an extremely virulent pathogen associated with aggressive and chronic periodontitis and may invade gingival epithelial cells (38). Few studies have investigated the effects of LTAP on known periodontal pathogens, and no studies to date were found investigating LTAP’s effect on Aa.

MATERIAL AND METHODS

Bacteria

Bacterial strains used in this study were purchased from the American Type Culture Collection (ATCC). Tryptic soy broth (TSB) and yeast extract were from Becton, Dickinson and Co. (Franklin Lakes, NJ). Aa (ATCC 43718) were grown for 24 h with gentle mixing at 37°C in 100 mL cultures of TSB that were sealed to limit oxygen. TSB for the culture of Aa, in liquid or on solid media, was sterilized by filtration through a 0.22 μm filter and prefilter (Corning Life Sciences, Corning, NY). Pg (ATCC 33277) were grown 24 h at 37°C under anaerobic conditions (GasPak™ 150 Anaerobic System; Becton, Dickinson) in 10 mL cultures of TSB supplemented with yeast extract (0.5%), hemin (5 μg/mL), menadione (1 μg/mL) and cysteine (0.5 mg/mL). Absorbance of the bacterial suspensions was measured at 600 nm. The correlation of absorbance to bacterial concentration was determined by diluting and plating and absorbance was used to determine bacterial concentrations.
Low-Temperature Atmospheric Argon Plasma (LTAP)

The LTAP device was purchased from Nanova Inc (Columbia, MO). Ultra high purity gases, Argon (Ar) (99.999%) and Oxygen (O2) (99.997%) were purchased from nexAir (Memphis, TN) and were used to create the LTAP. The argon gas passed through a discharge chamber at a flow rate controlled by a mass flow controller (MKS Instruments, Andover, MA). An electrical field was applied to the two electrodes located inside the chamber to ignite a DC glow discharge by a DC power supply (PD, 1556C, Power Design, New York, NY). This plasma source was operated under low electrical power (<6.5 W) generating very low temperature plasma. The plasma discharge formed a brush-shaped low temperature plasma flame (LTAP plasma brush). The plasma brush was positioned at a standardized and reproducible distance from the samples using a silicone stopper with the brush tip in contact with the samples. The following plasma brush settings were used for all LTAP experiments unless otherwise noted: Ar pressure 40 psig, O2 pressure 1.4 psig, Output current 10 mA, Output voltage 0.8kV. All plasma experiments were conducted in ambient air.

Plasma Treatment of Bacteria

Bacterial cultures were centrifuged and the desired number of bacteria were re-suspended in either Dulbecco's phosphate buffered saline (DPBS) (Invitrogen, Inc., Carlsbad, CA), or isotonic saline depending on assay instructions. 200 µl portions of bacterial suspension were added to wells of a 48 well plate (Corning Life Sciences) with the intention to prevent bacterial desiccation, that could lead to decreased bactericidal activity due to drying effect of the gas flow. Cultures of Aa and Pg were treated with the plasma-brush (Plasma; Test Group) or with un-
ignited gas flow (Gas; Control Group) for 0, 30, 60, 90 and 120 s. Cultures of the obligate anaerobe *Pg* were exposed to ambient air for 45 min (Air; Negative Control), as the relevant treatments for each 48-well plate lasted approximately 45 min.

**Metabolic Activity Assay**

Bacterial metabolic activity was assessed with a microbial WST assay kit (Dojindo Molecular Technologies Inc, Kumamoto, Japan), which measures the reduction of WST by bacterial dehydrogenases. Briefly, a 100µL suspension of plasma-treated bacteria at 2.5 or 5 x10^8 cells/mL in DPBS was transferred to a 96-well plate and diluted with an equal volume of bacterial growth media free of cysteine. The WST reagent/electron mediator was then added to the wells. Plates were incubated anaerobically (AnaeroGen™ Compact, Becton, Dickinson and Co.) at 37°C for 2-3 h. Following the incubation, the absorbance of reduced WST was measured at 450 nm with a Spectrostar Nano spectrometer (BMGLabTech, Offenburg, Germany). The bacterial concentration and time of incubation were chosen to give an absorbance of approximately 1.5 at 450 nm for untreated controls.

Bacteriological media contain substances that reduce the tetrazolium dyes such as WST in a time-dependent manner. Some of these substances are formed by chemical reactions during the autoclaving process. In our study, media used in WST assays was filter-sterilized rather than autoclaved, and the reducing agent cysteine was omitted. The filter-sterilized media was pre-reduced overnight in an anaerobic environment for WST assays with anaerobes. In all of our experiments, plasma treatment was performed on bacteria suspended in buffer or isotonic saline rather than bacteriologic media.
**Bacterial viability assays**

Bactericidal effect was assessed by two methods: Counting Colony Forming Units (CFU) and the live/dead BacLight method. *Aa* bacterial suspensions were treated at 2.5x10⁶ cells/mL or 2.5x10⁸ cells/mL and serially diluted 20-200,000 times with sterile DPBS, and 100 µL portions were then plated on filter sterilized TSB agar plates. Buffers used for dilution and plating of *Aa* bacteria were autoclaved in order to remove oxygen. Plates were incubated at 37°C in 95% air, 5% CO₂ for 24-48 h, and bacterial colonies were counted manually.

The bactericidal effect of plasma on *Aa* and *Pg* was also measured using the LIVE/DEAD BacLight™ bacterial viability kit (Invitrogen Inc, Carlsbad, CA) which utilizes a mixture of DNA binding dyes – green fluorescent SYTO 9 that enters and stains all bacteria, and red-fluorescent propidium iodide that enters and stains only dead bacteria with damaged membranes. Assays were performed according to the manufacturer’s protocol. Briefly, after the experimental treatments, 100µL of 10⁹ bacteria/mL of *Aa* or *Pg* in suspension were transferred to black 96-well plates (Corning Life), and 100 µL of BacLight dye mixture was added in each well. Plates were incubated for 15 min at 37°C in an AnaeroGen™ pouch in the dark. Immediately following removal of the plate from the pouch, fluorescence was measured in a fluorescence plate reader (SpectraMax Gemini EM, Molecular Devices, Sunnyvale, CA). Green fluorescence of SYTO 9 was measured with an excitation of 485 nm and emission at 538 nm. Red fluorescence of propidium iodide was measured with an excitation of 485 nm and emission at 612 nm. A portion of the bacteria were killed with 70% isopropanol, washed and re-suspended 1x10⁸ CFU/mL in saline. Standard curves were prepared with various ratios of live and dead bacteria, and the number of live bacteria in the samples was determined from the ratio of green to red fluorescence compared to standard curves.
**Effect of un-ignited gas and ambient air on bacteria**

The effect of plasma treatment was determined by comparison with gas flow treatment without igniting the plasma, since gas flow alone may have an effect on bacterial metabolic activity and survival. Suspensions of *Aa* and *Pg* were exposed to 40 psig argon/1.4 psig oxygen for 0-2 min without igniting the plasma. In addition, in order to determine the effect of O2 alone on suspensions of *Pg* which is an obligate anaerobe, *Pg* samples placed in anaerobic conditions were compared with *Pg* suspensions exposed to ambient air for 45 min.

**Statistical Analysis**

All experiments were conducted with duplicate or triplicate samples and each experiment was repeated at least 3 times. Data were analyzed with Prism 4 software (Version 4.0c, GraphPad Software Inc, San Diego, CA). One-way and two-way ANOVA with Bonferroni post hoc analyses were performed to detect statistical significance (p<0.05). The Wilcoxon Signed Rank test was performed when only two groups were available (live/dead viability assay for *Pg* exposed in ambient air and in anaerobic conditions).

**RESULTS**

**LTAP effect on Aggregatibacter actinomycetemcomitans**

**LTAP Plasma Brush Inhibits Aa Metabolic Activity**
Aa metabolic activity was significantly inhibited by the LTAP plasma brush for both bacterial densities tested, and this inhibition was directly correlated with the duration of the plasma treatment, reaching 88.72% and 91.87% after 120 s at the two bacterial densities tested. Both time and type of treatment resulted in significant reduction of the metabolic activity of Aa (p<0.001; figure 1). Aa metabolic activity was reduced significantly at all treatment times with the plasma brush when compared with the control un-ignited gas flow treatment and with no treatment (p<0.001) and the metabolic activity reduction reached 88.72±1.68% (mean±SD) after 120 s of plasma treatment vs only 35.55±18.86% for the un-ignited gas flow treatment of 2.5 x 10^8 bacteria/mL. Interestingly, the significant metabolic inhibition after 120 s of plasma treatment was maintained to 91.87±1.87% even when the bacterial density doubled to 5 x 10^8 bacteria /mL, while the un-ignited gas flow was only inhibited by 13.89±5.35%.

LTAP Plasma Brush has bactericidal effect against Aa

In order to determine whether the plasma brush is effective in killing Aa as opposed to merely impairing the bacterial metabolism, we tested the plasma brush’s bactericidal properties by live/dead staining assay and CFU analysis. Live/dead staining of 10^8 bacteria/mL revealed significant Aa killing with the plasma brush, achieving 81.31±15.27 % dead bacteria after 120 s of treatment (p<0.001; figure 2). In addition, Plasma brush treatment for 60 s was also effective in killing Aa, as shown by post hoc analysis (57.71±19.46% killing, p<0.05). The bactericidal effect of the LTAP plasma brush on Aa was confirmed by CFU analysis after 2.5x10^6 and 2.5x10^8 of Aa bacteria/mL were treated with either the LTAP plasma brush or the un-ignited gas flow. Samples were diluted, plated on solid media, and colonies were counted to determine the number of viable bacteria per mL. LTAP treatment for 120 s showed significant CFU reduction...
by 99.78% and 98.22% for 2.5x10^6 and 2.5x10^8 cells/mL respectively (p<0.001, figure 3). In addition, post hoc analysis indicated significant CFU reduction between all time points (p<0.05). Moreover, treatment with the LTAP brush was significantly more effective than treatment with un-ignited gas in CFU reduction (p<0.001).

**LTAP effect on *Porphyromonas gingivalis***

**LTAP Plasma Brush Inhibits *Pg* Metabolic Activity**

*Pg* metabolic activity was significantly inhibited by the LTAP plasma brush for both bacterial densities tested, and this inhibition was directly correlated with the duration of the plasma treatment, reaching after 120 s treatment, 61.22% and 64.78% at the two bacterial densities tested (figure 4). More specifically, the *Pg* metabolic activity was significantly reduced after 60 and 120 s of plasma brush treatment of 2.5 x 10^8 bacteria/mL (p<0.01) and after 30, 60 and 120 s of plasma brush treatment of 5 x 10^8 bacteria/mL (p<0.001). The metabolic activity reduction was moderate and reached 64.78±21.23 % and 61.22±5.79 % after 120 s of plasma treatment of 2.5 x 10^6 and 5 x 10^8 bacteria/mL respectively.

**LTAP Plasma Brush has bactericidal effect against *Pg***

The LTAP plasma brush’s bactericidal effectiveness on *Pg* was assessed by the live/dead staining viability assay. Live/dead staining of 10^8 bacteria/mL revealed significant *Pg* killing with the plasma brush, achieving 84.65±16.96 % dead bacteria after 120 s of treatment with the plasma brush (p=0.0016; figure 5 A). Plasma brush treatment for 60 s was also effective in killing *Pg*, as shown by post hoc analysis (51.02±29.16% dead *Pg* bacteria, p<0.05).
LTAP Plasma Brush bactericidal effect is not due to the O$_2$ in the un-ignited gas flow

The un-ignited gas failed to kill $Pg$ bacteria at any time point (figure 5 B, $Pg$ survival rate ranging 99.55±3.08% to 100%, p>0.05), while the LTAP plasma brush effectively killed $Pg$ and reached statistical significance after 60 and 120 s of treatment (37.52±13.33 % and 79.87± 10.21 % dead $Pg$ bacteria at 60 and 120 s respectively; p<0.001 for both 60 and 120 s of plasma brush treatment).

LTAP Plasma Brush bactericidal effect is not due to the O$_2$ in the ambient air

Finally, the effect of the ambient air on $Pg$ survival was tested, since $Pg$ is strictly anaerobic. Uncovered 96-well plates with 200 µL of $10^8$ cells/mL $Pg$ suspension were exposed to ambient air for 45 min, while control samples were sealed and kept in anaerobic conditions. Exposure to ambient air for 45 min had no effect on $Pg$ survival, and there was no difference in bacterial survival in suspension between the anaerobic conditions and the ambient air exposure (p>0.05; figure 5 C).

DISCUSSION

Our findings show that an LTAP plasma brush can be effective in inhibiting metabolic activity and viability of periodontal pathogens $Aa$ and $Pg$ (up to 99.78% and 84.65% respectively) after 2 min treatment. Furthermore, we have shown that the effects of LTAP are independent of gas blowing effect, or transient exposure to ambient air.
Many mechanisms have been proposed to explain the LTAP’s effect on bacterial cells including DNA damage by UV irradiation, erosion and weakening of chemical bonds by UV photons, and molecular structure erosion through etching (14, 39). In fact Yu et al reported that the energy of the various plasma-generated species is higher than the bonding energy of organic bacterial molecules, supporting the erosion through etching mechanism (25). Another mechanism that has been proposed for bacterial inhibition by low-temperature plasma treatment is via charge accumulation and destabilization of the cell wall leading to irreversible electroporation (40).

Previous studies have suggested many factors that may affect the efficacy of plasma treatment for bacterial inhibition, including the size and type of bacteria, the type of the surface or medium where the bacteria were treated, the bacterial density, as well as the bacterial growth pattern (planktonic vs biofilm, monoculture vs mixed culture). More specifically, the size of bacterial cells can affect the time needed for eradication. Yang et al demonstrated the removal of \textit{S mutans} in as little as 13 s, while it took 300 s to destroy \textit{L acidophilus} in the same conditions (24). Since \textit{L acidophilus} has a larger cell size than \textit{S mutans}, the authors proposed that it would take longer to achieve an equal amount of cell damage. Moreover, high bacterial density may reduce the plasma treatment effectiveness, since overlapping of the larger bacteria may act as a shield and limit the direct effect of the plasma flame. Accumulation of cell debris from the overlying bacteria can also be a factor as confirmed with SEM (40). Interestingly, in our study there was no decrease of the plasma brush effectiveness at the higher cell density to inhibit the \textit{Aa} metabolic activity, unlike the un-ignited gas flow that failed to inhibit \textit{Aa} when the \textit{Aa} cell density doubled.
The surface or medium where the bacteria grow may affect the plasma effectiveness. Yu et al suggested that plasma may achieve more direct contact with cells in broth than those seeded on porous filter paper, or colloid agar plates, as these surfaces may shield bacteria (25). Our preliminary data, with *E coli* and TSB medium, showed that plasma treatment of TSB promoted reduction of WST by media in the absence of *E coli*. Therefore, the composition of the medium might enhance or attenuate the effectiveness of plasma treatment.

Our data suggest a tentative adjunct role for plasma disinfection during routine dental procedures or periodontal surgery. In our methodology, we consciously limited treatment times to those considered relevant in a clinical scenario as any treatment longer that 1-2 min per tooth/periodontal site would be impractical and shorter treatment times would allow oral health practitioners to treat more sites per appointment. Our findings showed that transient exposure of the strictly anaerobic *Pg* (suspended in medium) to ambient air for 45 min did not affect viability. This implies that *Pg* exposed during periodontal surgery would be minimally affected by ambient air exposure. LTAP treatment at the time of surgery might offer a way to eradicate remaining *Pg*.

Many chronic infections, including periodontal diseases are caused by bacterial biofilms that are difficult to eradicate with conventional methods. Periodontal pathogens are protected by the biofilm’s extracellular matrix, rendering the biofilm up to 1,000 times more resistant to disinfection, and host defenses (41). Plasma treatment may offer an advantage to disrupt the biofilm, as previously suggested by the use of a He plasma on *S mutans* biofilms (42). A single 1 min treatment was reportedly sufficient to inhibit growth of *S mutans* biofilm, however re-growth was observed in biofilms grown with sucrose, most likely due to thicker biofilm that may impede plasma penetration. In contrast, Alkawareek et al treated *Pseudomonas aeruginosa*
biofilms with a helium/oxygen plasma jet and observed more than 99.99% reduction in viable cells after 4 min (41).

Two previous studies were found investigating the effect of low temperature atmospheric plasma on \( P_g \). Mahasneh et al treated \( P_g \)-inoculated agar plates with He plasma for 5, 7, 9 and 11 min, after which the plates were incubated and examined for zones of inhibition. The authors reported statistically significant inhibition zones in plates treated with plasma compared to those treated with He gas alone. They also found significantly larger zones of inhibition between each of the different treatment times, suggesting a dose-dependent inactivation of \( P_g \) (43). Liu et al evaluated the bacterial killing effect of a He/O\(_2\) atmospheric pressure non-equilibrium plasma jet (APNP) with\( P_g \) biofilms in vitro. Biofilms were treated for up to 5 min, after which they were fluorescently stained, and observed under a laser scanning confocal microscope. The \( P_g \) biofilms treated with APNP were mostly dead, while those that received no treatment, or treatment with gas only were alive (44).

The same report presented an animal study evaluating oral mucosa of rabbits for pathological changes following treatment with APNP. Rabbits were treated with plasma on the cheek and ventral surface of the tongue on the left side while the right side served as untreated controls. Histology showed no ulcers, or oral mucositis in rabbits treated either 1-day or 5-days previously, and mucus membrane irritation index scores indicated that no significant irritation occurred. The group concluded that APNPs could effectively kill \( P_g \) in biofilms without causing harm to healthy mucosa. More recent findings using mesenchymal stem cells, rat skin and pig gingiva support this (39, 45).

In conclusion, there were some limitations to this study that should be recognized. Although we were able to kill planktonic \( A_a \) and \( P_g \), in a clinical scenario these bacteria will
always be found co-aggregated in a mixed biofilm and in difficult areas to access. Further studies are needed to evaluate the bactericidal effect of LTAP on mature biofilms. We observed significantly more killing when Aa was evaluated with CFUs, compared to the Live/Dead assay. A possible reason is that the Live/Dead assay takes far less time to complete (15 min vs 48 h for CFUs). Some bacteria may have been damaged by LTAP, yet remained viable for a short time. Such damaged bacteria may stain as live, yet not remain viable long enough to grow on an agar plate. Although we observed statistically significant killing of both bacteria, we did not achieve total eradication. This may have been due to the study protocol, or it may be that LTAP cannot sufficiently destroy these bacteria within a clinically relevant time frame. Further studies of LTAP on periodontal pathogens are needed, however it can be inferred from this study that LTAP may be useful as an adjunctive therapy to mechanical removal of bacterial biofilms.

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**Figure Legends**

**Figure 1.** Metabolic Activity of *A. actinomycetemcomitans* is significantly inhibited by the LTAP plasma brush compared with un-ignited gas flow at (A) 2.5 x 10^8 bacteria/mL and (B) 5 x 10^8 bacteria/mL and at all time points (p<0.001).

(WST assay, data normalized for 100% metabolic activity of negative control)

**Figure 2.** Bactericidal effect of LTAP plasma brush on *A. actinomycetemcomitans* (p<0.001)

(Live/dead staining, 10^8 bacteria/mL)

**Figure 3.** *A. actinomycetemcomitans* Colony Forming Units (CFU) analysis after LTAP plasma brush treatment (compared with un-ignited gas flow) (p<0.001). CFUs as % of the untreated (negative control) for (A) 2.5x10^6 and (B) 2.5x10^8 bacteria/mL.

**Figure 4.** Metabolic Activity of *P. gingivalis* is significantly inhibited by the LTAP plasma brush at (A) 2.5 x 10^8 bacteria/mL and (B) 5 x 10^8 bacteria/mL with increasing time of plasma application (p<0.001).

(WST Assay, data normalized for 100% metabolic activity of negative control)

**Figure 5.** Bactericidal effect of LTAP plasma brush on *P. gingivalis*. (A) The LTAP plasma brush has a bactericidal effect on *Pg* with increasing time of treatment (p<0.001). (B) Comparison of bactericidal effect of the LTAP plasma brush with the un-ignited gas flow. (C) Ambient air has no short-term bactericidal effect on *Pg*.
(Live/dead staining, $10^8$ bacteria/mL)