Received: 11 June 2018 | Revised: 18 October 2018 | Accepted: 22 October 2018

DOI: 10.1002/JPER.18-0366

JOURNAL OF Periodontology

ORIGINAL ARTICLE

Low-temperature plasma on peri-implant–related biofilm and gingival tissue

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Abstract

Background: Evaluate the effect of low-temperature plasma (LTP) on an anaerobic biofilm and on the biological response of an in vitro reconstituted gingival epithelium tissue.

Methods: *Porphyromonas gingivalis* W83 biofilm was cultured on titanium discs and reconstituted gingival tissues were submitted to similar treatment conditions. Treatments: LTP1—plasma treatment for 1 minute, LTP3—plasma treatment for 3 minute, CHX—0.2% chlorhexidine for 1 minute, GAS—gas only (no plasma) for 3 minute, and NEGATIVE—no treatment. TRITON group was included as a positive control for tissue analysis. Counting of viable colony forming units (CFU/mL) and confocal laser scanning microscopy were performed to evaluate LTP's antimicrobial effect. EpiGingival tissue was evaluated through cytotoxocity, viability, histology, and imunnohistochemistry (Ki67, vascular endothelial growth factor-A vascular endothelial growth factor A [VEGF-A], and terminal deoxynucleotidyl transferase dUTP nick end labeling terminal deoxynucleotidyl transferase dutp nick end labeling [TUNEL] expression).

Results: LTP1 and LTP3 presented significantly different reduced CFU/mL reduction in comparison to the negative control ($P < 0.001$), but it was not as effective as the positive control (CHX). Low cytotoxicity and high viability were observed in gingival epithelium of NEGATIVE, GAS, CHX, and both LTP groups. The morphologic analysis of gingival epithelium revealed minor cell damage in the plasma groups (score 1). LTP1, LTP3, GAS, and NEGATIVE groups exhibited less than 5% of basal layer positive cells. LTP1, LTP3, GAS, and CHX groups were not positive for TUNEL assay. LTP1 and LTP3 showed the most positivity for VEGF.

Conclusions: LTP treatment can be considered as an effective method for reducing *P. gingivalis* biofilm on implant surfaces, while being safe for the gingival epithelium. Furthermore, plasma treatment may be associated with cell repair.

KEYWORDS

dental implants, microbiology, periodontium, *Porphyromonas gingivalis*, therapeutics

1 | INTRODUCTION

Even though implant-based dental rehabilitation has now become a routine treatment to replace missing teeth with high survival rates, a "surviving" implant is certainly not a "successful" implant because many of those implants may present surrounding infected tissue.[1] If conservatively 10% of all implants have a complication, 100 000 implants per year will need treatment.[2] Among these complications, peri-implant mucositis has a prevalence of 43% and 22% for peri-implantitis.[3] Therefore, the implant biologic complications, which can potentially lead to implant failure, have attracted the attention of researchers and clinicians.

A significant debate exists regarding the properties of different antimicrobial agents and to what extent each contributes to inhibit microbes, and novel therapies using low-temperature plasma (LTP) have been considered.[4] Plasma reactive species are transiently generated and represent an especially good source of reactive oxygen and nitrogen species, including singlet oxygen (O), ozone (O_3), hydroxyl radicals (OH), NO, and NO_2.[5–7] Oxygen and nitrogen-based radicals are considered the most significant contributors to the sterilizing effects.[8] These reactive species have strong oxidative effects on the outer structures of bacterial cells, whether it is a spore coat or cell membrane.[8]

Another advantage of LTP application for biologic decontamination is their well-targeted local application.[9] Therefore, LTP could reach the site of infected implants during peri-implantitis surgery.[9] Furthermore, plasma can be considered supportive for the treatment of peri-implant diseases, because plasma treatment reduced contact angle and supported spreading of osteoblastic cells.[10]

Although previous study investigating the inactivation of peri-implant biofilms has shown that plasma could be a useful adjuvant treatment modality for peri-implant disease, this study was not conducted on *Porphyromonas gingivalis* biofilm, the major etiologic agent that contributes to chronic peri-implantitis.[11] In addition, studies regarding the biologic safety of plasma are still limited, particularly on gingival tissue. Therefore, the applicability of this approach requires more extensive studies.

Previously, our research group demonstrated that LTP is effective against *Candida albicans* and *Staphylococcus aureus* mature oral biofilms.[12] Furthermore, the effective dose against these microorganisms was tolerable for the reconstituted oral epithelium, because no significant alterations and cytotoxicity were found.[12] Our hypothesis is that LTP treatment is effective against *P. gingivalis* biofilm without causing any significant tissue damage to the in vitro reconstituted human gingival epithelium. The hypothesis was tested by determining the effect of LTP against *P. gingivalis* biofilm, and by characterizing the tissue response to LTP treatment using an in vitro reconstituted gingival epithelium.

2 | MATERIALS AND METHODS

2.1 | Substrate

Sterile, sandblasted, and acid-etched titanium grade 4 discs (Singular Implants—Dmr Ind. e Com. de Materiais Odontologicos Ltda, Parnamirim, Rio Grande do Norte, Brazil) with 6 mm diameter, 2 mm thickness, and 0.8505 ± 0.128 μm of mean roughness (*Ra*) Surface roughness profilometer Surftest SJ-401 (Mitutoyo Corporation, Kanagawa, Japan) were used.

2.2 | Biofilm

Porphyromonas gingivalis W83 biofilms were formed on brain heart infusion (BHI) supplemented with hemin (0.5 mg/mL) and menadione (5 mg/mL), and anaerobically incubated at 37°C for 5 days. The biofilms that started from a culture were adjusted to optical density (OD_{660}) of 1.2 in a spectrophotometer, which were equivalent to 1×10^5 colony forming units (CFU)/mL. Titanium discs were then incubated in 3 mL of the culture solution and after every 48 hours of incubation, 1.5 mL of the medium was removed, and an equal volume of fresh medium was added. After 5 days of biofilms development,[13] they were treated according to the following groups: LTP1—plasma for 1 minute, LTP3—plasma for 3 minutes, CHX—positive control with 0.2% chlorhexidine for 1 minute, GAS—negative control with argon gas only (no plasma) for 3 minutes, and NEGATIVE—negative control without treatment. The experiment was conducted in duplicates in three independent occasions.

2.3 | Plasma treatment

The LTP was generated through ionization of argon gas (Ar) at atmospheric pressure using the device Kinpen (Leibniz Institute for Plasma Science and Technology—INP, Greifswald, Germany). The device consists of a hand-held unit for generation of a plasma jet at atmospheric pressure, a DC power supply (system power: 8 W at 220 V, 50/60 Hz), and a gas supply unit.[12] The plasma tip-to-sample distance was set to 7 mm. The samples were moved horizontally during plasma application to allow scanning of the overall surface.[12] The experiment was conducted by a calibrated operator. The argon (Ar) gas flow was set to 5 slm and the flow rate was controlled by a flow controller.

2.4 | Positive control

The discs with *P. gingivalis* biofilm were immersed in 3 mL of 0.2% CHX solution for 1 minute. The samples were washed with phosphate buffered saline (PBS—10 mM PO_4^{3-}, 137 mM NaCl, 2.7 mM KCl).

2.5 | Analyses

2.5.1 | Viable colony forming units (CFU/mL)

The discs were removed from the culture plates and the bottom surface was rubbed with a sterilized swab to remove nontreated biofilm. Each disc was inserted in PBS and subjected to ultrasound bath 2 minutes for serial dilution. Serially diluted aliquots were plated in Anaerobe 5% Sheep Agar Blood and incubated anaerobically at 37°C for 5 days.

2.5.2 | Confocal laser scanning microscopy (CLSM)

The biofilms were stained using the Live/Dead BacLight Viability kit (Invitrogen-Molecular Probes, Waltham, MA) and incubated in anaerobiosis for 15 minutes at room temperature. Live cells are stained in green while dead cells are stained in red. A series of images of the biofilm was obtained using a Leica TCS SP5 II confocal microscope (Leica, Germany) with Leica HCX APO L 40x/0.8 W U-V-I water dipping lens (Leica, Germany).[12] Five random optical fields were examined for each specimen.

2.5.3 | Tissue culture

In vitro reconstituted gingival epithelium EpiGingival (GIN-100 MatTek Corporation, Promega, USA) model is based on normal human oral keratinocytes differentiated into tissues with a cornified, gingival phenotype, part number GIN-100. It was used to test the biologic response, as previously described.[12]

2.5.4 | Tissue analyses

CytoTox-ONE (Promega, USA) Homogeneous Integrity Assay kit was used as a fluorometric method to estimate cell viability based on the release of lactate dehydrogenase (LDH) from cells with damaged membrane. LDH release into the culture medium was measured by an enzymatic assay that results in the conversion of resazurin into resorufin. The MatTek MTT toxicology kit (MTT-100 MatTek Corporation, Fitchburg, Wisconsin) was used to check tissue viability.[12]

2.5.5 | Histology

Samples were immersed in 10% formalin, washed with PBS, dehydrated with 50% and 70% ethanol and prepared for paraffin embedding, cutting, and Hematoxylin/Eosin (H/E) staining. For tissue analysis, the slides were scanned with Panoramic MIDI 1.15 SPI 3D HISTECH® (Budapest, Hungary) and captured in Panoramic Viewer 1.115.2 3D HISTECH® (Budapest, Hungary). The histopathologic parameters (epithelium thickness and modification, hyperkeratosis, hyperplasia, and changes in cell morphology) were classified by semiquantitative analysis into scores from 0 to 3 (0—no modification, 1—minimum modification, 2—medium modification, and 3—severe modification).[14]

2.5.6 | Immunohistochemistry

To identify actively proliferating cells, angiogenic activity (tissue repair activity) and apoptosis, immunohistochemistry was performed on 4 μm formalin-fixed paraffin-embedded samples using the following markers: Ki67, vascular endothelial growth factor (VEGF-A), and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (Thermo Scientific, Waltham, MA), respectively. The sections were deparaffinized in xylene, rehydrated through graded alcohols (100% and 95% ethanol), and rinsed in distilled water. Heat-induced epitoperetrieval was performed in a 1200 watt microwave oven at 100% power in 10 mM sodium citrate buffer, pH 6 for 20 and 10 minutes, respectively. Sections were allowed to cool for 30 minutes and then rinsed in distilled water. Antibody incubation and detection were carried out at 40°C on a Discovery instrument (Ventana Medical Systems, Tucson, AZ) using Ventana's reagent buffer and detection kits. Endogenous peroxidase activity was blocked with hydrogen peroxide. Antibody was diluted (1:400) in Dulbecco's PBS (Life Technologies, Waltham, MA). Samples were incubated overnight at room temperature. Antibody was detected with biotinylated goat anti-rabbit diluted at 1:200 (Vector Laboratories, Burlingame, CA) for 30 minute. This was followed by application of streptavidin-horseradish-peroxidase conjugate. The complex was visualized with 3,3-diaminobenzidene and enhanced with copper sulfate. Slides were washed in distilled water, counterstained with hematoxylin, dehydrated and mounted with permanent media. The experiment was conducted in duplicate in two independent occasions. Immunohistochemical reaction in epithelial cells for VEGF was also performed and analyzed.

2.6 | Statistical analyses

IBM SPSS v. 22 software (IBM Corp., Armonk, NY) was used for statistical analysis with a confidence level of 95%. CFU data were $\log_{10}$ transformed prior to analysis, and log CFU data were further rank transformed in some analyzes in order to homogenize within-group variances for analysis of variance (ANOVA). Group differences were compared using one-way ANOVA, and given a significant omnibus test, post hoc Tukey HSD tests were pursued. For tissue analyses, LDH data were analyzed again by one-way ANOVA and post hoc Tukey HSD tests were pursued using a pooled estimate of the standard error, and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) (MTT) data were analyzed by percentile differences. Descriptive analyses were conducted for histologic and immunohistochemistry results.

TABLE 1 CFU/mL (log10) and standard deviation obtained for each group

Treatments	CFU/mL (log_{10})	Standard deviation
LTP1	4.66[c]	2.03
LTP3	4.64[c]	0.76
GAS	5.92[a]	0.40
CHX	2.92[b]	1.49
NEGATIVE	6.02[a]	0.32

Means followed by the same letter in the column are not significantly different ($P > 0.05$).

3 | RESULTS

3.1 | Biofilm analysis

Table 1 shows means and standard deviations of CFU/mL (log_{10}) of all groups. The LTP groups showed a reduction in CFU/mL when compared to the NEGATIVE and GAS control groups ($P < 0.001$). Parallel analysis of a rank transformation of the log CFU data (which homogenized within cell variability) confirmed the results of the raw CFU/mL analysis. Figure 1 shows the CLSM biofilm images after treatment, in which live cells are shown in fluorescent green and dead cells are shown in fluorescent red. Plasma-treated samples visually showed less biofilm than the NEGATIVE and CHX groups, which could indicate a possible mechanical removal of dead bacteria, however, further studies would be necessary to prove this hypothesis.

3.2 | Tissue analysis

3.2.1 | Cytotoxicity and viability

Percent cytotoxicity of LDH release showed difference between TRITON and the others groups ($P < 0.05$). Post hoc *t*-tests showed that GAS (5.09 ± 2.56%), LTP1 (3.83 ± 1.42%), and LTP3 (5.44 ± 2.11%) were similar and different from control groups ($P < 0.05$). For MTT, except TRITON, all groups showed high viability (Figure 2).

3.3 | Histologic and immunohistochemistry analyses

Figure 3A–F shows the histologic analysis. Minor tissue alterations in plasma-treated samples (score 1) with slight keratinization in comparison to the NEGATIVE (score 0) group were seen. The immunohistochemistry analyzes are presented in Figure 4. VEGF was slightly more evident for LTP1 (A) and LTP3 (B) than for negative control (F) and was absent in the TRITON group (F); Ki-67 showed LTP1 (G), LTP3 (H), GAS (I), CHX (K), and control negative (L) groups with <5% of positive cells in basal layer and TRITON group with >15% of positive cells in superior stratum (J); TUNEL was negative for LTP1 (M), LTP3 (N), GAS (O), CHX (Q), and control negative (R) groups, however, TRITON group evidenced several positive cells (P).

4 | DISCUSSION

Porphyromonas gingivalis is considered the major etiologic agent involved in chronic peri-implantitis.[15,16] However, there is a lack of studies on the effect of LTP against *P. gingivalis* biofilm.

This study aimed to determine the effect of LTP on *P. gingivalis* biofilm cultured on titanium discs. Plasma-treated biofilms for 1 and 3 minute presented log_{10} CFU/mL reduction in comparison to negative control. The results confirm our hypothesis that LTP is effective against *P. gingivalis* biofilm. However, in terms of log_{10} CFU/mL reduction, 3 minute application did not provide any improvement when compared to 1 minute irradiation. These findings agree with previous study, where 60 and 120 s did not show different results when it was applied on *Streptococcus mitis* biofilm.[11] Considering biofilms have a strong extracellular matrix, the superficial layer of the dead biofilm could have blocked the plasma effect on the residual bacteria inside the biofilm. Tissue does not have as much protection as the biofilm extracellular matrix, so possibly this could be a reason why plasma was

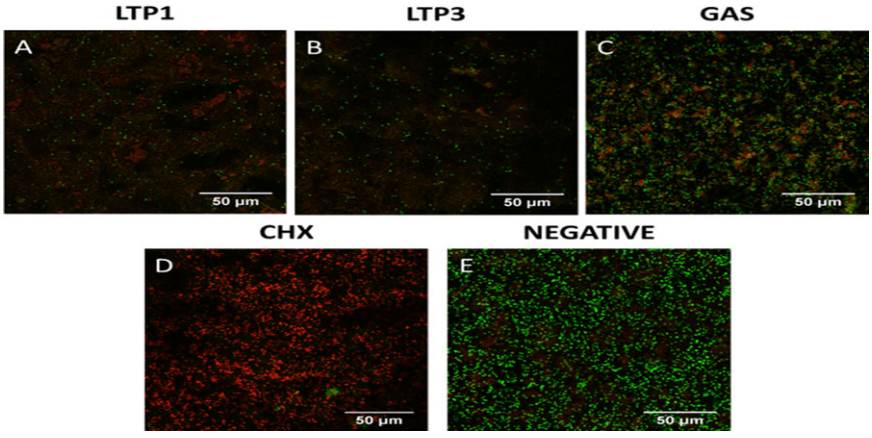

FIGURE 1 Confocal laser scanning microscopy of *P. gingivalis* biofilm after LTP treatment for 1 minute (**A**); LTP treatment for 3 minutes (**B**); GAS for 3 minutes (**C**); CHX-Positive Control for 1 minute (**D**); and NEGATIVE (**E**) 4 × zoom. Biofilm stained with Live/Dead BacLight Viability kit. Live cells in fluorescent green and dead cells in fluorescent red. Plasma-treated samples showed visually lower amount of biofilm than the NEGATIVE and CHX groups. After plasma treatment, images indicate a possible mechanical removal of death bacterium

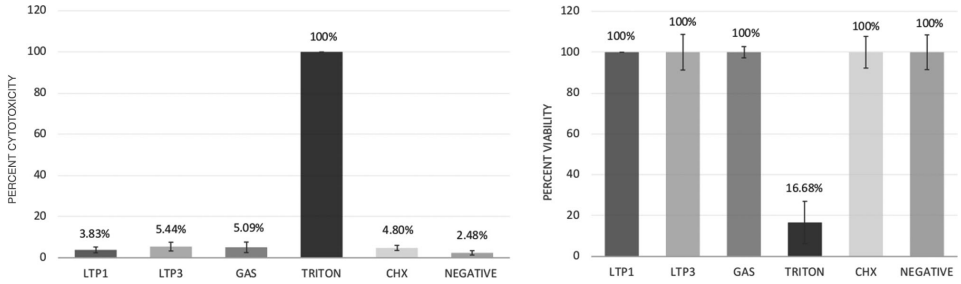

FIGURE 2 Mean (±SD) percent cytotoxicity (**left**) and viability (**right**) values of tissue. Considering the TRITON as a reference group for cytotoxicity calculation, the similarity between the results of the negative control and plasma groups indicates a minimum cytotoxic effect of plasma treatment on gingival epithelium within the dosage applied in this study. For MTT, considering the NEGATIVE control group as a reference for viability calculation, all groups showed 100% of percent viability, except TRITON

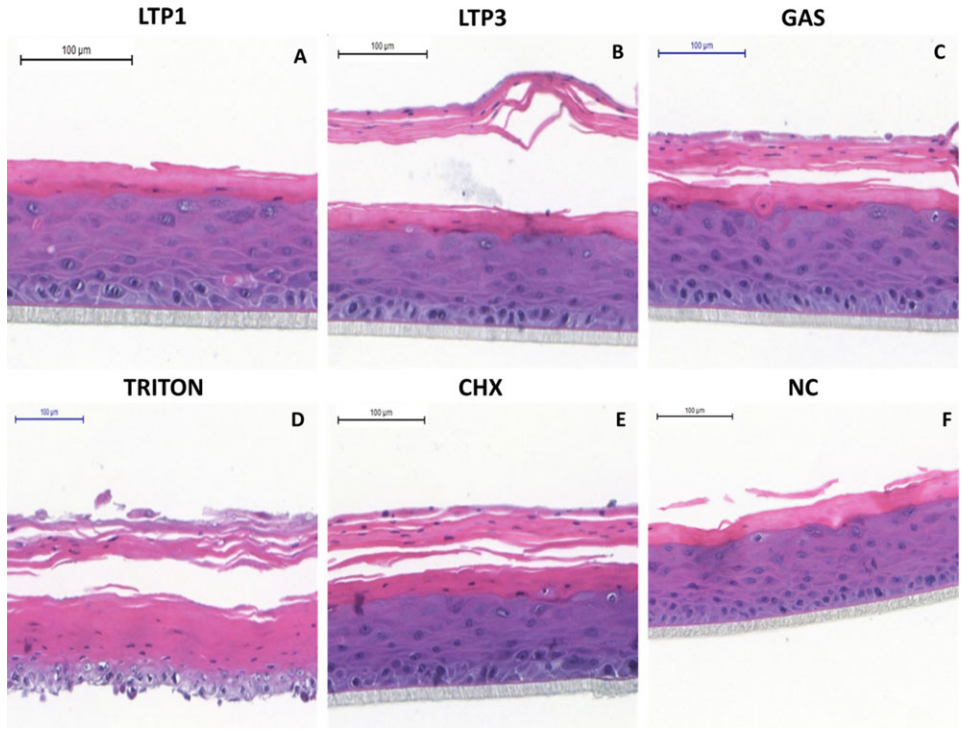

FIGURE 3 H/E: LTP1, score 1 (**A**); LTP3, score 1 (**B**); GAS Score 1 (**C**); Triton, score 3 (**D**); CHX, score 0 (**E**); and negative control, score 0 (**F**). TRITON exhibited significant tissue damage with cell vacuolization and nuclear shrinkage (ie, pyknosis) (score 3). LTP1 and LTP3 showed similar morphology

more effective after a higher exposure time. Further studies are needed to understand these outcomes.

In our study, a control GAS group (without plasma) was included assuming that gas application could have any possible effect on removing and killing bacteria. The results did not show any difference between GAS and NEGATIVE treatments, meaning that biofilm inactivation was promoted by plasma and not by the gas or the mechanical influence. For the gold standard treatment for anti-plaque therapy, chlorhexidine digluconate (CHX 0.2%) was used as a positive control, considering it shows antibacterial effects against periodontopathogens.[17–20]

Previous studies have shown the antimicrobial effect of LTP application on *Streptococcus sanguinis*, *C. albicans,* and *S. aureus* biofilm, and *S. Mitis.*[11,12,21] A study conducted in beagles also showed a significant decrease in detection of bacteria (*P. gingivalis* and *Tannerella forsythia*) when nonequilibrium plasma treatment was applied as an adjunct to the conventional therapy.[22] It has been shown that reactive oxygen species and reactive nitrogen species are the central players in its actions of antimicrobial and cancer therapies.[23] Furthermore, plasma-induced apoptosis has been clearly demonstrated in a recent paper that investigated cellular signaling related to an apoptotic process.[24]

In addition to oral biofilm inactivation, the study in beagles observed a larger amount of new bone formation in the bone-to-implant contact surface area in the plasma group.[25] In another study, the ability to remove naturally grown biofilms

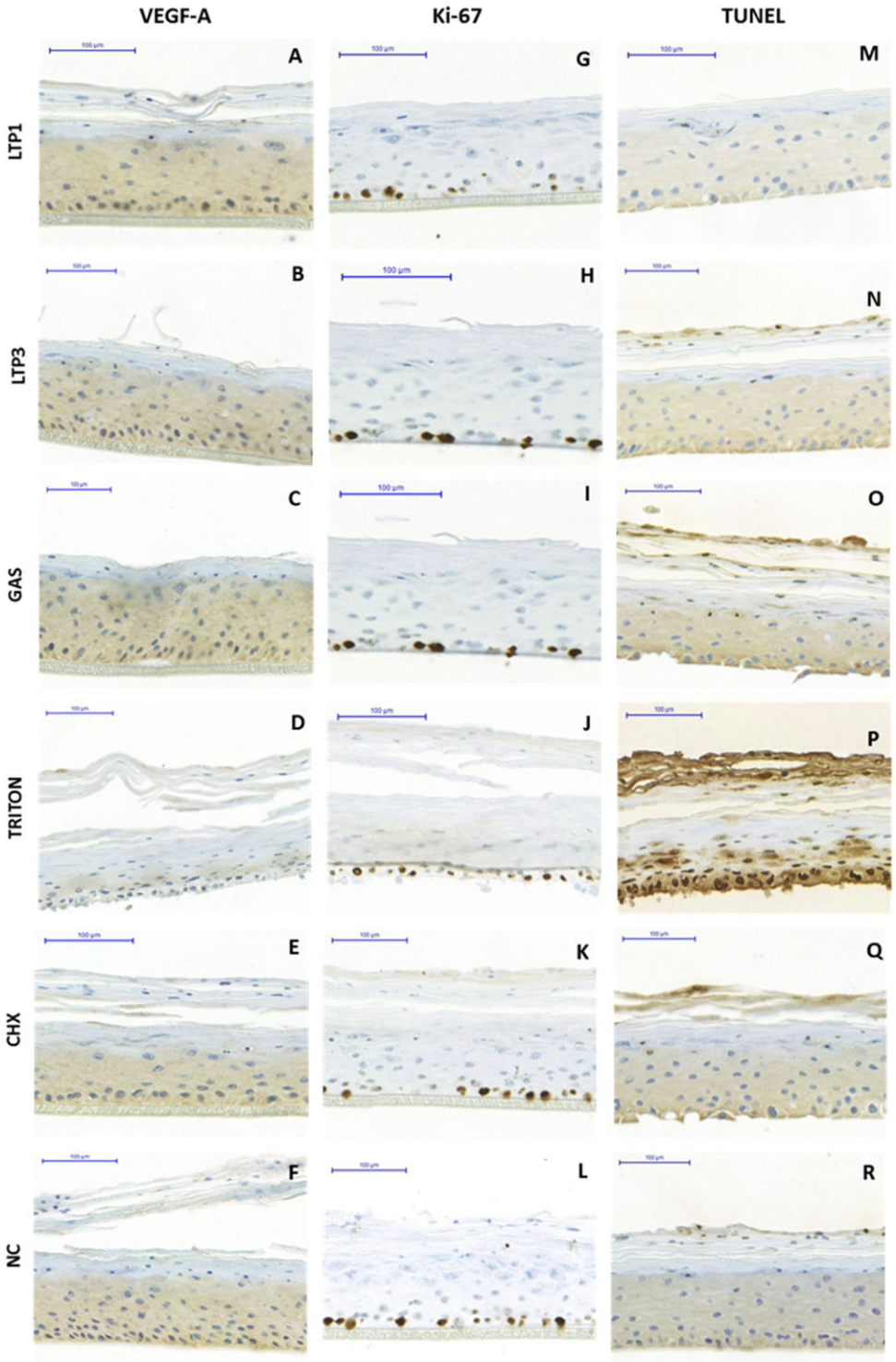

FIGURE 4 VEGF: Intense and diffuse immunostaining in LTP1, LTP3, GAS, CHX groups, and negative control (**A, B, C, E, F**) group. It shows discreetly more evident for LTP1 and LTP3. Absence of marking in the TRITON group (**D**); Ki-67: LTP1 (**G**), LTP3 (**H**), GAS (**I**), CHX (**K**), and control negative (**L**) groups with <5% of positive cells in basal layer; TRITON group with >15% of positive cells superior stratum (**J**); TUNEL: LTP1 (**M**), LTP3 (**N**), GAS (**O**), CHX (**Q**), and control negative (**R**) groups were negatives; TRITON group evidenced several positive cells (**P**)

on teeth with plasma treatment was compared to sonic brush, and biofilm removal was comparable for both treatments.[25]

However, it is important to note that killing the biofilm is not enough for its removal, considering there are other biofilm products that play a role in peri-implantitis, including cytokines.[26] It was demonstrated that removal of dead microbial residues was equally necessary to promote cell adhesion.[27] The visual analysis of the confocal images of the

present study showed fewer microorganisms in plasma-treated samples than in negative control groups. Although these findings indicate a possible mechanical removal of dead biofilm, the LTP potential to remove dead bacterium is still controversial. A recent study used 7-day-old biofilms to investigate the cleaning efficacy of air polishing with or without additional cold plasma treatment and concluded that concomitant use of air polishing and plasma treatment did not enhance osteoblast spreading.[28]

Despite the effective antimicrobial effect of plasma treatment, the fundamental nature of the interaction between plasma and human cells is still unknown to a large extent. So, a detailed knowledge of these interactions is essential for the evaluation of plasma effects in relation to cytotoxic and the establishment of new therapeutic plasma tools.

Various toxic responses or cellular and molecular functions are affected by LTP treatment in terms of up or down regulation of their associated proteins. Most of the toxicity responses are linked to oxidative stress response emphasizing oxidative stress as a possible key event in the regeneration process of epithelial cells, as well as in the adaptation to plasma exposure.[29] It is interesting to note that some recent studies have already revealed dose-dependent cellular effects in response to cold plasma treatment.[30,31] These studies indicate that increasing plasma doses could heighten the proportion of cells which are forced to undergo apoptosis, leading to a progressive negation of positive effects. Therefore, the biologic response should be tested for specific conditions.

To evaluate possible toxic responses by LTP application, plasma treatment was conducted on an in vitro reconstituted human gingival tissue containing normal and human-derived gingival cells. The cells have been cultured to form multilayered, highly differentiated models of gingival phenotypes. The tissues are cultured on specially prepared cell culture inserts using serum-free medium and attain levels of differentiation on the cutting edge of in vitro cell culture technology. The EpiGingival tissue models exhibit in vivo-like morphologic and growth characteristics, which are uniform and highly reproducible.[32] As gingival tissue has similar morphologic and physiologic characteristics of peri-implantitis soft tissue, testing plasma biologic response on gingival tissue is a distinction of this study. For our acknowledgment, no previous study with plasma was conducted on gingival tissue.

The results of the present study showed low cytotoxicity (Figure 2), high viability (Figure 3) levels after LTP application. It agrees with results of previous study that was conducted with the same plasma device, but in a different tissue, a reconstituted oral epithelium.[9] Regarding LDH, although negative control group showed statically significant difference in comparison to LTP groups, clinical relevance has to be further studied. Results for LTP1 were similar to GAS and CHX groups. However, LTP1 and LTP3 were different. Three minutes of plasma application was more cytotoxic than 1 minute. This data is in accordance to some recent studies that demonstrated dose-dependent cellular effects in response to cold plasma treatment.[33,34] Furthermore, LTP treatment is able to get selective killing effect between normal gingival cells and cancer cells due to sensitivity against reactive species.[30,35]

The histologic and immunohistochemical results also showed safety biologic response for LTP treatment. H/E stained sections showed minimal cell damage for plasma-treated groups, similar to the NEGATIVE and CHX groups. It means that the damage was repaired since the cellular changes were unobtrusive. Through labeling for Ki-67, it was possible to detect that LTP1 and LTP3 groups showed proliferative index similar to the NEGATIVE and CHX groups (<5%), similar to what was described previously, which illustrated that plasma was not able to act deep in the reconstituted oral epithelium tissue structure. Keratinized epithelium may also have a protective barrier against plasma reactive species.[12] TUNEL marker also showed that TRITON group showed diffuse positivity, different from all others groups. The epithelial cell analysis for anti-VEGF-A antibody demonstrated that LTP1 and LTP3 evidenced intense and diffuse cytoplasmic labeling in almost all epithelial layers. VEGF is a highly conserved, disulfide-bonded dimeric glycoprotein of 34-45 kDa and it is produced by several cell types, including fibroblasts, neutrophils, endothelial cells and peripheral blood mononuclear cells, macrophages, activated T-cells, and epidermal keratinocytes. VEGF binds to specific receptors expressed in endothelial cells.[36] VEGF plays a fundamental role in tissue repair, considering revascularization is a dynamic event necessary for the complete healing process. During angiogenesis, several growth factors are expressed and regulated. However, it is known that VEGF is the main factor in this process.[37,38] When the level of VEGF released in the culture media of human gingival epithelial cells from patients with generalized chronic periodontitis was analyzed, it was concluded that VEGF in periodontal disease may predict a greater regeneration capacity of gingival tissue.[39,40] Thus, the results of the present study indicate that VEGF-A immunoexpression identified in the epithelial cells of plasma-treated groups may be an induced repair factor.

Another possible advantage of LTP treatment is that it can potentially enhance peri-implant soft-tissue seal on titanium abutments, possibly preventing infection from the oral environment and decreasing implant failure. A recent study demonstrated that LTP treatment for 10 s improved the attachment of human gingival fibroblasts to titanium disks, perhaps due to oxygen present in functional groups on the surface, and/or decreased levels of carbon contamination.[4]

Even though significant reduction of viable biofilm was found in plasma-treated groups when compared to negative control, the magnitude of the reduction was less than 2 $\log_{10}$ CFU/mL. Further studies should be conducted in order to show whether this is a clinically relevant microbial reducing.

As we know, tip-to-sample distance, biofilm thickness, and treatment duration can influence plasma effect.[30,31] Furthermore, as CHX had the highest antimicrobial effect, further studies could consider additive or synergistic effects resulting from the combination of both treatments (CHX + LTP) or (LTP + CHX). This hypothesis would have to be further studied. In addition, biologic responses should be evaluated for each parameter. Despite the proven antimicrobial effect of CHX on titanium bound biofilms, CHX may compromise the biocompatibility of titanium surfaces, and its use is not recommended to detoxify implants. Then, LTP could be an alternative method for peri-implantitis treatment.[41] Further studies are also needed to determine the safety and efficacy of LTP application in vivo and in the clinical settings.

As a limitation, even if plasma treatment was effective in the studied *P. gingivalis* biofilm, in vivo peri-implantitis biofilms are much more structured than the one simulated here, which might affect the antimicrobial potential of LTP. So, future experiments should be conducted in more complex biofilm models aiming to understand the effect of the specific treatment on the biofilm structure, similar to the clinical peri-implant biofilm.

5 | CONCLUSION

Within the limitations of the present in vitro study, it can be concluded that LTP is a promising approach in peri-implant infection treatment. LTP treatment safely reduced *P. gingivalis* biofilm. In addition, plasma treatment may be associated with cellular report within a reconstituted gingival epithelium. Further studies are required to investigate the impact of LTP on clinical parameters, such as bleeding on probing and probing depth, and on the regeneration of gingival tissues surrounding titanium implants.

ACKNOWLEDGMENTS

This study was performed in the Department of Basic Science & Craniofacial Biology at New York University College of Dentistry where the supervisor Dr. Simone Duarte was working as Clinical Associate Professor before moving to Indiana University School of Dentistry, where she is currently working as an Associate Professor. We would like to thank Singular Implants—Dmr Ind. e Com. de Materiais Odontologicos Ltda, Parnamirim, RN, Brazil for providing the titanium discs. The authors report no conflicts of interest related to this paper.

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How to cite this article: Carreiro AFP, Delben JA, Guedes S, et al. Low-temperature plasma on peri-implant–related biofilm and gingival tissue. *J Periodontol*. 2019;90:507–515. https://doi.org/10.1002/JPER.18-0366