

Electrical enhancement of chlorhexidine efficacy against the periodontal pathogen *Porphyromonas gingivalis* within a biofilm

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SUMMARY

Electric currents have been shown to promote the antimicrobial effectiveness of several biocides against microbial biofilms. Therefore, the objective of this work was to test the null hypothesis that low electric direct currents (DC) do not influence chlorhexidine (CHX) efficacy against the periodontal pathogen *Porphyromonas gingivalis* within a biofilm. A brain heart infusion medium inoculated with *Streptococcus gordonii* and *P. gingivalis* was perfused for 7 days in anaerobiosis through two modified Robbins devices (MRD) assembled in parallel. Biofilms grew on hydroxyapatite discs placed at the bottom of the MRD plugs, and were then treated for 10 min with either CHX or CHX/DC (1.5 mA or 10 mA). The bactericidal effect against biofilms was then evaluated by comparing the mean proportions of *P. gingivalis* killed. In the first series of experiments (CHX +/- 1.5mA), the proportions of *P. gingivalis* killed were 81.1% for biofilms undergoing CHX and 79.1% when they were additionally treated with 1.5mA ($p>0.05$). In the second series (CHX +/- 10mA), the viability of *P. gingivalis* was reduced by 87.3% with CHX and 98.9% when CHX was supplemented with 10mA ($p<0.01$). The null hypothesis was rejected, since a significant enhancement of the chlorhexidine 0.2% efficacy against *P. gingivalis* was observed when applying 10mA currents.

KEY WORDS: Bacteria, Bioelectric effect, Biofilm(s), Chlorhexidine, Modified Robbins device, *Porphyromonas gingivalis*.

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INTRODUCTION

Periodontitis is a common polymicrobial inflammatory disease responsible for the progressive destruction of the tooth-supporting tissues (Pihlstrom *et al.*, 2005). Without any treatment, this disease finally results in tooth loss (Lamont

et al., 1998) and potentially systemic complications (Genco *et al.*, 2010). The mechanisms by which periodontal homeostasis is disrupted are not precisely understood but the inflammatory pathogenesis of periodontal diseases involves a breakdown of the host-microbe equilibrium (Socransky *et al.*, 1992). It is currently believed that the disease is the consequence of synergistic and dysbiotic microbial communities whose detrimental activities could be coordinated by some keystone pathogens (Hajishengallis *et al.*, 2012). The microorganisms of these communities which make up the very complex human oral microbiota (more than 700 prokaryote species), settle over the mucosa and the dental surfaces on which they grow and develop as dental biofilms (Dewhirst *et al.*, 2010).

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A biofilm is a highly structured community of adherent microbial colonies embedded in an extracellular polymeric matrix whose cells express genes that differ profoundly from their planktonic counterparts (Stoodley *et al.*, 2002). One of the consequences of this bacterial mode of growth is the development of structural and physiological properties that result in a number of chronic infections including periodontitis (Donlan *et al.*, 2002).

Biofilm infections are more difficult to cure essentially because biofilm microorganisms have the ability to withstand the host immune response and to resist antimicrobial agents (Stewart *et al.*, 2001).

Indeed, bacteria in biofilms are thus 500 to 5000 times more resistant to biocides than their planktonic homologues (Costerton *et al.*, 1994). Controlling the oral biofilm is fundamental for the maintenance of periodontal health and for the treatment of periodontal diseases (Scheie *et al.*, 2004). Thus, the majority of periodontal therapies consist of non-surgical and surgical debridement of periodontal pockets in order to remove calculus and disorganize mechanically the subgingival biofilm from the dental root surface. In general, these therapeutic procedures, associated with optimal plaque control, are successful in controlling the inflammatory reaction and periodontal disease evolution. However, in some clinical situations, mechanical debridement of periodontal pockets, even if carefully performed, is not sufficient, and leads to clinical failures. For example, this may occur in deep and/or narrow lesions, when access for instruments is limited (Socransky *et al.*, 2002). For that reason, some authors have recommended subgingival antimicrobial irrigations, during or after scaling and root planing to improve bacterial biofilm reduction and improve the clinical outcomes (Jorgensen *et al.*, 2005). Unfortunately, the results of clinical studies are controversial and in some situations, even with the addition of antimicrobials the periodontal treatment proves insufficient (Greenstein *et al.*, 2005). This might be explained by the physiological and morphological properties of the microbial biofilms that confer a strong resistance to antimicrobial agents and to the host immune system.

Therefore, new strategies to eliminate biofilms have to be developed in order to improve the

outcome of periodontal treatments. One interesting approach could be the addition of low intensity direct electric currents (DC) to antimicrobial agents. This was first suggested in the industry by Blenkinsop *et al.* (Blenkinsop *et al.*, 1992), who observed that the bactericidal efficacy of several industrial biocides was significantly increased against bacterial biofilms when DC were applied concomitantly. This phenomenon, called the “bioelectric effect”, has to our knowledge neither been investigated on periodontal pathogens like *Porphyromonas gingivalis* (*P. gingivalis*) nor in association with oral antiseptics. Since the bacteria from the “red complex” (*P. gingivalis*, *Tannerella forsythia*, *Treponema denticola*) are strongly associated with chronic periodontitis (Socransky *et al.*, 1998), it is of great interest to test the bioelectric effect on these in association with antiseptics already used clinically for such purposes, notably chlorhexidine (CHX). More specifically, *P. gingivalis* is considered one of the major pathogens associated with severe forms of periodontitis (Griffen *et al.*, 2012). This bacterium has also recently been described as a key-stone pathogen because it has been shown to be able, even in low abundance, to transform a symbiotic microbiota to a dysbiotic one leading subsequently to a breakdown of host-microbe homeostasis (Hajishengallis *et al.*, 2011; Darveau *et al.*, 2012). This modification of the overall load and composition of the commensal oral microbiota is then responsible for complement-dependant destructive inflammation and bone loss (Hajishengallis *et al.*, 2014).

Hence, the aim of this study was to test the null hypothesis that the application of low electric direct currents (DC) does not influence the efficacy of chlorhexidine 0.2% (CHX) against the periodontal pathogen *Porphyromonas gingivalis* grown within a dual-species biofilm.

MATERIALS AND METHODS

Bacterial strains, growth conditions and biofilm formation

P. gingivalis is unable to form a biofilm by itself. It needs primary colonizers like the commensal nonperiodontopathogenic *Streptococcus gordonii* (*S. gordonii*) to develop and grow

as a biofilm (Cook *et al.*, 1998). In order to form dual-species biofilms composed of *P. gingivalis* W83 and *S. gordonii* ATCC10558, two modified Robbins devices (MRD, LPMR-12 E, Tyler Research, Edmonton, Alberta, Canada), each containing 12 cylindrical steel plugs, were used in this experiment. These apparatuses allow for dynamic biofilm growth on the lower surfaces of the plugs on which ceramic hydroxyapatite discs (5 mm diameter/2 mm width) (Clarkson chromatography products Inc., South WilliamSPORT, PA, USA) were previously attached (12 hydroxyapatite discs on each MRD). The two devices were assembled in parallel and the circuit was sterilized with glutaraldehyde 2% for 1 hour. Thereafter, the circuit was rinsed thoroughly with sterile saline (NaCl 0.9%) during 3 hours. A 500 mL culture broth medium (brain heart infusion (BHI) supplemented with 5 g/L of yeast extract, 0.5 g/L of cysteine, 5 mg/L of hemine, 1 mg/L of vitamin K) (pH=7.3, DDP=-97 mV), inoculated 1 hour before with *S. gordonii* (as to be adjusted to 0.5 McFarland units) was then perfused for 1 hour through the circuit at

48 mL/h to initiate biofilm formation. Like the previous disinfection step, this procedure was allowed by the use of a Masterflex® peristaltic pump (Barnant company, Barrington, IL, USA). The *S. gordonii* culture broth was then replaced by 500 mL of a similar broth previously inoculated with *P. gingivalis* approximately 6h before to obtain a 2McFarland turbidity units. Biofilm growth was continued over a 7 day period in anaerobiosis (80% N₂, 10% H₂, and 10% CO₂) at 37°C on the 24 hydroxyapatite discs according to a protocol previously described by Bercy and Lasserre (Bercy *et al.*, 2007). All assembly elements were linked to each other by means of silicone tubes (Tygon® Tubing, Cole Parmer Instrument Company, Vernon Hills, IL, USA) presenting 1.6 mm internal diameter and 3.2 mm external diameter.

To adhere to an oral solid surface, *S. gordonii* uses its surface proteins, which allow its irreversible attachment to the acquired pellicle components such as α -amylase, prolin-rich proteins, silylated mucins or salivary agglutinins (Kolenbrander *et al.*, 2002). Thus, in order to

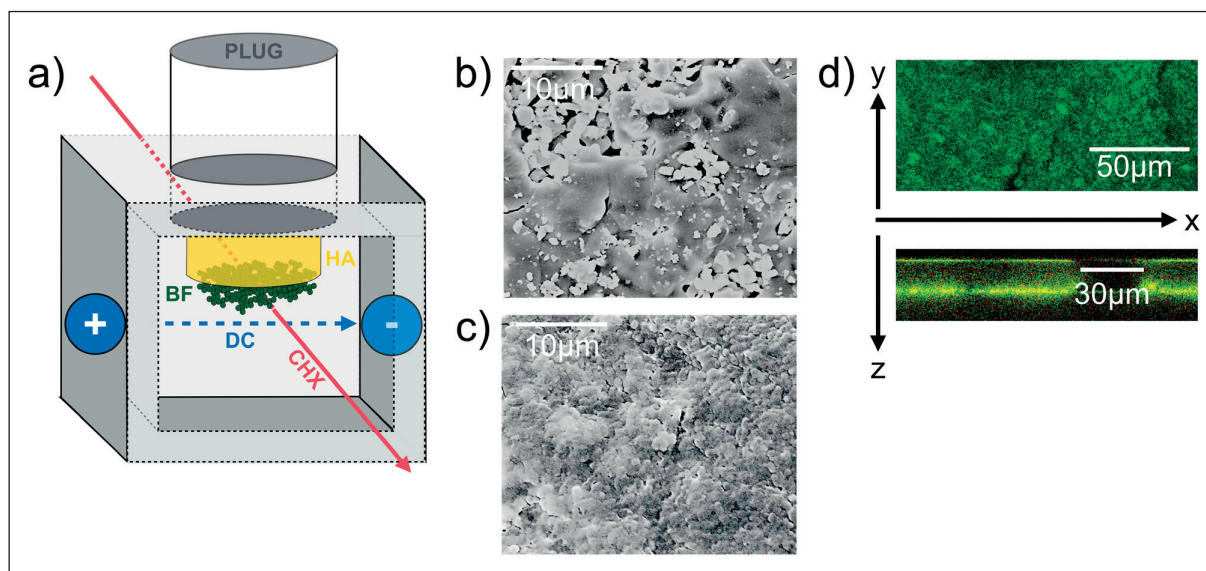


FIGURE 1 - a) Schematic representation of the electrified modified Robbins device during the antibiofilm treatment. Thanks to the platinum electrodes and to the DC supply, the biofilms grown on hydroxyapatite discs and composed of *P. gingivalis* W83 and *S. gordonii* ATCC10558 undergo at the same time the action of CHX 0.2% and the electric DC of 1.5 or 10mA; b) SEM micrograph of a pristine hydroxyapatite disc (2.98kX, 15kV); c) SEM micrograph of the same disc after the formation of a 7-day period dual-species biofilm (3.32kX, 15kV); d) CLSM observation of a 7-day dual-species biofilm through XY and XZ plans. Biofilm was previously stained for 20 minutes with BacLight Live/Dead staining. Live bacteria appear green and dead bacteria red. This type of observation allows an appraisal of the 3D bacterial distribution within the biofilm.

simulate an acquired pellicle *in vitro*, 1L of saliva was collected from 18 volunteer students of the school of dentistry from the Université catholique de Louvain. This saliva was then centrifuged (10 min, 600 rpm) (VWR®, compact star CS4, USA) to separate cells from salivary molecules. The supernatant obtained was then sterilized through a 0.2-µm-pore-size cellulose filter. The final volume obtained was divided into 10mL portions and kept at -18°C. Before each experiment, hydroxyapatite discs were soaked for 30 minutes at 37°C in 10mL of this saliva supernatant. The aim of this process was to allow salivary glycoprotein adsorption on the inner surfaces of the discs in order to promote, via this conditioning film, a better adhesion of the *S. gordonii*. Indeed, even though *P. gingivalis* seems to have salivary receptors and an *in vitro* capacity to adhere to saliva coated hydroxyapatite (Cimasoni *et al.*, 1987), it has been demonstrated with confocal microscopy that it presents many difficulties in surface adhesion and self-based biofilm formation (Cook *et al.*, 1998). However, *P. gingivalis* will adhere firmly to a solid surface when it is associated with an early plaque colonizer like *S. gordonii*. Together these two species will then rapidly form biofilm structured micro-colonies. This is relevant to what happens *in vivo*. In this context, the Cook *et al.* (1998) and Larsen *et al.* (1995) methods were modified to create a simple and reliable *in vitro* technique which allows the formation of *P. gingivalis*-containing biofilms (Larsen *et al.*, 1995; Cook *et al.*, 1998). In order to distribute bacteria homogeneously, the BHI-enriched broth was mixed continuously using a magnetic stirrer. Every 24 hours, the BHI broth was renewed. During each 24 hours of culture, the broth pH and DDP decreased on average from 7.3 to 6.2 and from -97 mV to -150 mV, respectively. After 7 days of biofilm formation, the circuit was opened, purged and finally rinsed, under low shear forces, with a sodium chloride (NaCl 0.9%) solution for 5 minutes to remove loosely attached bacteria. Prior to the killing assays, biofilms were characterised by means of scanning electron microscopy (SEM) (3.32kX, 15kV) and confocal laser scanning microscopy (CLSM). For the latter, biofilms were previously stained during 20 minutes with BacLight Live/Dead staining.

Biofilm killing assay

Three series of experiments were conducted (in anaerobiosis), i.e. CHX vs CHX + 1.5 mA, CHX vs CHX + 10 mA, and NaCl vs NaCl + 10 mA, with a total of 18 replicates (five, five and eight replicates, respectively).

To apply DC to the antiseptic solution, one of the two MRDs was connected to a DC power supply (Velleman® HQ power™ PS1503SB, 0/15V-3A, Gavere, Belgium) via two platinum electrodes (each strand with a 0.25 mm diameter and 216 mm length) running through the MRD and allowing the application of the DC (1.5 mA or 10 mA) on the concerned biofilms (Figure 1a). This connection was possible thanks to two pin jacks emerging from opposite sides of the main body of the appliance. The solutions NaCl or CHX (pH=7.3 and 5.6 respectively), both non degassed nor reduced as is usual in clinical practice, were then perfused through the two MRDs. The duration of the killing assays was 30 minutes.

For each replicate, the 12 discs of each MRD were pooled three by three at T0 (controls), T=10 and T=30 min of antimicrobial procedure. The three remaining discs were used for microscopy imaging. Afterwards, each pool was processed and cultured as described below. During the first series of experiments (CHX vs CHX + 1.5 mA) (five replicates), the intensity of the currents was adjusted to 1.5 mA with the power supply and all along verified using a digital multimeter (Hapé® - MI-6 MK7). In the second series (CHX vs CHX + 10 mA) (five replicates), the intensity was increased to 10 mA. Biofilms that formed on hydroxyapatite discs at the bottom of the MRD plugs were treated in the two first series with CHX on the first MRD and CHX associated with low intensity DC (1.5 or 10 mA) on the second one. In order to evaluate the self effect of the currents on bacterial viability, a third series of experiments (eight replicates) was performed in which biofilms underwent either sodium chloride (NaCl 0.9%) or sodium chloride plus 10mA direct electric currents (NaCl +10 mA DC).

Culture and bacterial counts

During each experiment (replicate), biofilm-covered discs from each MRD were aseptically collected, rinsed of planktonic germs with

10 mL of saline and pooled three by three in 1 mL of PBS for analysis after T0, T=10 min and T=30 min of treatment. They were then vortexed during 1 minute and ultrasonicated for 1 minute (100W, 42KHz) to detach adherent bacteria as proposed by several authors (Larsen *et al.*, 1995; Mohn *et al.*, 2011). Finally these recovered bacteria were cultured as described below, in order to evaluate the antibacterial effect of each treatment modality on the periodontal pathogen *P. gingivalis*.

More precisely, the collected biofilm bacteria were inoculated after serial ten-fold dilutions (from 10^0 to 10^{-3}) on enriched blood agar plates. One hundred microliters of each dilution were thus cultured on Columbia agar plates (pH=7.3) (BBL™ Columbia Agar Base, Becton, Dickinson and Company, Sparks, MD, USA) enriched with 5mg/L of hemine and 1mg/L of vitamin K. All plates were then transferred for incubation within 15 minutes into the Bugbox® anaerobic workstation (LED Techno NV, manufactured by Ruskin Technology Limited: Leeds - UK) of which the atmosphere was composed of 80% N₂, 10% H₂ and 10% CO₂ (ANAERO 10, AIR LIQUIDE Medical, Liège, Belgium). Incubation was then allowed for 10 days at 37°C. Afterwards, the number of *P. gingivalis* CFUs was calculated by assessing colony morphology and Gram staining. Prior to the experiments, the *P. gingivalis* W83 strain had been isolated from a clinical subgingival plaque sample, cultured and identified after 16S rRNA gene sequencing. Hence, the number of black-pigmented Gram negative CFUs of *P. gingivalis* per cm² was calculated as well as standard deviations. Data were collected for T0, T=10 and T=30 min and for each treatment modality. Microbiological analyses were performed by an independent examiner blinded to the nature of the project.

Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS PASW, statistics 18.0 for PC, SPSS Inc., Chicago, IL, USA). The mean proportions of *P. gingivalis* killed after 10 minutes of antimicrobial procedures were used as data. The normal distribution of the data was not assumed. The non-parametric Mann-Whitney and Kruskal-Wallis tests were thus used to compare the

groups. Bonferroni correction was applied for multiple comparisons. As a consequence, results were considered significant at $p < 0.05$ for the Mann-Whitney analysis and at $p < 0.017$ for the Kruskal-Wallis tests.

RESULTS

After seven days of growth under controlled anaerobiosis, the dual-species biofilms were formed and characterised by SEM (Figures 1b and 1c) and CLSM (Figure 1d). In the latter, live bacteria appear green and dead bacteria red. This type of observation allows an appraisal of the 3D bacterial distribution within the biofilm. CLSM analysis also showed that thickness of the seven-day dual-species biofilms on Mylar® supports was on average 30 micrometers (Figure 1d). For each experiment and each modality of treatment (CHX; CHX +1.5mA; CHX +10mA; NaCl; NaCl +10mA) the mean numbers of *P. gingivalis* CFUs/cm², log₁₀ densities (LD) and standard deviations were calculated at T0, T=10 min and T=30 min. Regarding the effects observed in the first two series of experiments, after 30 minutes, regardless of the bactericidal modality used (CHX; CHX + 1.5 mA; CHX + 10 mA), the mean levels of *P. gingivalis* CFUs were found to be 1.9, zero and 0.6 Log₁₀, respectively. Hence, the biofilm bacteria were almost totally killed by CHX 0.2% with or without the electric current without any statistical difference between the treatment modalities. In the light of this, and since 30 minutes of treatment is longer than what would be reasonable clinically, it was decided to focus on the T=10 min results to compare the different treatment modalities on the microorganisms viability. The mean proportion of *P. gingivalis* killed with each modality was thus calculated and compared after T=10 min of antimicrobial procedure.

In the first series of experiments (CHX +/-1.5 mA), after 10 minutes of treatment, the proportions of *P. gingivalis* killed were respectively 81.1% (+/-10.0) for biofilms perfused with CHX only and 79.1% (+/-8.6) when adding 1.5 mA DC (Figure 2a) (Kruskal-Wallis: $p > 0.017$).

In the second series (CHX +/-10mA), a proportion of *P. gingivalis* killed of 87.3% (+/-3.8) was observed with CHX and 98.9% (+/-0.4)

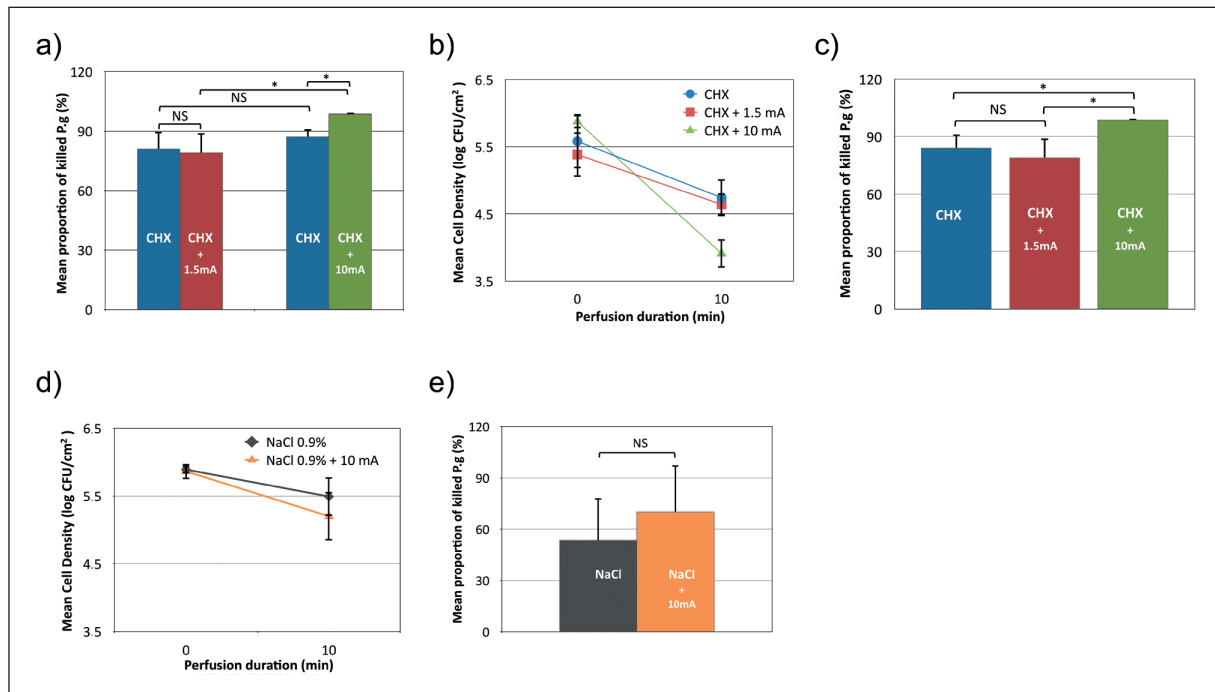


FIGURE 2 - a) Bactericidal effect of 10 min perfusion of chlorhexidine 0.2% on *Porphyromonas gingivalis* W83 in dual-species biofilms when used with or without low electric DC. A Kruskal-Wallis test adjusted with Bonferroni correction was used to define the significant differences between the samples (*: $p < 0.01$); b) Reduction of *Porphyromonas gingivalis* W83 viability after treatment with chlorhexidine 0.2% +/- low direct electric currents; c) Bactericidal effect of chlorhexidine 0.2% on *Porphyromonas gingivalis* W83 in dual-species biofilms when used with or without low electric DC; A Kruskal-Wallis test adjusted with Bonferroni correction was used to define the significant differences between the samples (*: $p < 0.01$). Perfusion Duration: 10 min; d) Reduction of *Porphyromonas gingivalis* W83 viability after treatment with sodium chloride 0.9% +/- low direct electric currents; e) Proportion of *Porphyromonas gingivalis* killed after 10 min undergoing NaCl 0.9% +/- 10 mA DC; A Mann-Whitney test was used to compare the two samples (NS: $p > 0.05$). Experimental Duration: 10 min.

when supplementing with DC (Figure 2a). This difference was statistically significant (Kruskal-Wallis: $p < 0.017$). The statistical analysis also demonstrated that the results obtained between the CHX + 1.5 mA and CHX + 10 mA antiseptic procedures were also significantly different ($p < 0.017$). These experiments with CHX and the various DC are summarized in the Figures 2b and 2c.

In the third series of experiments (NaCl +/- 10 mA), after T=10 min, 53.4% (+/- 24.6) of *P.gingivalis* were killed in the control group (NaCl) and 69.9% (+/- 27.3) in the test group (NaCl + 10mA) (Figure 2d and 2e). This difference was found non significant (Mann-Whitney U test: $p > 0.05$). Those results indicate that 10 mA DC do not have an additional bactericidal effect on *P.gingivalis* within the biofilms compared to NaCl alone. In order to see whether another

isotonic and stable (regarding the pH) solution could change those results, an additional experiment (19th) was carried out applying the same design but changing NaCl 0.9% by non degassed PBS (pH=7.2). It was observed in this experiment that using PBS the bacterial viability decreased very slightly during the 30 minutes of treatment with a maximum of 0.1 log₁₀ reduction from baseline whatever the modality used (PBS or PBS + 10 mA). Moreover, as was the case with NaCl, the use of the current did not have any additional impact on bacterial vitality. Finally, during the killing assays, gas formation was always observed all around the DC-connected platinum electrodes. This formation only manifested on the MRD submitted to the DC. It could be the consequence of electrolysis since this gas production was still observed after a few minutes when the experiment was

conducted without bacteria. This phenomenon was noted regardless of the solution used (CHX, NaCl 0.9% or PBS).

DISCUSSION

The null hypothesis was rejected, since a significant enhancement of chlorhexidine 0.2% efficacy against *P. gingivalis* was observed when applying 10 mA currents during 10 min, thereby highlighting the existence of a bioelectric effect against *P. gingivalis in vitro* for this specific combination of CHX and DC.

On the contrary, no effect of DC were observed either with CHX + 1.5 mA or with the other solutions (NaCl or PBS) associated with 10 mA. These observations suggest that the electrical enhancement observed with chlorhexidine against *P. gingivalis* within the biofilms is more likely a synergistic effect than an additional effect since no extra killing effect was shown using NaCl or PBS. It was noticed that CHX 0.2% was also effective by itself in killing *P. gingivalis* within a biofilm but needed as much as 30 minutes to eliminate most of the biofilm bacteria. It was also observed that *P. gingivalis* viability reduction appeared quite substantial with NaCl (53.4%). This observation might be explained by the fact that even if the experiment was conducted anaerobically, the saline had not been degassed/reduced before being added to the biofilm as is the case in a clinical setting. As a consequence, residual oxygen as well as pH modifications could have adversely affected *P. gingivalis*. This information shows that *P. gingivalis* vitality decreases when it is stressed by the absence of ideal growth conditions.

Galvanic currents have already been demonstrated to occur *in vivo* between amalgams and other metallic restorations but their intensity did not seem to reach more than a few microamperes (Sutow *et al.*, 2004), which is 1 to 10000 times lower than that used in the present study. Electric currents have previously been used in other medical fields. For instance, they have been used in oncology research to reduce the activity of certain brain tumour cells (Hernandez-Bule *et al.*, 2012). The generation of a cytotoxic and/or cytostatic effect in the target tissues was observed without damaging the

healthy surrounding cells. Another clinical application of electric currents has been proposed in orthopedics to improve bone healing in the treatment of non-union fractures (Hughes *et al.*, 2010). However, very few researchers have investigated the use of low electric currents on their potential to increase the efficacy of antimicrobial agents. The first electrical enhancement of industrial biocides was reported in 1992 (Blenkinsoop *et al.*, 1992). That study showed that various common biocides (kathon, glutaraldehyde, quaternary ammonium compound) presented an increased killing action against *Pseudomonas aeruginosa* biofilms when supplemented with low-intensity currents. This phenomenon, called the bioelectric effect, was then confirmed several times but on non oral bacteria and with antimicrobials that are rarely used in oral health (Costerton *et al.*, 1994; Jass *et al.*, 1996). Although some theories have been proposed to explain the way by which electric currents could enhance antimicrobial efficacy, the phenomenon is still not clearly elucidated. Some have suggested that the bioelectric effect could depend on electrophoretic forces that would facilitate biocide diffusion within the biofilm and/or through the cell membranes (Costerton *et al.*, 1994). Others reported that it could be explained by pH modifications (Stoodley *et al.*, 1997), electrochemically generated antimicrobial agents (like chlorine-based substances) (Davis *et al.*, 1994) or hyperoxygenation (Stewart *et al.*, 1999). What currently appears is the fact that even weak electric currents could have a range of physical and biological effects that could be responsible for the enhanced antimicrobial activity.

To our knowledge, only one publication investigated whether this electrical enhancement of antimicrobials could be emphasized with oral biofilm bacteria (Wattanakaroon *et al.*, 2000). The authors showed an increased efficacy of gentamicin in killing *S. gordonii* compared to the antibiotic alone. This enhancement was observed with 2 mA direct currents but after 24 hours treatment duration which cannot be transferred to a clinical procedure. Nevertheless, the idea of improving the antibacterial activity of commonly used antimicrobials against oral biofilms could be of interest in oral healthcare. This is particularly true for the treatment

of periodontal disease and oral periimplantitis, which are well recognised as pathologies due to the presence of bacteria organised in polymicrobial biofilm, the latter being much more resistant to antibacterial molecules than are their planktonic counterparts (Stewart *et al.*, 2001). Currently, the clinical benefits of adding antiseptic irrigations to conventional mechanical treatment of periodontal disease is still controversial (Greenstein *et al.*, 2005). Hence, the use of low DC might improve the results of antimicrobials and justify their use in a clinical setting since their application appears beneficial in some conditions in the present clinically relevant *in vitro* model. Two potential applications could result from the present research. The first would be to develop an ultrasonic hand-piece that could provide at the same time a mechanical action to disorganize the dental biofilm through a tip and an antiseptic irrigation combined with low DCs to optimize the antimicrobial strategy. This kind of appliance could be of particular clinical relevance for the treatment of severe or refractory periodontal diseases as well as for oral periimplantitis management. Furthermore, it has been shown that effective supragingival plaque control has a pronounced effect on the nature (percentage of sites infected with *P. gingivalis*) and the load of the subgingival microflora of moderate periodontal pockets (Hellström *et al.*, 1996). As a consequence, another possible clinical application would be the use of these electric currents through a special tray designed for home care to improve supragingival plaque control during supportive periodontal therapy.

As a conclusion, within the limitations of this *in vitro* model, the present work presents proof of concept that a 10 mA DC applied during a clinically relevant 10-minute period is able to enhance significantly the efficacy of 0.2% CHX in killing *P. gingivalis* within dual-species biofilms. Evidence of such a bioelectric effect in the context of periodontal biofilms and antiseptics paves the way for new approaches for taking care of periodontally compromised patients and oral periimplantitis infections. Nevertheless, further preclinical experimentations are warranted to validate the concept and apply it to clinical investigations. Finally, it is worth mentioning that some concerns could emerge

from the idea of submitting a human body to currents ranging from 1.5 to 10mA. Nevertheless, according to Dalziel's reference investigations (Dalziel, 1961), this range of direct electric currents, if possibly perceptible, should not be detrimental to patients. However, if the bioelectric effect is confirmed in further works, the safety of such currents needs to be verified.

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