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Effects of a novel gel containing 5-aminolevulinic acid and red LED against bacteria involved in peri-implantitis and other oral infections

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ABSTRACT

Antibiotic resistance is a major public health problem worldwide and the finding of alternative methods for eliminating bacteria is one of the prerogatives of medical research. The indiscriminate use of antibiotics in dentistry, especially for the treatment of peri-implantitis, could lead to superinfections.

Alternative methods, like photodynamic therapy mediated by the use of aminolevulinic acid and a red light has been largely described, especially in dentistry, but results were encouraging against Gram-positive bacteria, but limited against Gram-negative.

The effectiveness of photodynamic therapy mediated by a novel product containing aminolevulinic acid, Aladent (ALAD) has been tested in this *in vitro* study, against different types of bacteria particularly involved in the infections of the oral cavity and peri-implantitis. The novelty of ALAD is the marked hydrophilicity that should increase the passage of the molecule through the membrane pores of Gram-negative bacteria.

Considering the novelty of the product a preliminary experiment permitted to test the effectiveness against *Enterococcus faecalis* after 1 h of ALAD incubation at different concentrations, with or without different timings of LED irradiation. The count of CFUs and the live/dead observation with fluorescent microscopy showed a significant reduction and killing of bacterium.

Then, in the second stage, that could meet the necessity of effectiveness and the clinician's requests to reduce the timing of treatment, ALAD, with and without irradiation, was tested on *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Veillonella parvula* and *Porphyromonas gingivalis*.

In particular, the efficiency of different concentrations of the product after a 25 min incubation was tested with and without the adjunctive LED irradiation for 5 min.

A slight ALAD bactericidal effect was reported for all bacteria, also without LED irradiation, however, the most effective treatment was 25 min of 50% ALAD incubation followed by 5 min of a red LED.

The *in vitro* tests demonstrated that ALAD gel with LED irradiation exerts a potent antibacterial activity on different bacteria, both Gram-positive and Gram-negative.

1. Introduction

One of the most important challenges of recent times is antibiotic resistance, a major public health problem worldwide [1,2]. Bacteria, using different mechanisms like mutational adaptations, alterations of gene expression and the high ability to acquire genetic material, are able to develop resistance against antibiotics. As a consequence, a recent work highlighted an increased burden of infections in EU countries since 2007 [3]. Novel studies have highlighted the role of dentistry in contributing to antibiotic resistance and pointed out that more than 80% of antibiotic prescriptions were unnecessary [4,5].

Peri-implantitis is a bacterial inflammatory process that causes progressive bone loss around implants, that is difficult to solve [6]. We have previously shown the importance of bacterial colonization on long

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time success of dental implants, and this novel treatment could represent a potential solution against peri-implantitis [7–9]. There is still debate in literature about the similarity of the microbiota in sites affected by periodontitis and peri-implantitis, but there are no doubts about the negative role of *Porphyromonas gingivalis* in promoting bone breakdown in both diseases [6]. The use of antibiotics for the treatment of peri-implantitis is questionable, but literature agree in the necessity of additional instruments or therapies, that could achieve sufficient implant decontamination and disinfection, especially in sites difficult to reach and treat [10]. Indeed, a recent article highlighted the risk of bacterial superinfections and an exacerbate disease progression associated with the indiscriminate use of antibiotics, without a microbiological follow-up, for the treatment of peri-implantitis [11].

The use of light devices, like LEDs or LASERs represents a valid tool for bacterial reduction: their mechanisms of action is based mainly on photophysical and/or photochemical reactions [12,13]. In previous studies we have shown the ability of 880 nm LED light to promote a significant reduction of *Enterococcus faecalis* and *Pseudomonas aeruginosa*, both planktonic than in biofilm [14–16].

It has been shown that bacterial photoinactivation, can occur with different mechanisms: endogenous direct, in which high-energy photons, like UVs damage directly the bacterial DNA or other vital cellular parts, and indirect in which the photo-activation of endogenous or exogenous photosensitizers form reactive species that consequently cause cell death [17,18].

In particular in the indirect mechanisms, the light-activation of the specific targets produces free radicals and ROS that promotes cytotoxic, apoptotic and autophagic signals, particularly important in oncology and microbiology [19].

Among the exogenous molecules, a precursor of the natural photosensitizer protoporphyrin IX (PpIX), the aminolevulinic acid (ala), is particularly important. It can be synthesized endogenously in mitochondria but can be also added exogenously in form of a prodrug [20]. The use of a precursor, instead of directly adding the protoporphyrin, permits better intracellular absorption of the molecules, and finally the irradiation provides a cytotoxic stimulus [21]. All cells can adsorb and deactivate ala, but the enzymatic reactions involved are different in healthy cells and tumors; moreover, ala accumulation and the consequent photoactive porphyrins (PAPs) production is more pronounced in cells with high rates of metabolic activity, like cancer cells, inflammatory cells and bacteria [22]; this higher PAPs accumulation permits a selective and specific killing of neoplastic and/or bacteria after the irradiation [22].

Several authors reported that ala-antimicrobial photodynamic therapy is more effective in the inactivation of Gram-positive bacteria, since their cell wall, composed of peptidoglycan and lipoteichoic acid, is relatively more porous, allowing photosensitizer to reach the cytoplasmic membrane. In contrast, Gram-negative bacteria have a much more complex morphology [23]. Their cell wall contains negatively charged lipopolysaccharide, lipoproteins and proteins with a porin function, in addition to peptidoglycan [24]. This structural organization forms a physical and functional barrier that hinders the incorporation of the photosensitizer.

In turn, PAPs production is dependent also by other parameters, like lipophilicity, pH value, concentration and duration of exposure of ala [25]. In particular, it has been shown that the ppi production is represented by a biphasic bell-curve: until a certain intracellular ala concentration, the ppi production and fluorescence increase in directly proportional manner, but when it is reached a certain threshold, the production decreases [25].

A new gel containing 5% ala, characterized by higher hydrophilicity, was produced. The important parameters for microorganism-photosensitizer-interaction include: its relative solubility in water and lipids, ionization constant and other more specific factors, such as, light absorption characteristics and the efficiency of formation of the excited state triplet and singlet oxygen production [26].

The aim of this work was to evaluate the effect of the association of a novel product containing 5-aminolevulinic acid (ala) at 5%, commercialized as Aladent (Alphastrumenti, Italy) (ALAD), with a red LED irradiation on different types of bacteria, particularly important in dentistry and implantology.

In the first stage, we performed preliminary tests in order to find the better protocol that exerted an antimicrobial activity, following the manufacturer guidelines; we also evaluated the effect of different concentrations of ALAD and different irradiation times of red LED on *Enterococcus faecalis*, alone or in combination.

In the second stage, we tested the effectiveness of a protocol that responded to the clinicians' requests for reducing the time of treatment and the cost of materials. Moreover, we verified the effects of ALAD on different types of bacteria particularly important for dentistry and involved in the antibiotic resistance, both Gram-positive and Gram-negative, like the periodontopathogen *Porphyromonas gingivalis*.

2. Materials and Methods

2.1. ALADENT gel

Aladent (ALAD) is a gel containing 5% of 5-aminolevulinic acid (ala); it is covered by a patent (PCT/IB2018/060368, 12.19.2018) and the object is “pharmaceutical preparation comprising a topically released active ingredient and a heath sensitive carrier, method of obtaining same, and use of same in the treatment of skin and mucosal infections”. The full text is registered on patentscope/wipo/int con PubNo WO2019123332.

2.2. Light Source and Irradiation Parameters

An AlGaAs power Led device (TL-01) characterized by 630 nm ± 10 nm FHWM nm-wavelength was used as light source (Alphastrumenti, Italy).

The hand-piece was constituted by 1 LED with 6 mm diameter at the exit and a surface irradiance of 380 mW/cm^2. During the experiments, the LED hand-piece was mounted perpendicularly to the wells at 0.5 mm of distance with a particular polystyrene box to maintain a constant distance from light source (Fig. 1). At these conditions, the exit irradiance surface was 380 mW/cm^2 and the total specific dose was 23 J/cm^2 for each minute of irradiation (Fig. 1).

As shown in our previous studies, the irradiation was performed under a laminar flow hood in the dark under aseptic conditions in all the experiments [14–16].

2.3. Test 1

The objective of Test 1 was to:

- determine the effectiveness of different concentrations of ALAD, incubated for 1 h, as suggested by Manufactures with and without LED irradiation after the incubation,

2.4. Bacterial Strains and Growth Conditions

The reference strain *Enterococcus faecalis* ATCC 29212 was used for this study. *E. faecalis* was cultured at 37 °C for 24 h in Brain Heart Infusion (BHI) broth. After incubation, the bacterial suspension was centrifuged at 3000 rpm for 10 min, the supernatant was discarded, the pellet was suspended in Phosphate buffer saline (PBS) (Sigma Aldrich, MO, USA) and vortexed.

The bacterial suspension was evaluated using a spectrophotometer (Agilent Technologies 8453 UV, Santa Clara, USA) to assure an optical density of OD_{600} = 0.12 corresponding to 10^8 Colony Forming Units (CFU)/mL.

For several experiments, aliquots of 1 mL of standardized broth

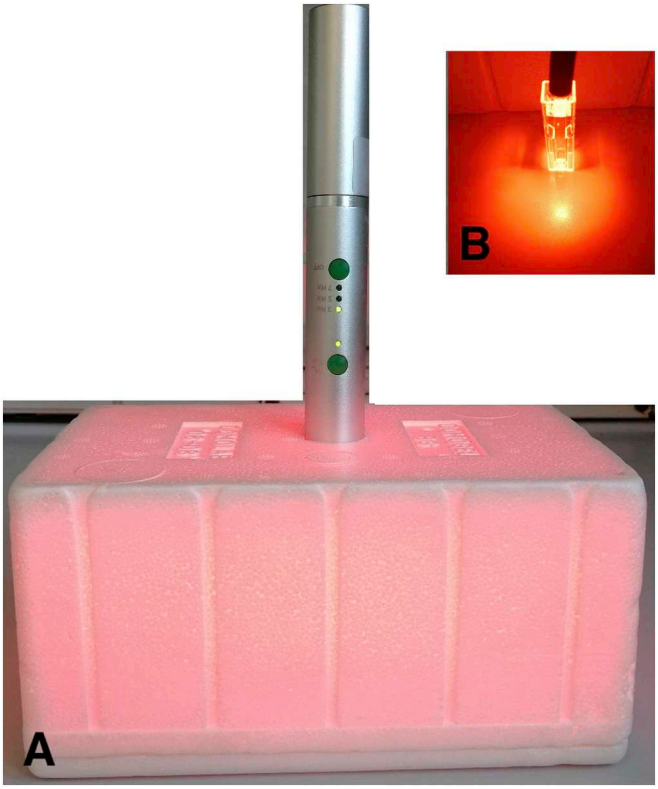

Fig. 1. A) The polystyrene apparatus utilized to obtain a uniform LED irradiation of the samples, maintaining a constant distance (d). B) the vision inside the box: a sample during the irradiation.

culture were dispensed in 24-well (dimension: 20 ∗ 20 mm) flat-bottom plates with lids separately and all of the tests included a positive control, containing bacteria and buffer, but no active substance (C+) and a negative one, containing the buffer without bacteria (C−).

The ALAD gel was added so the final concentration was 50% *v*/v and 10% v/v. The suspensions were incubated at 37 °C for 1 h, as suggested by Manufactures and after were seeded on agar plates. The remaining suspensions with 10% ALAD were irradiated with the LED device for 7 and 20 min; the suspension 50% ALAD was irradiated with LED device for 7 min. The suspensions were seeded on agar plates also after exposure to LED light.

Three independent experiments in triplicate were performed.

2.5. Bacterial Analysis

After treatments, the samples were checked, serially diluted in Phosphate buffer saline (PBS) (Sigma Aldrich, MO, USA) and Colony Forming Units per millilitre (CFU/mL) were determined, as previously described [16].

The cells viability was evaluated with a BacLight LIVE/DEAD Viability Kit (Molecular Probes, Invitrogen detection technologies, USA), as previously described [14].

2.6. Test 2

The objectives of test 2 were:

- to find a protocol able to reduce the time of treatment: so we used a reduced time of ALAD incubation (25 min) and we tested a reduced light exposure (5 min);
- to find a protocol able to reduce the costs, without influence the effectiveness: so we tested increasing concentrations of ALAD (10%, 25% and 50%).

2.7. Bacterial Strains and Growth Conditions

The microorganisms used in the test 2 were reference strains of *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Porphyromonas gingivalis* ATCC 33277 and *Veillonella parvula* ATCC 10790 (KWIK-STIK Microbiologics, Manassas, VA, USA).

E. coli, E. faecalis and *S. aureus* were cultured on blood agar supplemented with 5% sheep blood (HiMedia, Mumbai, India) at 37 °C for 24 h. Then 3–4 colonies of each bacterial strain were transferred to Brain Heart Infusion broth and incubated for 24 h. The strains of obligate anaerobe species *P. gingivalis* and *V. parvula* were grown on Brucella agar with hemin and vitamin K1 supplemented with 5% sheep blood (Sigma Aldrich, MO, USA) in anaerobic conditions at 37 °C for 48 h. Then 3–4 colonies of each bacterial strain was transferred to Schaedler broth with vitamin K (BD BBL, USA) and incubated for 24 h.

After incubation, the bacterial suspension was centrifuged at 3000 rpm for 10 min, the supernatant was discarded, the pellet was suspended in PBS and vortexed. The bacterial suspension was evaluated using a spectrophotometer (Agilent Technologies 8453 UV, Santa Clara, USA) to assure an optical density of OD_{600} = 0.12 and then diluted so the number of CFU/mL was around 10^5 for the facultative anaerobe, and 10^8 for the obligate anaerobe species. The exact number of CFU/mL in each bacterial suspension was obtained by culture on agar plates.

2.8. Photodynamic Treatment

Aliquots of 1 mL of bacterial suspensions were transferred into 24-well (dimension: 20 ∗ 20 mm) flat-bottom plates and the ALAD gel was added, so the final concentration of the gel was 50% *v*/v, 25% v/v and 10% v/v. The suspensions were incubated at 37 °C for 25 min (the obligate anaerobes were incubated in an anaerobic chamber), and after were seeded on agar plates. The suspensions were then irradiated with LED device for 5 min and the bacterial viability was determined by measuring the CFU/mL of each sample.

The number of CFU/mL was determined as previously described [14].

2.9. Statistical Analysis

Results were recorded on Data Sheet (Microsoft Office Excel, Microsoft, USA) and then the statistical analysis was performed by using SPSS for Windows version 21 (IBM SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) and the LSD test was used to compare the parameters analysed in the study. The significance threshold was set at 0.05.

3. Results

3.1. Test 1

The results of the CFU/mL count on agar plates are showed in Fig. 2.

1 h of dark incubation with 10% ALAD promoted a significant reduction of CFUs in respect to the positive control. However, the combination with LED irradiation significantly increased the antibacterial effect, proportionally to irradiation duration. A total inactivation of *E. faecalis* was achieved with 50% ALAD + LED 7 min. This effect was confirmed by live/dead observation (Fig. 3): significant differences were found among all groups, and the association between 50% ALAD + LED 7 min produced an evident killing effect on the *E. faecalis* cells, characterized by the presence of 95% of dead cells stained in red ($p < .05$).

3.2. Test 2

25 min of ALAD at different concentrations, without LED

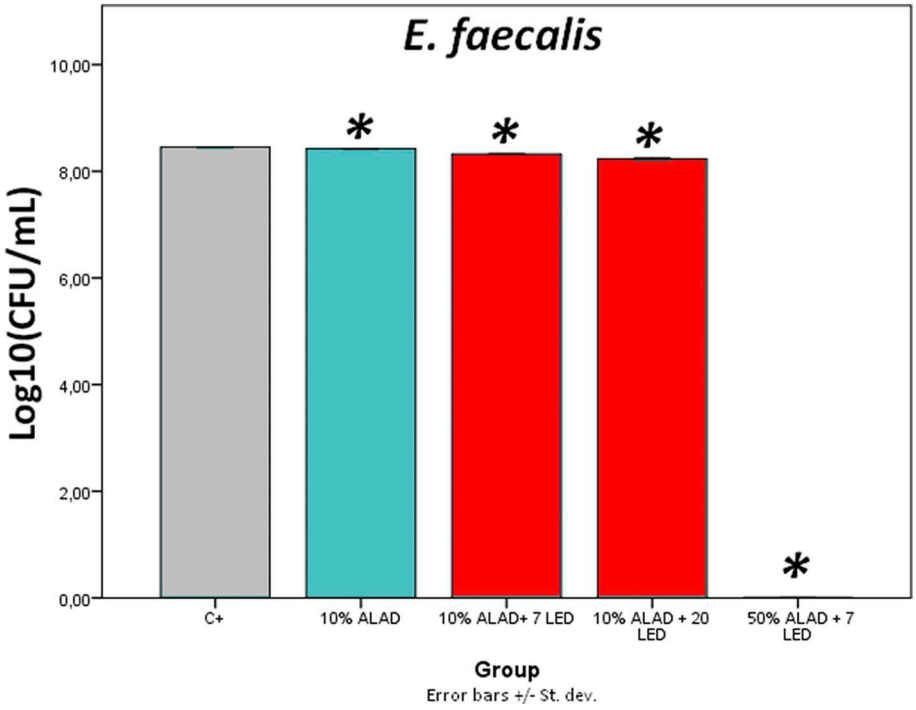

Fig. 2. Results of the preliminary Test 1: one hour of incubation with ALAD 10% permitted to achieve a significant reduction of *E. faecalis* respect C+.

irradiation, did not show an antibacterial effect against *E. faecalis* (Fig. 4). 25 min of incubation with 25% and 50% ALAD plus 5 min of red LED exerted a significant reduction of *E. faecalis*, but no significant differences have been found between the two different concentrations followed by the irradiation (for more details about significant p- values, please see the supplementary file, Table 1–5).

A significant reduction of *E. coli* has been detected after 25 min of incubation with 50% ALAD (Fig. 5). If the incubation with ALAD, was followed by 5 min of LED irradiation, the bacterial reduction was significant also with lower concentrations of ALAD, 10 and 25%, with a bactericidal effect that was proportional to the gel concentration. The highest bactericidal effect was recorded using 50% ALAD plus LED irradiation: this technique permitted to achieve statistically significant reduction compared to the other protocols tested in this study.

S. aureus was characterized by a significant reduction after 25 min of incubation with 25 and 50% ALAD (Fig. 6). The incubation with ALAD, followed by LED irradiation for 5 min, allowed to obtain a significant bacterial reduction also with lower concentrations of ALAD, as 10 and 25%, with a bactericidal effect proportional to the gel concentration. The highest effect was recorded using 50% ALAD plus LED irradiation: this association permitted to achieve statistically significant reduction in respect to the other combinations tested in this study, as well as to the control.

25 min of incubation with ALAD at different concentrations, 10, 25 and 50%, independently from LED irradiation (Fig. 7) led to a significant reduction of *V.parvula*. The combination with a LED irradiation allowed a further significant bacterial decrease. The use of a higher concentration of ALAD, 50%, followed by 5 min of LED irradiation, showed the best antibacterial results, with a significant reduction with respect to 25% ALAD + LED.

P. gingivalis was characterized by a significant decrease in bacterial growth only after 25 min of incubation with 50% ALAD (Fig. 8). The incubation with ALAD, followed by LED irradiation for 5 min, led to a significant bacterial reduction also with lower concentrations of ALAD (10 and 25%), with a bactericidal effect proportional to the gel concentration.

Statistically significant differences among the different ALAD concentrations followed by LED irradiation have been detected. The highest bactericidal effect was recorded using 50% ALAD plus LED irradiation: this protocol showed a statistically significant CFUs reduction in respect to other combinations analysed in this study.

4. Discussion

The effectiveness of photodynamic therapy mediated by the concurrent use of a novel product containing aminolevulinic acid (ALAD) and red led irradiation has been tested in this *in vitro* study, against different types of bacteria involved oral infections.

The novelty of ALAD, that is covered by an international patent, is that it was produced based on previous studies, in order to maximize the penetration in gram-negatives membrane.

In the first part of this study, we have performed a preliminary test on *E. faecalis*, a bacterium particularly resistant against chemical, mechanical and physical stimuli, and usually the cause of endodontic failures and secondary infections [27,28]. The use of increased concentrations of ALAD incubated for 1 h led to significant reductions of CFUs, also without LED irradiation. These results are in accordance with previous studies that have shown dark toxicity of the aminolevulinic acid, that is the ability of this substance to induce a certain amount of ROS production and promoting an antibacterial activity also without light activation [29]. Moreover, ALAD, contains also potassium sorbate and sodium benzoate as preservatives, and these could contribute to the bactericidal effect [30,31].

However, adding 7 min of LED irradiation after the incubation of ALA at a final concentration of 50%, achieved an almost total inactivation of *E. faecalis*. These results were very encouraging and were confirmed also by live/dead analysis. In previous studies, we showed that NIR-LED irradiation was able to promote a significant bacterial reduction, but the total inactivation of the bacteria was achieved only by adding 1% of sodium hypochlorite [14,16]. However, one of the advantages of the use of LED devices, is that the antimicrobic effect seems to be maintained over time, contrary to chemical substances that bacteria could inactivate over time, due to the presence of certain enzymes [14,15]. The use of ALAD permitted us to obtain similar results

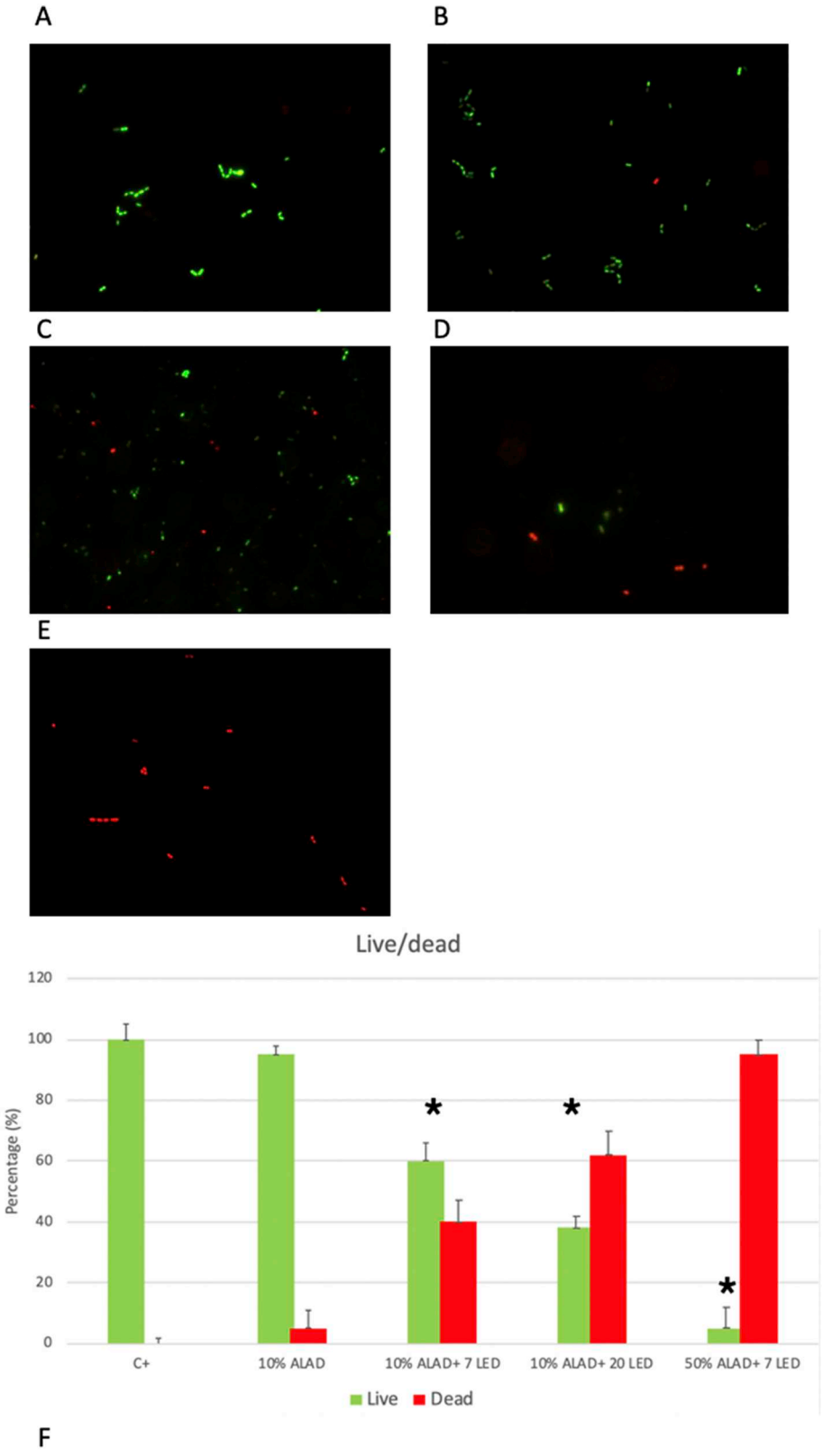

Fig. 3. Live/dead images of Test 1: A = C+; B = 10% ALAD; C = 10% ALAD+ LED 7; D = 10% ALAD+LED 20; E = 50% ALAD+LED 7; F = living and death cells in the analysed groups. Results showed that there were significant differences among all test groups and controls ($p < .05$).

of those obtained with NIR-LED, without the necessity to add NaOCl, which, although in low concentrations, could have a certain toxicity [32].

The second outcome of this study was to find a protocol that was more suitable for clinical practice by reducing the treatment duration and costs of materials, that still had an efficient and effective antimicrobial effect on bacteria particularly the ones involved in dental infections. For this reason, we decided to continue our study,

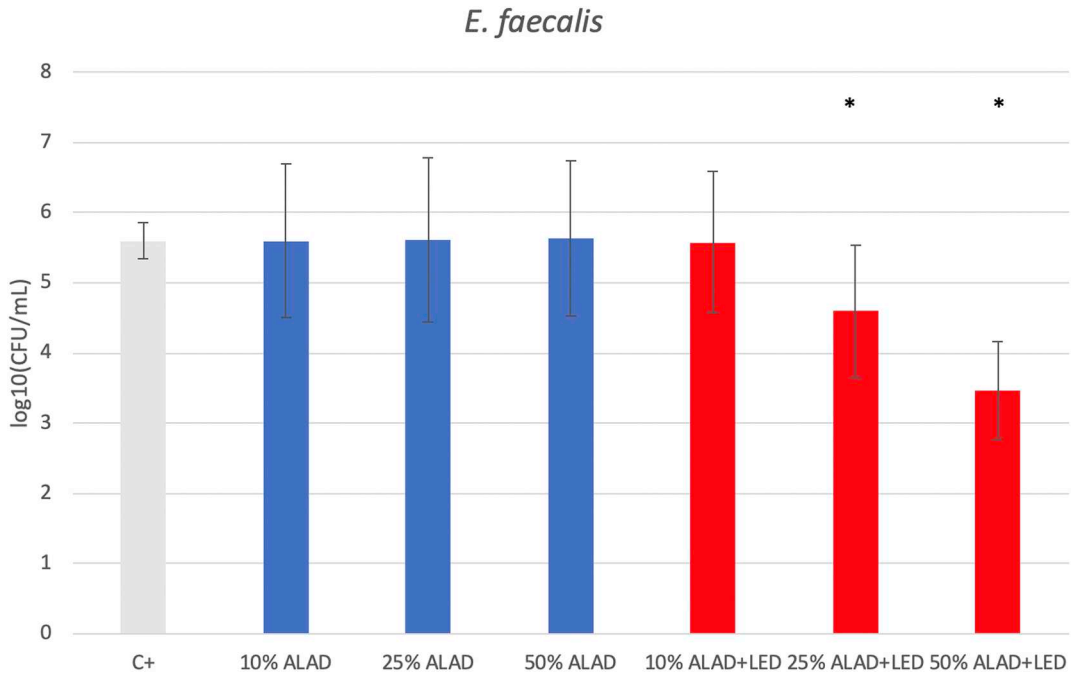

Fig. 4. Results of the Test 2: 25 min of incubation with ALAD 25% and 5 min of LED irradiation permitted to achieve the better *E. faecalis* reduction ($p < .001$). Error bars standard deviation. *p-value < .001 respect C+. In Table 1 of supplementary file is reported the table containing all the statistically significant results ($p < .05$), after LSD test.

decreasing the time of incubation of ALAD to 25 min, and the time of LED irradiation to 5 min, and by testing various concentrations of ALAD on different types of bacteria important for dentistry. As expected, at 10% ALAD alone or with LED irradiation was not effective in reducing the number of *E. faecalis* CFUs, but by increasing the concentration to 25% and adding LED treatment a significant reduction was recorded. Using a final concentration of 50% ALAD + LED 5 min the bacteria were almost completely inactivated.

The effects of ALAD on *V. parvula* were even more encouraging: 25 min of increasing concentrations of 10%, 25%, and 50% ALAD alone were able to induce a significant bacterial reduction, and combined with LED irradiation the effect was more evident. So for this bacterium, the gel contains certain toxicity by itself that is increased by the light activation (Fig. 7). *V. parvula* is a gram-negative bacterium, strictly anaerobic, that is a very important constituent of the dental biofilm. On day 4 after professional cleaning and removal of dental biofilm, *V. parvula* amounts to 5% of the newly formed biofilm, and this percentage rises to almost 10% by day 7. *V. parvula* is considered as a critical species that guides the development of multispecies communities when saliva is the main nutritional source [33–35]. So, the presence of this bacterium promotes the development of a complex bacterial community.

Similarly to *V. parvula*, ALAD alone had an intrinsic antibacterial effect also against *S. aureus.* A significant reduction of the CFU number was recorded with 25% and 50% of gel alone. When combined with a 5 min exposure to red LED light, the ALAD concentration of 50% almost completely eliminated the bacteria in the suspension. These results are not only important for dentistry, but also for medicine, because *S.*

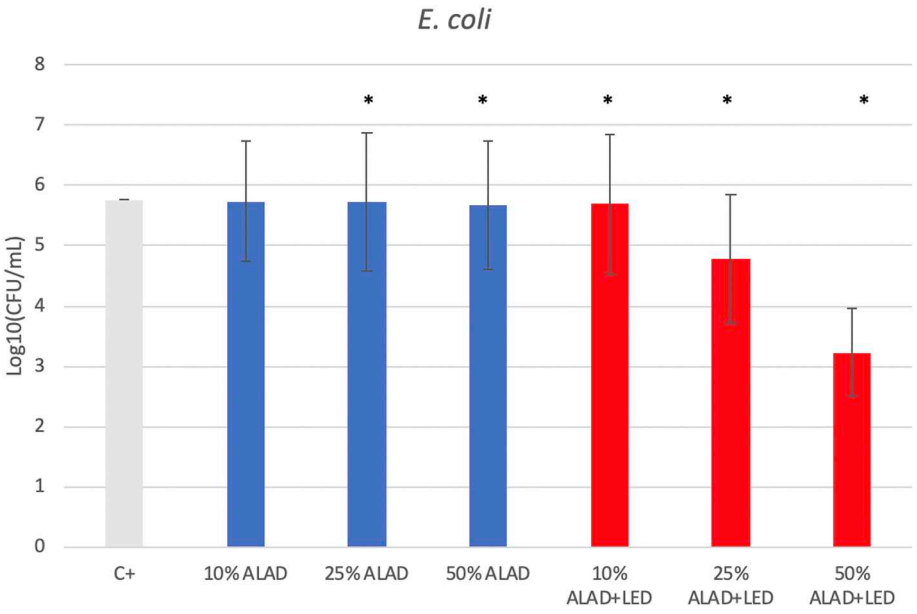

Fig. 5. Results of the Test 2: 25 min of incubation with ALAD 25% and 5 min of LED irradiation permitted to achieve the better *E. coli* decrease ($p < .001$). Error bars ± standard deviation. *p-value < .001 respect C+. In Table 2 of supplementary file is reported the table containing all the statistically significant results ($p < .05$), after LSD test.

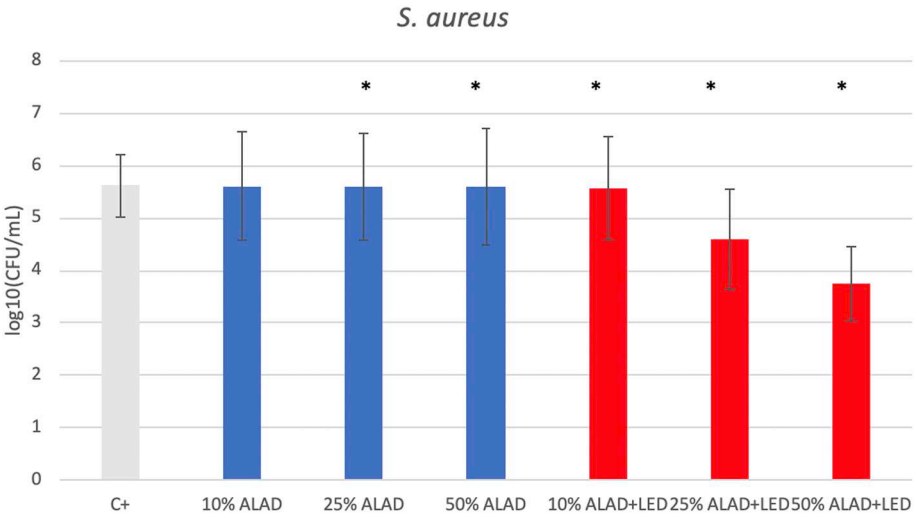

Fig. 6. Results of the Test 2: 25 min of incubation with ALAD 25% and 5 min of LED irradiation permitted to achieve the better *S. aureus* reduction ($p < .001$). Error bars ± standard deviation. *p-value < .001 respect C+. In Table 3 of supplementary file is reported the table containing all the statistically significant results ($p < .05$), after LSD test.

aureus is considered a major human pathogen, thanks to many virulence factors and the ability to acquire resistance to most antibiotics, including methicilline [36]. It is considered both a commensal but also a pathogen, able to cause bacteremia and infective endocarditis, as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections [37]. *S. aureus* has a great importance also in dentistry, because, together with *S. epidermidis,* it is considered the putative cause of 80–90% of jaw osteomyelitis [38].

Considering the high presence of *S. aureus* in the whole population and the connected risks of infection, the effectiveness of ALAD plus LED irradiation to provide an antibacterial activity, could encourage this protocol also for the disinfection of medical devices or prosthesis. As shown by Fotinos et al. *S. aureus* incubated with ala produces protoporphyrin IX (PPIX), a well-known photosensitizer [39]. Our results were partially in accordance with Hsieh et al., that found no dark toxicity for ala in *S. aureus* [29]. However, as specified by Eckl et al. dark toxicity depends on various parameters, like the sensitizer concentration and bacterial mode of life [40]. Moreover, different bacteria are able to produce different types of porphyrins after ala exposure [39].

E. coli is a facultative anaerobic Gram-negative bacillus that lives in the large intestine of humans and warm-blooded animals. It is usually not pathogenic but some strains could cause intestinal and extraintestinal diseases, sometimes also with fatal consequences [41]. *E. coli* has been associated to 10% of jaw osteomyelitis, especially in infants and older patients suffering from underlying medical illnesses [38].

This bacterium was more resistant to respect *V. parvula* and *S. aureus*, and it was necessary to increase ALAD to 50% to have a significant reduction of the number of CFUs without LED irradiation. These results are in accordance with Fotinos et al. that showed that, without irradiation, bacterial survival was not affected until the 5-ala concentration reached 40 mM. Contrarily irradiated samples have showed a drastic decrease in bacterial viability [39]. Ala incubation induces the production of two main photosensitizers by *E. coli*: lipophilic porphyrins carboxyporphyrin and PPIX [39].

P. gingivalis is an anaerobe gram-negative bacterium that, thanks to the ability to produce cysteine proteases and gingipains, is one of the major causes of periodontal and bone breakdown, causing periodontal and peri-implant disease [6,42]. Moreover, recent literature has also highlighted a potential role of this bacterium in the pathogenesis and proliferation and invasion of neoplastic cells of oral squamous cell and gastric carcinoma and immune diseases like rheumatoid arthritis [43–45]. The effects of ALAD incubation on this bacterium followed a trend similar to *E. coli*: the 50% ALAD alone permitted a significant reduction of CFUs, but adding LED irradiation for 5 min we had a

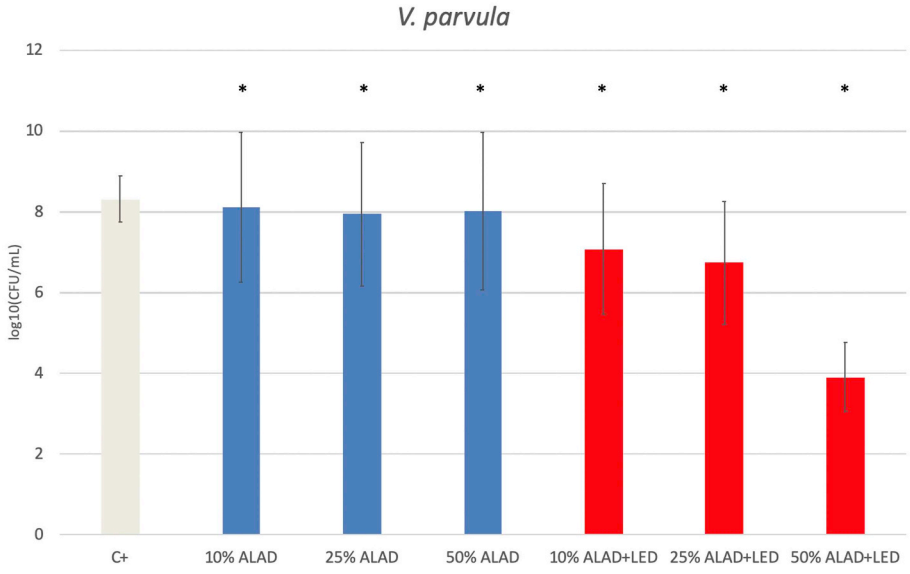

Fig. 7. Results of the Test 2: 25 min of incubation with ALAD 25% and 5 min of LED irradiation permitted to achieve the better *V. parvula* reduction ($p < .001$). Error bars ± standard deviation. *p-value < .001 respect C+. In Table 4 of supplementary file is reported the table containing all the statistically significant results ($p < .05$), after LSD test.

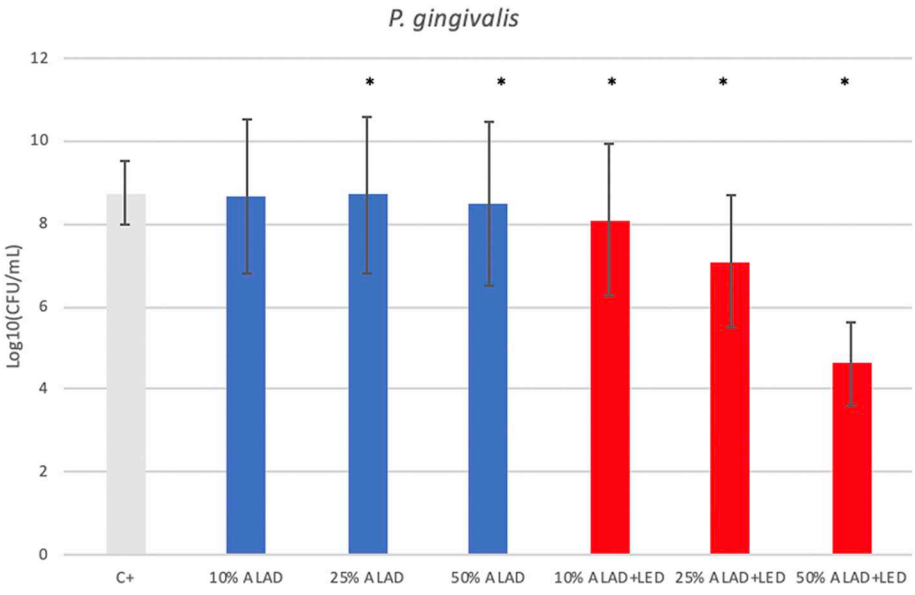

Fig. 8. Results of the Test 2: 25 min of incubation with ALAD 25% and 5 min of LED irradiation permitted to achieve the better P. gingivalis decrease $p < .001$). Error bars ± standard deviation. *p-value < .001 respect C+. In Table 5 of supplementary file is reported the table containing all the statistically significant results ($p < .05$), after LSD test.

boosted antibacterial effect.

In conclusion, both purposes of this study were confirmed: ALAD with LED irradiation exerted a potent antibacterial activity, that was confirmed also reducing the time of treatment. However, other *in vivo* and *in vitro* studies, with biofilm or multispecies bacteria, are necessary to confirm that this treatment could be used as a substitute of antibiotic therapy for localized dental infections and for the treatment of peri-implantitis.

5. Conclusions

This preliminary study showed that ALAD gel with LED irradiation exerted a potent antibacterial activity on different bacteria involved in antibiotic resistance and oral infections, both Gram-positive and Gram-negative.

The more effective protocol that shows a greater inhibitory effect on *E. faecalis* consisted of 1 h of gel incubation at a final concentration of 50% followed by 7 min of LED irradiation, but by decreasing the time of ALAD incubation to 25 min with 5 min of LED irradiation, encouraging results were obtained.

A significant bacterial reduction of *V. parvula, S. aureus, E. coli,* and *P. gingivalis* was recorded after 25 min of ALAD gel alone at 50% but combined with 5 min of red LED irradiation a stronger antibacterial activity was achieved.

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Declaration of Competing Interests

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphotobiol.2020.111826.

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