Photodiagnosis and Photodynamic Therapy 27 (2019) 1–6

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

The impact of *Aggregatibacter actinomycetemcomitans* biofilm-derived effectors following antimicrobial photodynamic therapy on cytokine production in human gingival fibroblasts

Hadi Peeridogaheh[a,1], Maryam Pourhajibagher[b,1], Hamid Reza Barikani[c,*], Abbas Bahador[d,**]

[a] Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran
[b] Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran
[c] Dental Implant Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran
[d] Oral Microbiology Laboratory, Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Aggregatibacter actinomycetemcomitans
Indocyanine green
Biofilms
Periodontitis
Peri-implantitis
Cytokines
Antimicrobial photodynamic Therapy

ABSTRACT

Background: Antimicrobial photodynamic therapy (aPDT) is an effective adjunctive therapeutic modality for the treatment of local infections, including periodontitis and peri-implantitis. After receiving aPDT, microbial cells in the biofilm structure may produce and/ or release soluble biofilm-derived effectors (BDEs), which may affect the biology of the host cells in the community context of their surrounding microenvironment. Given the fact that no study has investigated the role of BDEs following aPDT in the pathogenesis of infectious diseases, the aim of the current study was to determine the effect of BDEs of *Aggregatibacter actinomycetemcomitans* following exposure to sub-lethal doses of indocyanine green (ICG)-aPDT on human gingival fibroblasts (HGFs) in terms of cytokines produced.

Materials and methods: In this study, we evaluated the effect of biofilm-conditioned medium (BCM) resulting from the treatment of *A. actinomycetemcomitans* biofilm with a sub-lethal dose of aPDT on cytokines production, including IL-6, IL-8, CXCL10, TGF-β, and bFGF of HGFs using enzyme-linked immunoassays (ELISA). The sensitivity of cytokines to BDEs was determined by micro-titer plates.

Results: The maximal sub-lethal dose of ICG-PDT was 20.15 μM/mL ICG at a fluence of 31.2 J/cm^2. The BCM of ICG-PDT-treated viable *A. actinomycetemcomitans* significantly reduced IL-6, IL-8, and CXCL10 levels compared to the BCM of untreated viable *A. actinomycetemcomitans* (78-, 93-, and 61.6-fold reduction, respectively; all $P < 0.01$). TGF-β and bFGF were strongly induced by BCM of ICG-PDT treated viable *A. actinomycetemcomitans* (by 57.7 and 36.1 folds, respectively; both $P < 0.05$). The BCM of untreated viable *A. actinomycetemcomitans* degraded most of the CxCL10, TGF-β and bFGF (58.8, 61.5, and 71.6%, respectively) in 24 h, while it degraded 9.3% of IL-6 and 15.1% of IL-8 after 24 h.

Conclusion: The results of the current study revealed that a sub-lethal dose of ICG-aPDT through the effect of BCM on HGFs could not only significantly reduce the production of pro-inflammatory cytokines but also promoted their role in periodontal regeneration due to increasing the bFGF level. Altogether, ICG-aPDT, with it's antimicrobial effects reduces inflammation and induces of tissue regeneration resulting from BCM, can be considered an efficient adjunctive therapeutic method for the treatment of local infections.

1. Introduction

Peri-implantitis and periodontal diseases (periodontitis) are chronic inflammatory diseases characterized by interactions between multi-species biofilms of periodontopathic bacteria, including *Aggregatibacter actinomycetemcomitans*, and the host immune system, resulting in the inflammatory destruction of the supporting structures around osseointegrated implants and the teeth, respectively [1–3]. Several studies

* Corresponding author.
** Correspondence author: Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Keshavarz Blvd, 100 Poursina Ave., Tehran, Iran.
E-mail addresses: h.piridogahe@gmail.com (H. Peeridogaheh), mphb65@yahoo.com, m-pourhajibagher@alumnus.tums.ac.ir (M. Pourhajibagher), hr-barikani@farabi.tums.ac.ir (H.R. Barikani), abahador@sina.tums.ac.ir (A. Bahador).
[1] Equal contribution.

https://doi.org/10.1016/j.pdpdt.2019.05.025
Received 31 October 2018; Received in revised form 18 May 2019; Accepted 20 May 2019
Available online 21 May 2019

have highlighted the necessity of biofilm-derived effectors (BDEs) in association with the regulation of microbial virulence features [4–7]. Furthermore, the expression of human genes can be affected by BDEs [8–12]. Currently, microbial biofilm removal by frequent debridement during scaling and root planning (SRP) along with antibiotic therapy is one of the most clinically effective treatments applied to chronic periodontitis and peri-implantitis [13]. Antimicrobial photodynamic therapy (aPDT) has been recently used as a new adjunctive therapeutic modality for treatment of periodontitis and peri-implantitis to enhance the disinfection of the diseased sites [14].

In aPDT, several types of dyes (photosensitizers [PS]) are activated by LED light (light emitting diode) or laser at a specific wavelength, resulting in the production of highly reactive oxygen species (ROS) and singlet oxygen, which inactivate microbial cells without the risk of inducing microbial resistance [15]. If aPDT is used as a treatment modality, it is likely that PSs would reach the target site at sub-lethal concentrations, especially if the target tissue does not dry out well, and might subsequently be activated by the corresponding spatial light at sub-lethal doses due to the long distance of sensitized microorganisms from the LED or laser beam sources [16]. As a result, the microbiome is not eliminated and remains viable in the infected tissue exposed to doses of aPDT that would not result in microbial cell death, i.e. sub-lethal doses of aPDT (sPDT) [17]. Recently, *in-vitro* studies have shown that sPDT also has the potential to change the expression of microbial virulence factors [15–18]. Indocyanine green (ICG) is a PS widely used in PDT. It is also reported to be an effective near-infrared (NIR) light absorber for laser-mediated photothermal therapy (PTT). NIR laser-induced ICG based PTT converts optical energy to thermal energy and has the potential to act as an effective local, minimally invasive, anti-microbial treatment [19].

Recent reports have shown that in periodontitis and peri-implantitis, *A. actinomycetemcomitans*can promote osteoclast formation and bone loss due to inducing the secretion of interleukin 6 (IL-6) from human gingival fibroblasts (HGFs) [20]. Interleukin 8, which is upregulated in response to *A. actinomycetemcomitans* infection, correlates positively with the severity of periodontal inflammation and destruction in periodontal diseases [20]. Interferon-inducible protein-10 (CXCL10) resulting from infection with *A. actinomycetemcomitans* plays an important role in inflammatory periodontal bone resorption [21]. *A. actinomycetemcomitans* infection–mediated induction of Transforming Growth Factor-beta (TGF-β) negatively correlates with the level of inflammation, reinforcing its protective role against tissue destruction [22]. Basic fibroblast growth factor (bFGF), also known as FGF2, FGF-β, or heparin-binding growth factor, has shown positive effects on the promotion of periodontal regeneration [23]. However, particular gene (s) being switched on or off such as cytokine encoding genes and the gene(s) involved in the periodontal regeneration has been linked to exposure to exogenous agents such as antimicrobial agents during aPDT [24–27]. The indirect inductive effects of aPDT on periodontium cells, which may be stimulated by released BDEs of aPDT treated microbiomes, are not fully understood, especially when the microbiome receives sPDT doses. Thus, the aim of this study was to determine the effects of released BDEs of sPDT treated *A. actinomycetemcomitans* on IL-6, IL-8, CXCL10, TGF-β, and bFGF released from HGFs. In the present study, we investigated the response of HGFs exposed to biofilm-conditioned medium (BCM) of ICG-PDT treated and untreated *A. actinomycetemcomitans* to identify the indirect effects of aPDT on HGFs in terms of cytokines and bFGF produced. We correlated the data from enzyme-linked immunoassays (ELISA) to delineate the BCM of ICG-PDT treated *A. actinomycetemcomitans* specific responses associated with suppression of inflammation and induction of periodontal healing. We also presented evidence for formulating a hypothesis that BDEs of PDT treated *A. actinomycetemcomitans* might induce treatment in peri-implantitis and periodontitis.

We correlated data from all the tests under consideration to provide definitions of the `critical' point at which dispersion becomes a problem for land managers.

2. Materials and methods

2.1. Definition

In this study, a sub-lethal dose was defined as a dose inducing no significant reduction in the CFU/mL of *A. actinomycetemcomitans.* The sub-minimum inhibitory concentration (sMIC) of ICG was defined as the concentration of ICG in the last well of the microtiter plate showing bacterial growth. The irradiation time of diode laser in the last well showing growth was defined as the (maximal) sub-lethal dose of diode laser irradiation time. The lowest concentration of ICG with the shortest diode laser irradiation time in the last well showing growth was defined as the (maximal) sPDT. The biofilm-derived effectors (BDEs) were defined as molecules secreted/released into the surrounding environment by bacteria in the microbial biofilm growth culture.

2.2. Cell culture

Human gingival fibroblast (HGFs; IBRC C10459) cells were grown in a monolayer culture and maintained in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, USA) with 10% fetal bovine serum (FBS; Gibco, UK), 1% penicillin/streptomycin (Mast Group Ltd., UK) solution (10,000 Unit/mL penicillin and 10 mg/mL streptomycin) and 2 mM L-glutamine. HGF cells were grown until full confluence was reached in culture flasks (SPL, Korea) at 37 °C in a humidified atmosphere containing 5% CO_2. For independent experiments requiring HGF cells in polystyrene cell culture microplates, the third passages were placed in flat-bottom microplates (Greiner Bio-One, Germany) [23].

2.3. Preparation of A. actinomycetemcomitans

A. actinomycetemcomitans ATCC 33384 was cultured in the brain heart infusion broth (sBHI) (Merck, Darmstadt, Germany) supplemented with 5 mg/L hemin, 1 mg/L menadione (both purchased from Sigma-Aldrich, Steinheim, Germany) and 0.6% (wt/vol) yeast extract (Merck, Darmstadt, Germany) in microaerophilic conditions at 37 °C. The bacterial cells were harvested by centrifugation, washed, and resuspended in phosphate buffered saline (PBS).

2.4. Photosensitizer and light source

A stock solution of 2580 μM/mL ICG (Serva, Heidelberg, Germany) was prepared in PBS solution. The ICG solution was sterilized using a disposable 0.22 μm filter and stored at 4 °C in the dark before use [23]. An 810 nm diode laser (DenLase; Daheng Group Inc., China) with a power output of 200 mW was applied in this study.

2.5. ICG-PDT treatment

The sub-minimum inhibitory concentration (sMIC) of ICG, maximal sub-lethal dose of diode laser irradiation time, and maximal sub-lethal dose of ICG-PDT were determined according to a previous study [28].

To find out the sMIC of ICG, after adding 2X sBHI broth (100 μL) to each well of a sterile flat-bottomed, 96-well microtiter plate, ICG at 2 × MIC (100 μL) was serially diluted twofold. Finally, *A. actinomycetemcomitans* suspension (100 μL) was added to each well at a final concentration of 5.0×10^5 CFU/mL. The microtiter plate was then incubated in a microaerophilic atmosphere at 25 ± 2 °C in the dark for 24 h. Bacterial growth was quantified as CFU/mL by plating 10-fold dilutions on the sBHI agar.

To determine the sub-lethal dose of diode laser irradiation time, an 810-nm diode laser was irradiated to 200 μL of *A. actinomycetemcomitans* suspension at a final concentration of 1.5×10^5 CFU/mL in the wells of a round-bottomed 96-well microtiter plate at room temperature at a fluency of 15.6, 31.2, and 62.5 J/cm^2 for 0.5, 1, and 2 min, respectively. The number of CFU/mL was determined according to the methods mentioned above. The irradiation time of the diode laser showing growth was defined as the (maximal) sub-lethal dose of diode laser irradiation time.

To find out the maximal sub-lethal dose of ICG-PDT, 100 μL of ICG at 2 × MIC was serially diluted two-fold to 1/8 × MIC in 100 μL of 2X sBHI broth in a flat-bottomed 96-well microtiter plate. The microtiter plate was then inoculated with 100 μL/well of fresh *A. actinomycetemcomitans* culture at a concentration of 1.5×10^6 CFU/mL in the dark for 5 min and then quickly exposed to diode laser irradiation.

The lowest concentration of ICG with the shortest diode laser irradiation time in the last well showing growth was defined as the maximal sub-lethal dose of ICG-PDT. In addition, the wells containing *A. actinomycetemcomitans* suspension without ICG, and sBHI without *A. actinomycetemcomitans* and ICG were used as the positive and negative control, respectively.

2.6. *A. actinomycetemcomitans biofilm culture conditions and preparation of BCM*

Cell culture inserts (35 mm diameter, Thermo Fisher Scientific) were placed into six-well plates with 2.1 mL DMEM supplemented with 10% FBS in each well. Then, 50 mL of ICG-PDT treated *A. actinomycetemcomitans* added to individual culture inserts and the biofilms were allowed to mature for 96 h. The media were collected, pH was adjusted to 7.2, filter was sterilized, and the wells were re-filled with fresh DMEM supplemented with 10% FBS. The collected medium was referred to as the biofilm-conditioned medium (BCM). *A. actinomycetemcomitans* BCM was pooled to provide sufficient quantities of BCM for assays and to help remove day-to-day variations that might occur during biofilm formation.

The BCM of untreated *A. actinomycetemcomitans* was prepared as described above with the exception that *A. actinomycetemcomitans* was not treated with ICG-PDT. Confluent HGFs in a 24-well plate (Greiner Bio-One, Germany) were cultured in the presence of BCMs of ICG-PDT treated and untreated *A. actinomycetemcomitans* for 24 h. Cell culture supernatants were collected for detection of cytokines and bFGF by ELISA. DMEM was used as a negative control.

2.7. *Assessment of cytotoxicity of BCM using XTT reduction assay*

The cytotoxicity of BCE resulting in maximal sub-lethal dose of ICG-PDT was determined using the XTT (2,3-bis [2-methyloxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide) (Sigma Aldrich, Steinheim, Germany) reduction assay. The XTT solution was prepared in PBS (1 mg/mL) and then sterilized by a 0.22 μm filter and stored at −80 °C before use. Sterile menadione (1 mM; Sigma-Aldrich, Steinheim, Germany) solution was prepared in acetone immediately before each assay. Following treatment of HGFs with BCM of ICG-PDT treated *A. actinomycetemcomitans*, the HGFs were collected into 1.5-mL polypropylene tubes from microplate wells and centrifuged. The resulting cell sediment was dissolved in 12 μL aliquots of XTT-menadione solution (12.5:1 v/v) and 100 μL PBS in microplate wells. The microplate was then placed in the dark at 37 °C for 180 min. Next, 100 μL of the solution was transferred to the wells of a new microplate and the color intensity was measured by a microplate reader (492 nm). The wells without HGFs were used as negative controls. The percentage of cell viability was calculated using the equation ([OD of non-treated HGFs as growth control _ OD sample]/OD growth control) × 100 − 100.

2.8. *Cytokines detection by ELISA*

Supernatants collected from HGF cultures were centrifuged at 12,000 *g* for 3 min at 4 °C and then analyzed using Boster's Picokine™ enzyme-linked immunoassays (ELISA) kits (BosterBio, CA) for IL-6, IL-8, CXCL10, and TGF-β as well as Quantikine Human FGF basic Immunoassay kit for bFGF according to the manufacturer's instructions. Cytokines and bFGF were detected as pg/mL in the HGF culture supernatant.

2.9. *Degradation of IL-6, IL-8, CXCL10, TGF-β, and bFGF by BCMs*

To determine the degradation of IL-6, IL-8, CXCL10, TGF-β and bFGF by BCM, the cytokines and bFGF (30 pg/mL) supplied in the Boster's Picokine™ ELISA Kits and Quantikine Human FGF basic Immunoassay kit for the standard curves were incubated with BCM at 37 °C for 24 h, respectiively. The concentrations of residual cytokines and bFGF were measured using Boster's Picokine™ and Quantikine Human FGF basic ELISA kits respectively and compared with baseline concentrations of controls, which were mixed with DMEM alone.

2.10. *Data analysis*

Immunoassays were carried out in three independent experiments. Statistical analysis was done with student's *t*-test in SPSS version 20. The data are presented as mean ± standard deviation (SD). P values less than 0.05 were considered significant.

3. Results

3.1. *Maximal sub-lethal dose of ICG-PDT*

ICG at concentrations of 80.62–1290 μM/mL reduced *A. actinomycetemcomitans* growth significantly (28.8–51.2%) when compared to the control group (untreated *A. actinomycetemcomitans*; $P < 0.05$), but there was no significant reduction in growth when ICG decreased from 40.31 to 2.51 μM/mL ($P > 0.05$). Therefore, the maximal sMIC of ICG was 40.31 μM/mL. Treatment of *A. actinomycetemcomitans* cells with 810 nm diode laser for up to 2 min did not completely inhibit *A. actinomycetemcomitans* growth. However, a significant decrease in the bacterial cell survival observed was at 2 min with an energy density of 62.5 J/cm^2 (87.1%; $P < 0.05$) compared to untreated bacteria. On the other hand, an irradiation time of 1 min with an energy density of 31.2 J/cm^2 was defined as the maximal sub-lethal dose of diode laser irradiation time at 810 nm. As shown in Table 1, the maximal sub-lethal dose of ICG-PDT was 20.15 μM/mL ICG at a fluence of 31.2 J/cm^2.

3.2. *BCMs was not cytotoxic for HGFs*

BCMs of ICG-PDT treated *A. actinomycetemcomitans* alone had no significant effect on the reduction of cell viability compared to the control group (1.93%; $P > 0.05$; Fig. 1).

Table 1
Detection of maximal dose of ICG-PDT contributing to the sub-significant reduction of CFU/mL of *A. actinomycetemcomitans*.

Energy fluency (J/cm^2)	ICG concentration (μM/mL)			
	40.30	20.15	10.07	5.03
	Cell survival (% of control)			
15.6	58.37*	6.81	3.13	0.26
31.2	64.26*	9.67#	7.91	2.11
62.5	71.68*	43.65*	47.18*	3.33

* Significantly different from the control, with a P value of < 0.05.
# Maximal dose of ICG-PDT contributing to the sub-significant reduction of CFU/mL of *A. actinomycetemcomitans*.

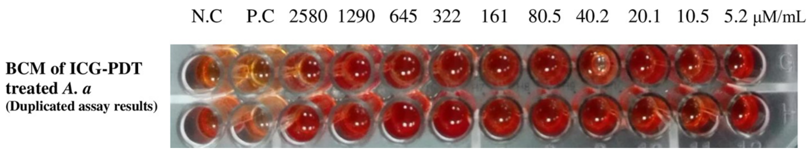

Fig. 1. Evaluation of cell cytotoxicity of BCM of ICG-PDT treated *A. actinomycetemcomitans* (*A.a*) against Human gingival fibroblast cells using XTT assay.

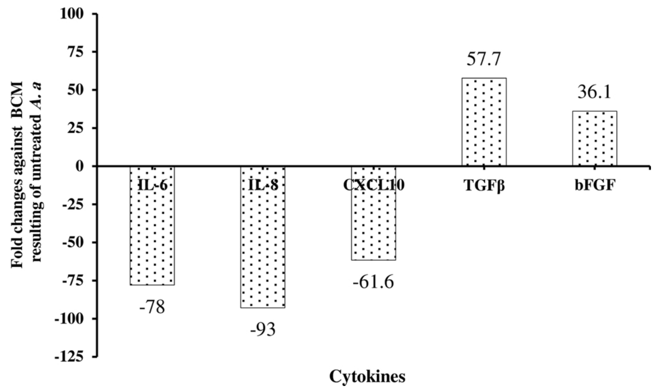

Fig. 2. Fold changes of cytokines production by Human gingival fibroblast cells against BCM resulting of untreated *A. actinomycetemcomitans*.

3.3. Regulation of cytokines and bFGF production in HGFs by BCMs

As shown in Fig. 2, the levels of IL-6, IL-8, and CXCL10 were significantly down-regulated by the BCM of ICG-PDT treated *A. actinomycetemcomitans* (by 78, 93, and 61.6 folds, respectively), whereas TGF-β and bFGF levels were significantly induced by the BCM of ICG-PDT treated *A. actinomycetemcomitans* (by 57.7 and 36.1 folds, respectively).

Interestingly, the BCM of ICG-PDT treated *A. actinomycetemcomitans* induced significantly lower levels of IL-6, IL-8, and CXCL10 compared to the BCM of untreated *A. actinomycetemcomitans* ($P < 0.01$). TGF-β and bFGF were significantly induced by the BCM of ICG-PDT treated *A. actinomycetemcomitans* versus untreated *A. actinomycetemcomitans*.

3.4. Ability of BCMs to degrade cytokines and bFGF

The BCM of untreated *A. actinomycetemcomitans* tended to have higher degradation ability for cytokines compared to the BCM of ICG-PDT treated *A. actinomycetemcomitans*; however, in general, IL-6 and IL-8 were less susceptible to degradation compared to CXCL10, TGF-β, and bFGF (Fig. 3). The BCM of untreated *A. actinomycetemcomitans* degraded most of the CXCL10, TGF-β and bFGF (58.8, 61.5 and 71.6%, respectively) in 24 h, while it only degraded 9.3% of IL-6 and 15.1% of IL-8 after 24 h.

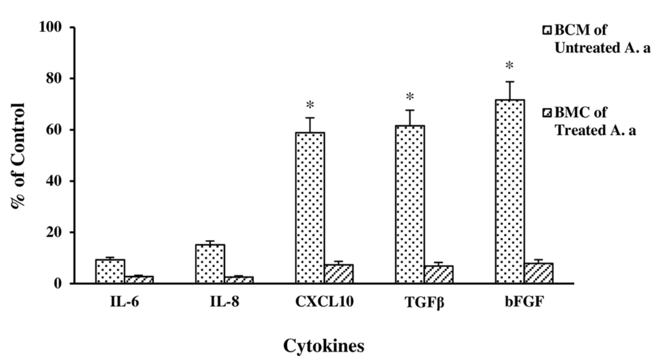

Fig. 3. Comparison of change in the cytokines levels, after exposure with BCMs of ICG-PDT treated and untreated *A. actinomycetemcomitans*. *$P < 0.05$.

4. Discussion

Cytokines are important for maintaining healthy periodontium, indicating the functional attributes of the innate immune defense system and its effectiveness against oral pathogens. To our knowledge, this is the first report of the effect of the BCM of ICG-PDT treated *A. actinomycetemcomitans* on IL-6, IL-8, CXCL10, TGF-β and bFGF released from HGFs. Our study showed interesting results such as a much lower expression of pro-inflammatory cytokines (IL-6 and IL-8) and CXCL10 from HGFs when activated with the BCM of ICG-PDT treated *A. actinomycetemcomitans* compared to the BCM of untreated *A. actinomycetemcomitans*. Indeed, IL-6 and CXCL10 play an important role in inflammatory periodontal bone resorption [20,21]. It has been shown that IL-8 levels correlate positively with the severity of periodontal inflammation and periodontal destruction in the diseased sites [29]. We also presented some evidence suggesting that TGF-β and bFGF were strongly induced by BCM of ICG-PDT treated *A. actinomycetemcomitans*. It should be noted that TGF-β and bFGF correlate with resolution of inflammation and periodontal regeneration processes [14,23].

This study found that the BCM of *A. actinomycetemcomitans* highly upregulated the expression of proinflammatory mediators (IL-6 and IL-8), which is in agreement with the reports indicating that the extracellular DNA, cell wall, and cytolethal distending toxin extracted from *A. actinomycetemcomitans* potently stimulate the production of IL-6 and IL-8 in HGFs [30–33], while the BCM of ICG-PDT treated *A. actinomycetemcomitans* minimally induced their expression. Our study showed higher expression of CXCL10 by HGFs when activated with the BCM of untreated *A. actinomycetemcomitans* compared to the BCM of ICG-PDT treated *A. actinomycetemcomitans*. These results suggest that the BCM of ICG-PDT treated *A. actinomycetemcomitans* can modulate the immune response of HGFs. Shaddox et al [34] found that CXCL10 is involved in generating and recruiting activated and effector T helper 1 cells into sites of tissue inflammation where it is thought to play an important role in inflammatory periodontal bone resorption [21]. Kawai et al showed that *A. actinomycetemcomitans* lipopolysaccharide and outer membrane protein (29-kDa) activation of T helper 1 cell appeared to trigger inflammatory bone resorption in periodontal lesions, leading to tissue damage [35]. This result may also be attributed to the finding that the BCM of ICG-PDT treated *A. actinomycetemcomitans* poorly activates Toll-like receptor 2 and 4 [36,37] and that BCM of ICG-PDT treated *A. actinomycetemcomitans* showed weaker nucleotide-binding oligomerization domain (NOD)1- and NOD2- stimulatory activities which result in lower expression of CXCL10 from HGFs [38]. Leakage of the BCM of untreated *A. actinomycetemcomitans* into the gingival tissue can induce the expression of antimicrobial chemokine CXCL10, resulting in direct killing of bacteria by these chemokines via their antimicrobial activity and their removal by infiltrated adaptive immune cells [24]. The results of the current study showed that the expression of IL-8 was strongly induced by the BCM of untreated *A. actinomycetemcomitans*. However, the BCM of ICG-PDT treated *A. actinomycetemcomitans* tended to induce low levels of IL-8 expression compared with the control. Wilson et al. [39] found that by triggering IL-8, osteoblastic cells may recruit inflammatory cells into the peri-implant tissue and induce a further increase in oxidative stress mediators, and thus increase the inflammatory responses. Our finding showed that the BCM of ICG-PDT treated *A. actinomycetemcomitans* might modulate the connection with adaptive immunity. However, leakage of the BCM of untreated *A. actinomycetemcomitans* into the gingival tissue can induce the expression of proinflammatory mediators such as IL-6 from HGFs, resulting in the infiltration of various immune cells and induction of osteoclast formation, which leads to periodontal bone loss. However, it is vital to determine whether the total immune response is directed toward a destructive or protective effect *in-vivo*.

Researchers have shown that higher TGF-β concentrations in the gingival crevicular fluid (GCF) may suggest better resolution of inflammation and repair processes [14]. As mentioned previously, our findings may only be due to the additional application of low-level laser, as it is an effective tool in reducing inflammation and enhancing wound repair [40–42]. The TGF- β level expression was in agreement with other studied reporting higher TGF- β levels after low-level He-Ne laser irradiation [41]. Moreover, it has been shown that higher TGF-β expression levels in response to the BCM of ICG-PDT treated *A. actinomycetemcomitans* treatment may suggest a better resolution of inflammation and repair process. Several studies have shown the regenerative effects of aPDT *in-vitro* and *in-vivo* assay systems. For instance, Franco et al. [43] concluded that aPDT significantly upregulated FGF2 gene expression, which has a key role in the periodontal healing process. Our findings were consistent with the results of the study conducted by Franco et al. [43] and other published studies [40–42] indicating the positive effect of the BCM of ICG-PDT treated *A. actinomycetemcomitans* on the expression of mediators related to periodontal regeneration. For the first time, our study found a significant increase in the expression of bFGF in HGFs in response to the BCM of ICG-PDT treated *A. actinomycetemcomitans*. The results of the present study suggest that the BCM of ICG-PDT treated *A. actinomycetemcomitans* may also have a role in tissue regeneration due to significant upregulation of bFGF. With the limitations of this study, the results suggest that ICG-PDT treatment of *A. actinomycetemcomitans* can modify bacterial antigenic determinants resulting in modulation of the regulation of HGFs cytokines and innate immune response. Therefore, PDT can result in an enhanced therapeutic response in most cases of peri-implantitis.

5. Conclusions

The results of this study showed an additional effect of aPDT in the treatment of biofilm-based chronic infections. The processes related to tissue damage healing improved following inactivation of host pro-inflammatory cytokine/chemokines by BCM of ICG-PDT treated *A. actinomycetemcomitans*, and periodontal repair was induced more through increased TGF-β and bFGF levels induced by BCM of ICG-PDT treated *A. actinomycetemcomitans*. Specifically, the BCM of ICG-PDT treated *A. actinomycetemcomitans* was shown to act in an anti-inflammatory manner. The production of IL-6, and IL-8 by human gingival fibroblasts decreased after exposure to the BCM of ICG-PDT treated *A. actinomycetemcomitans*. The noted reduction in the level of cytokines produced by fibroblasts exposed to the BCM of ICG-PDT treated *A. actinomycetemcomitans* may reduce polymorphonuclear leukocyte chemotaxis and subsequently recruitment in the inflammatory processes of periodontitis and peri-implantitis. Whether this denotes a critical effect with regards to an anti-inflammatory property remains to be established.

Collectively, however, ICG has been reported to be an effective NIR light absorber for laser-mediated PTT and PDT, our results suggest that PDT effect of ICG can be of therapeutic benefit for periodontium tissue regeneration and, according to its antimicrobial effect, aPDT can be considered adjunctive therapy to SRP for periodontal disease treatment. In future studies, it is also imperative to determine the temperature of ICG treated cell suspensions following exposure to laser light to detect radiation activated the photothermal effect of ICG.

References

[1] A. Hanif, S. Qureshi, Z. Sheikh, H. Rashid, Complications in implant dentistry, Eur. J. Dent. 11 (2017) 135–140.
[2] M. Pourhajibagher, A. Monzavi, N. Chiniforush, M.M. Monzavi, S. Sobhani, S. Shahabi, et al., Real-time quantitative reverse transcription-PCR analysis of expression stability of Aggregatibacter actinomycetemcomitans fimbria-associated gene in response to photodynamic therapy, Photodiagnosis. Photodyn. Ther. 18 (2017) 78–82.
[3] L.F. Brown, P.J. Ford, A.L. Symons, Periodontal disease and the special needs patient, Periodontol. 74 (2017) (2000) 182–193.
[4] T.D. Scherr, M.L. Hanke, O. Huang, D.B. James, A.R. Horswill, K.W. Bayles, et al., Staphylococcus aureus biofilms induce macrophage dysfunction through leukocidin AB and alpha-toxin, MBio 6 (2015) 1–13.
[5] R.O. Mattos-Graner, M.J. Duncan, Two-component signal transduction systems in oral bacteria, J. Oral. Microbiol. 9 (2017) 1400858.
[6] D. Passos da Silva, M.C. Schofield, M.R. Parsek, B.S. Tseng, An update on the sociomicrobiology of quorum sensing in gram-negative biofilm development, Pathogens 6 (2017) E51.
[7] P. Krzyzek, G. Gosciniak, A proposed role for diffusible signal factors in the biofilm formation and morphological transformation of Helicobacter pylori, Turk. J. Gastroenterol. 29 (2018) 7–13.
[8] K.R. Kirker, G.A. James, P. Fleckman, J.E. Olerud, P.S. Stewart, Differential effects of planktonic and biofilm MRSA on human fibroblasts, Wound. Repair. Regen. 20 (2012) 253–261.
[9] A. Scalise, A. Bianchi, C. Tartaglione, E. Bolletta, M. Pierangeli, M. Torresetti, et al., Microenvironment and microbiology of skin wounds: the role of bacterial biofilms and related factors, Semin. Vasc. Surg. 28 (2015) 151–159.
[10] S.D. Goodman, K.P. Obergfell, J.A. Jurcisek, L.A. Novotny, J.S. Downey, E.A. Ayala, et al., Biofilms can be dispersed by focusing the immune system on a common family of bacterial nucleoid-associated proteins, Mucosal. Immunol. 4 (2011) 625–637.
[11] C.M. Gries, E.L. Bruger, D.E. Moormeier, T.D. Scherr, C.M. Waters, T. Kielian, Cyclic di-AMP released from Staphylococcus aureus biofilm induces a macrophage type I interferon response, Infect. Immun. 84 (2016) 3564–3574.
[12] P.R. Secor, G.A. James, P. Fleckman, J.E. Olerud, K. McInnerney, P.S. Stewart, Staphylococcus aureus biofilm and planktonic cultures differentially impact gene expression, mapk phosphorylation, and cytokine production in human keratinocytes, BMC. Microbiol. 11 (2011) 143–156.
[13] C.V.G. Roman-Torres, M.S. Bryington, S.T. Kussaba, A.C. Pimentel, R. Jimbo, J.R. Cortelli, et al., Comparison of full-mouth scaling and quadrant-wise scaling in the treatment of adult chronic periodontitis, Braz. Dent. J. 29 (2018) 296–300.
[14] L. Beytollahi, M. Pourhajibagher, N. Chiniforush, R. Ghorbanzadeh, R. Raoofian, B. Pourakbari, et al., The efficacy of photodynamic and photothermal therapy on biofilm formation of Streptococcus mutans: an in vitro study, Photodiagnosis. Photodyn. Ther. 17 (2017) 56–60.
[15] M. Pourhajibagher, L. Beytollahi, R. Ghorbanzadeh, A. Bahador, Analysis of glucosyltransferase gene expression of clinical isolates of Streptococcus mutans obtained from dental plaques in response to sub-lethal doses of photoactivated disinfection, Photodiagnosis. Photodyn. Ther. 24 (2018) 75–81.
[16] M. Pourhajibagher, N. Chiniforush, R. Raoofian, B. Pourakbari, R. Ghorbanzadeh, F. Bazarjani, et al., Evaluation of photo-activated disinfection effectiveness with methylene blue against Porphyromonas gingivalis involved in endodontic infection: an in vitro study, Photodiagnosis. Photodyn. Ther. 16 (2016) 132–135.
[17] M. Pourhajibagher, N. Chiniforush, R. Ghorbanzadeh, A. Bahador, Photo-activated disinfection based on indocyanine green against cell viability and biofilm formation of Porphyromonas gingivalis, Photodiagnosis. Photodyn. Ther. 17 (2017) 61–64.
[18] M. Pourhajibagher, N. Chiniforush, R. Raoofian, R. Ghorbanzadeh, S. Shahabi, A. Bahador, Effects of sub-lethal doses of photo-activated disinfection against Porphyromonas gingivalis for pharmaceutical treatment of periodontal-endodontic lesions, Photodiagnosis. Photodyn. Ther. 16 (2016) 50–53.
[19] N. Chiniforush, M. Pourhajibagher, S. Parker, S. Shahabi, A. Bahador, The in vitro effect of antimicrobial photodynamic therapy with indocyanine green on Enterococcus faecalis: influence of a washing vs non-washing procedure, Photodiagnosis. Photodyn. Ther. 16 (2016) 119–123.
[20] D. Kawamoto, E.S. Ando-Suguimoto, B. Bueno-Silva, J.M. DiRienzo, M.P. Mayer, Alteration of homeostasis in pre-osteoclasts induced by Aggregatibacter actinomycetemcomitans CDT, Front. Cell. Infect. Microbiol. 6 (2016) 1–10.
[21] Y. Hosokawa, I. Hosokawa, S. Shindo, K. Ozaki, T. Matsuo, Calcitriol suppressed inflammatory reactions in IL-1β-stimulated human periodontal ligament cells, Inflammation 38 (2015) 2252–2258.
[22] G.P. Garlet, Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints, J. Dent. Res. 89 (2010) 1349–1363.
[23] R. Mokhtari Asl, P. Ghoraeian, A. Monzavi, A. Bahador, Analysis of gene expression of basic fibroblast growth factor (bFGF) following photodynamic therapy in human gingival fibroblasts, Photodiagnosis. Photodyn. Ther. 20 (2017) 144–147.
[24] G. Sivaramakrishnan, K. Sridharan, Photodynamic therapy for the treatment of peri-implant diseases: a network meta-analysis of randomized controlled trials, Photodiagnosis. Photodyn. Ther. 21 (2018) 1–9.
[25] A. Mistry, R. Pereira, V. Kini, A. Padhye, Effect of combined therapy using diode laser and photodynamic therapy on levels of IL-17 in gingival crevicular fluid in patients with chronic periodontitis, J. Lasers. Med. Sci. 7 (2016) 250–255.
[26] F.M. Pillusky, R.C.S. Barcelos, L.T. Vey, L.M. Barin, V. de Mello Palma, R.M. Maciel, et al., Antimicrobial photodynamic therapy with photosensitizer in ethanol improves oxidative status and gingival collagen in a short-term in periodontitis, Photodiagnosis. Photodyn. Ther. 19 (2017) 119–127.
[27] M. Bassetti, D. Schär, B. Wicki, S. Eick, C.A. Ramseier, N.B. Arweiler, et al., Anti-infective therapy of peri-implantitis with adjunctive local drug delivery or photodynamic therapy: 12-month outcomes of a randomized controlled clinical trial, Clin. Oral. Implants. Res. 25 (2014) 279–287.

[28] M. Pourhajibagher, N. Chiniforush, S. Shahabi, S. Sobhani, M.M. Monzavi, A. Monzavi, et al., Monitoring gene expression of rcpA from Aggregatibacter actinomycetemcomitans versus antimicrobial photodynamic therapy by relative quantitative real-time PCR, Photodiagnosis. Photodyn. Ther. 19 (2017) 51–55.
[29] M.K. Noh, M. Jung, S.H. Kim, S.R. Lee, K.H. Park, D.H. Kim, et al., Assessment of IL-6, IL-8 and TNF-α levels in the gingival tissue of patients with periodontitis, Exp. Ther. Med. 6 (2013) 847–851.
[30] J. Im, J.E. Baik, K.W. Kim, S.S. Kang, J.H. Jeon, O.J. Park, et al., Enterococcus faecalis lipoteichoic acid suppresses Aggregatibacter actinomycetemcomitans lipopolysaccharide-induced IL-8 expression in human periodontal ligament cells, Int. Immunol. 27 (2015) 381–391.
[31] C. Nonnenmacher, A. Dalpke, S. Zimmermann, L. Flores-De-Jacoby, R. Mutters, K. Heeg, DNA from periodontopathogenic bacteria is immunostimulatory for mouse and human immune cells, Infect. Immun. 71 (2003) 850–856.
[32] N. Pahumunto, P. Chotjumlong, A. Makeudom, S. Krisanaprakornkit, G. Dahlen, R. Teanpaisan, Pro-inflammatory cytokine responses in human gingival epithelial cells after stimulation with cell wall extract of Aggregatibacter actinomycetemcomitans subtypes, Anaerobe 48 (2017) 103–109.
[33] U. Soto-Barreras, G. Cortés-Sandoval, R. Dominguez-Perez, A. Loyola-Leyva, P.R. Martinez-Rodriguez, J.P. Loyola-Rodriguez, Stimulatory effect of Aggregatibacter actinomycetemcomitans DNA on proinflammatory cytokine expression by human gingival fibroblasts, Arch. Oral. Biol. 82 (2017) 127–133.
[34] L.M. Shaddox, P.F. Gonçalves, A. Vovk, N. Allin, H. Huang, W. Hou, et al., LPS-induced inflammatory response after therapy of aggressive periodontitis, J. Dent. Res. 92 (2013) 702–708.
[35] T. Kawai, R. Eisen-Lev, M. Seki, J.W. Eastcott, M.E. Wilson, M.A. Taubman, Requirement of B7 costimulation for Th1-mediated inflammatory bone resorption in experimental periodontal disease, J. Immunol. 164 (2000) 2102–2109.
[36] V. Gelani, A.P. Fernandes, T.H. Gasparoto, T.P. Garlet, T.M. Cestari, H.R. Lima, et al., The role of toll-like receptor 2 in the recognition of Aggregatibacter actinomycetemcomitans, J. Periodontol. 80 (2009) 2010–2019.
[37] H.R. Lima, V. Gelani, A.P. Fernandes, T.H. Gasparoto, S.A. Torres, C.F. Santos, et al., The essential role of toll like receptor-4 in the control of Aggregatibacter actinomycetemcomitans infection in mice, J. Clin. Periodontol. 37 (2010) 248–254.
[38] T. Okugawa, T. Kaneko, A. Yoshimura, N. Silverman, Y. Hara, NOD1 and NOD2 mediate sensing of periodontal pathogens, J. Dent. Res. 89 (2010) 186–191.
[39] T.G. Wilson Jr., P. Valderrama, M. Burbano, J. Blansett, R. Levine, H. Kessler, et al., Foreign bodies associated with peri-implantitis human biopsies, J. Periodontol. 86 (2015) 9–15.
[40] S.M. Safavi, B. Kazemi, M. Esmaeili, A. Fallah, A. Modarresi, M. Mir, Effects of low-level He-Ne laser irradiation on the gene expression of IL-1beta, TNF-alpha, IFN-gamma, TGF-beta, bFGF, and PDGF in rat's gingiva, Lasers. Med. Sci. 23 (2008) 331–335.
[41] A.M. Rocha Junior, B.J. Vieira, L.C. de Andrade, F.M. Aarestrup, Low-level laser therapy increases transforming growth factor-beta2 expression and induces apoptosis of epithelial cells during the tissue repair process, Photomed. Laser. Surg. 27 (2009) 303–307.
[42] M. Akrami, S. Balalaie, S. Hosseinkhani, M. Alipour, F. Salehi, A. Bahador, et al., Tuning the anticancer activity of a novel pro-apoptotic peptide using gold nanoparticle platforms, Sci. Rep. 6 (2016) 1–12.
[43] E.J. Franco, R.E. Pogue, L.H. Sakamoto, L.L. Cavalcante, D.R. Carvalho, R.V. de Andrade, Increased expression of genes after periodontal treatment with photodynamic therapy, Photodiagnosis. Photodyn. Ther. 11 (2014) 41–47.