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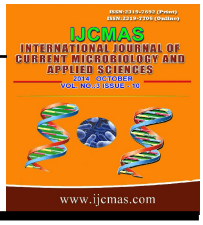

Original Research Article

Effect of Antibacterial agents for treatment of peri-implantitis in vitro against *Aggregatibacter actinomycetemcomitans*

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Keywords

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A B S T R A C T

Periodontitis is an oral disease that leads to the destruction of the supporting tissues of the teeth. The therapy of periodontitis is based on the elimination of these periodontal pathogens. The aim of this research was to study the influence of different treatment approaches against bacterium grown on the surface of implant. The effect of antibacterial agents *against Aggregatibacter actinomycetemcomitans* ATCC 43718 was evaluated. Thirty implants were inoculated with *A. actinomycetemcomitans* in tryptic soy broth and were incubated for 48 hours. The implants were then subjected to 2% chlorhexidine gel, Tetracycline and photodynamic therapy. Following treatment, the implants were sampled with a sterile curette. The samples were dispersed in transport medium, serially diluted, and cultured on tryptic soy serum agar to determine the number of colony forming units (CFU). The photodynamic therapy (PDT) with 50% and 2% Chlorhexidine gel with 41% achieved maximum reduction in the number of viable bacteria.

Introduction

Periodontal diseases are those affecting the supporting structures of the teeth. That is an infectious disease is identificated by alveolar bone destruction and tooth loss. Periodontal diseases are started by a wrapped network of molecular interactions between growing as a biofilm in the subgingival crevice and the host tissues that support the teeth (Kheur *et al.*, 2014).

Bacteria present on implant surface may lead to an inflammation of the peri-implant mucosa, and, if left untreated, the inflammation spreads apically and results in bone loss, a process that has been named peri-implantitis (Garcia *et al.*, 1998). So, the removal of bacterial biofilms seems to be a prerequisite in the therapy of peri-implant infections (Schwarz *et al.*, 2005). Periodontal diseases Prevention is often based on plaque control. The aim of traditional dentistry is based on the therapy of oral diseases. During the previous studies, great progress has been made in the field of oral microbiology, particularly as it relates to diagnosis. Some of this obtained knowledge can already be used in the

clinical work today (Buehni and Guggenhheim, 1996). Peri-implantitis is an inflammatory reaction together with loss of supporting bone in the tissues around the implant (Prathapachandran and Suresh, 2012). Bacterial infections have maximum impact in the failure of dental implants. (Heydenrijk *et al.,* 2002). The microorganisms most commonly related to the failure of an implant are the Gram-negative anaerobes, such as *Prevotella intermedia, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Bacterioides forsythus, Treponema denticola, Prevotella nigrescens, Peptostreptococcus micros,* and *Fusobacterium nucleatum* (Heydenrijk *et al.,* 2002; Shibli *et al.,* 2003). *A. actinomycetemcomitans* is a Gram-negative facultative anaerobic microorganism. Antimicrobial chemotherapeutic methods can be used to prevent growth of *A. actinomycetemcomitans*. Chlorhexidine digluconate (CHX) is effective against a large spectrum of Gram-positive and Gram-negative oral microorganisms. Short-term adjunctive use of CHX gel and mouth rinses decreases periodontal pathogen counts and limits subgingival biofilm re-growth after mechanical removal of dental plaque (Silva *et al.,* 2013; Lamell *et al*, 2000; Fine *et al.,* 2010; Perinetti *et al.,* 2004; Sekino *et al.,* 2004). Tetracyclines are a family of broad-spectrum antibiotics to treat systemic infections has been used since the 1940s. Topical and systemic tetracycline derivatives doxycycline and minocycline, as well as the most common antimicrobial drugs used to treat infections of periodontal. In the treatment of periodontal disease, they are often resistant to treat periodontitis and localized aggressive periodontitis were used. Tetracycline has the ability to focus on periodontal tissues and may prevent the growth *A. actinomycetemcomitans.* Specific microbial information regarding the presence of putative pathogens is indispensible to make a meaningful decision regarding systemic or local antibiotic therapy (Silverstein *et al.,* 1988). The elimination of bacterial biofilm is a definite component in the prevention and treatment of these diseases. The usage of mechanical factors is a cost-effective method that has been demonstrated to be efficient in the control (Nayak *et al.,* 2010; Tyagi *et al.,* 2011). Many antimicrobials can be used in local drug delivery forms as tetracycline fibers, such as metronidazole, and chlorhexidine in bio absorbable polymer (Wilson *et al.,* 1992).

Photodynamic therapy has also been explored for periodontal therapy (Pfitzner *et al.,* 2004) using a variety of photosensitizing dyes methylene blue and chlorine, (Chan and Lai, 2003) and with lasers of various wavelengths (Novaes *et al.,* 2012). Mechanical removal of the biofilm and additional use of antibacterial disinfectants or antimicrobial drugs is the conventional approach to treat aggressive periodontitis; however, there have been reports of bacterial strains becoming resistant due to the frequent use of antimicrobial drugs (Raab, 1900). PDT could be an alternative to the conventional therapeutic methods. The principle of PDT is a photoactivatable compound binds the target cell and becomes activated by light of appropriate wavelength. The activation process, free radicals are created, which have a toxic effect on the cell. The term photodynamic therapy was established as early as 1900 by Raab. Several in vitro investigations have shown that tetracycline hydrochloride is highly effective against the major suspected periodontal pathogens (Beiswanger *et al.,* 1992; Mattiello *et al.,* 2011). Chlorhexidine gluconate (chlorhexidine) is a broad-spectrum bactericidal agent against all kinds of microbes. (Vaziri *et al.,* 2012).

The aim of this study was to compare the effectiveness of Chlorhexidine gel, Tetracycline gel and PDT against *A. actinomycetemcomitans* on the surface of dental implants in vitro.

Materials and Methods

A clinical strain of *A. actinomycetemcomitans* ATCC 43718 was used for experiment. This bacterium was maintained in tryptic soy agar (TSA) supplemented with 10% horse serum (Mattiello *et al.,* 2011).

Experimental groups

Implants were autoclaved for sterilization and were randomly divided into three experimental groups.

Photodynamic therapy

Implants infected with *A. actinomycetemcomitans* was subjected to lethal photosensitization with toluidine blue with concentration 15 g/ml and diode laser for 60 seconds. The irradiation source was a diode laser (Laser Gmbh, Germany) with a total power 810nm of Wavelength (Wilson *et al.,* 1992; Vaziri *et al.,* 2012).

Tetracycline gel

The implants were inoculated with tetracycline (Playstation Ron, Japan) for 5 min, removed with sterile paper points (Raab, 1900).

Chlorhexidine (CHLOSITE) irrigation

The implants were injected with Chlorhexidine (2% v/v) (Casalecchio di Reno Italy) for 5 min, removed with sterile paper points, and irrigated with normal saline solution (0.85% v/v) (Silverstein *et al.,* 1988; Nayak *et al.,* 2010).

Control groups

Controls consisted of no treatment (positive control) and without inoculation of bacterium (negative control) for three experimental groups (Mattiello *et al.,* 2011).

Sampling procedures

Following all treatments, the liquid content of the implants was carefully absorbed with paper points. Implants were filled with sterile 0.85% normal saline solution, and sample was taken by the use of 11/12 Hufriedy's Gracey curette. Curettes were transferred to tubes containing 1 ml of 0.85% normal saline solution and agitated for 1 minute. After 10-fold serial dilutions in normal saline, aliquots of 0.1 ml were plated onto TSA plates and plates were incubated in anaerobic conditions with 5% CO_2 at 37℃ for 3 days. The colony forming units (CFUs) grown were counted and then transformed into actual counts based on the known dilution factors. Data were analyzed by Mann-Whitney U test at 5% significance level. The significance level for all analyses was determined at P <.05. (Mattiello *et al.,* 2011; Vaziri *et al.,* 2012).

Results and Discussion

Table 1 shows the mean, median, range, and reduction percent of CFUs for all groups. The effects of antibacterial materials are reported in Table 1. Counting of all CFU of *A. actinomycetemcomitans* remaining in the control (no treatment) and groups was done to studied efficacy of antibacterial agents. There was a significant of reduction in the CFU counts (P < 0.05) compared with the initial numbers recorded in positive control. A maximum of viable bacterial reduction was observed with PDT that resulted in 50% bacterial kill (Table 1). The group analyses were carried to study the ability of each

treatment for reducing the bacterial counts when compared with the positive control. A reduction in the number of CFUs was statistically significant for all groups ($P <$ 0.05 for all groups). The reduction factors were significantly higher in group PDT compared with the other groups ($P <.05$). The Mann-Whitney U test showed significant differences when comparing samples between groups of Tetracycline gel and Chlorhexidine ($P < 0.05$). Highest CFU of viable bacteria was from positive control group, while the negative control group was without of microorganisms under the experimental conditions (Figure 1).

This study attempted to examine whether lethal photosensitization, Tetracycline gel and chlorhexidine gel which has been shown to be effective in eliminating bacteria in vitro conditions. Therefore was studied the in vitro effect of antibacterial agent on *A. actinomycetemcomitans* on the surfaces of dental implants. The results of This study indicate that all treatment procedures result in a significant decrease in *A. actinomycetemcomitans* grown on implants, however PDT by with toluidine blue and diode laser with wavelength 810nm is a better effect in compared with was used other antibacterial agents tetracycline and chlorhexidine gel. Francis *et al* (1987) demonstrated efficient inhibition of dental plaque and control of gingival bleeding in patients treated with 4-week tray application of 1% CHX gel. Sekino *et al.,* (2004) demonstrated that the combination of a 0.2% CHX mouth rinse and a 1% CHX gel decreased salivary microorganisms. Slot *et al.* (2010) reported an improvement of dental plaque control after tray application of 1% CHX gel compared to 0.12% CHX dentifrice gel. Silva *et al.* (2013) reported that CHX gel of 2% reduced *A. actinomycetemcomitans* in vivo conditions. Previous studies were showed that significant improvements from baseline usually occur for clinical indicators following treatment with tetracycline (Silverstein *et al.,* 1988). Perinetti *et al.,* (2004) studied clinical and microbiological effect of gels of chlorhexidine and metronidazole on persistent pockets of chronic periodontitis patients and reported reduction of periodontal bacteria as *A. actinomycetemcomitans, P. gingivalis, P. Intermedia* After treatment with the gels. Several studies showed reduction of microorganisms of below the gum during and after treatment with tetracycline shave been associated with clinical improvement (Silverstein *et al.,* 1988). Kulic *et al.* (2008) showed that tetracycline is the most efficient antibiotic against *A. actinomycetemcomitans.* In the literature, tetracycline fibers are reported suitable for local application.

The previous researchers reported the susceptibility of *A. actinomycetemcomitans* to tetracycline vary (Eick *et al.,* 1999; Muller *et al.,* 2002; van Winkelhoff *et al.,* 2000). In this research PDT was more effective at removing *A. actinomycetemcomitans* biofilms than chlorhexidine and tetracycline gel. That PDT can be used as a disinfection method successfully. However, clinical application of PDT in the treatment of periodontitis in non-surgical control experiment is aggressive periodontitis.

However, other studies have shown that it is not able to completely eliminate periodontal pathogens in periodontal pockets, that the results obtained in this study also confirm it. The peak of absorption of a photosensitizer should match the wavelength of the light used for irradiation in order to promote formation of singlet oxygen responsible for PDT-mediated killing of bacterial (Souza *et al.,* 2010; Onopka and Goslinski, 2006; Bonsor *et al.,* 2006).

The diode laser is a soft tissue laser having its target chromospheres as the tissue pigments (hemoglobin and melamine). It is cheapest and most extensively used laser by the dentist (Khandge *et al.,* 2013). Significant bacterial reduction was seen even at low levels of irradiance with low energy diode lasers (Ahmed *et al.,* 2011). Thus with the previous research and results of this study can be concluded that diode lasers can be used safely and efficiently on the implant surfaces.

The use of various laser systems in implantology as compared to the conventional methods or the other newer methods has high privileges. The laser has bactericidal and anti-inflammatory activity, it can be used for managing soft tissues and hard tissues (Khandge *et al.,* 2013). The laser effect on the implant topography and temperature rise during the procedure are important factors which have to be taken under consideration. The diode laser does not cause any irreversible damage to the implant surface in therapeutic dosage parameters (Ahmed *et al.,* 2011).

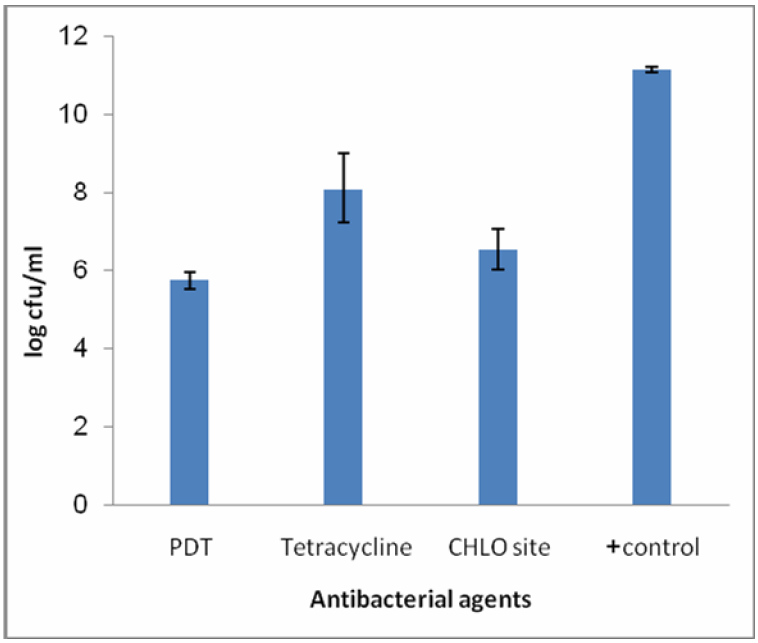
Periodontal diseases will be one of the main applications for PDT within the oral cavity. The reasons for such a use are the multiple-choice infection and the multi factorial genesis, the localization of the bacteria and, probably the fact that the method is easy to perform. By photosensitization even multi-resistant, Gram-negative hospital strains are killed (Nitzan *et al.,* 1998). Besides killing, important virulence factors of Gram-negative bacteria are diminished by PDT, endotoxins and proteases (Komerik *et al.,* 2000). Methylene Blue (MB), toluidine blue (TBO), and acridine orange are potent photosensitizers. The photo induced killing efficacy increases in the order MB < monomethyl-MB < dimethyl-MB, again in relation to cellular uptake. MB and TBO are used as vital dyes for oral mucosa. Besides treatment of periodontitis, also peri-implantitis, endodontic treatment and caries came into the focus. The spectrum of pathogens inducing peri-implantitis is largely similar to periodontitis (Meisel and Kocher, 2005). In our study, tetracycline was less efficient than PDT and chlorhexidine in reduction of *A. actinomycetemcomitans* colonies; with regard to the emergence of bacteria resistant species that reported in clinical trials and difficulty in clinical application, it seems that PDT as a new method of adjunctive periodontal treatment can be a suitable alternative to older methods.

Despite the successes achieved in the Medical, Dental Implants, implant failures owing to peri-implant diseases have on the implant surface significant role in osseointegration. Removal of bacteria from the surface of the implant is necessary in order to eliminate the source of infection and disrupt the formation of biofilm. In previous studies reported influence of surface modification of implants on antibacterial activity in vitro and in vivo. But in research was studied the effect of antibacterial agents and comparison them against bacteria was cultured on the surface of the implant. The results of this study show local antimicrobial procedure such as PDT can be prevent implant-associated infections by impeding bacterial adherence to the implant surface or reducing the concentration of bacteria in the immediate vicinity of the implant.

Table.1 Counts of *A. actinomycetemcomitans* colony-forming units after effect of antibacterial agents

Groups	Mean	Median	Range	Percentage
PDT	1.11×10^{11}	5.5×10^{5}	1.58×10^{5}-1.05×10^{6}	50%
Chlorhexidine	1.42×10^{11}	3.5×10^{6}	7.2×10^{5}-4×10^{7}	41%
Tetracycline gel	1.83×10^{11}	1.25×10^{8}	3.8×10^{6}-4.1×10^{8}	27%

Figure.1 Effect of antibacterial agents against *A. actinomycetemcomitans* on the implant surface



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