Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 7s, https://carcinogenesis.com/

Disinfection potential of 1% CHX Gel Compared to 10% Doxycycline for Titanium Implants Infected with P. gingivalis

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ABSTRACT

Background: Chlorhexidine is among various antimicrobial agents assessed for managing peri-implantitis at different concentrations; however, it is considered ineffective at concentrations of 0.05-2% against P. gingivalis. Doxycycline has also been reported to have short-term benefits in cases of peri-implantitis. However, existing literature data is scarce comparing these two agents.

Aim: The study was conducted with an aim to assess and compare the disinfection potential of 1% CHX Gel compared to 10% Doxycycline for sandblasted acid-etched Titanium Implants Infected with P. gingivalis.

Methods: The study assessed sandblasted acid-etched titanium implants that were divided into two groups, namely Group I and II. Both groups were further subdivided into three subgroups, labeled A-I, B-I, and C-I, and A-II, B-II, and C-II. All the implant surfaces in both groups were disinfected with P. gingivalis. Group I implants were managed with 1% chlorhexidine, and Group II with 10% doxycycline gel. Following decontamination, residual counts of viable P. gingivalis were evaluated using from culture method.

Results: The study results showed that at baseline, mean values from Groups I and II were 110,000,000 CFU (colony-forming unit). On day 1, mean CFU values from Groups B-I and A-I were 0 and 3289.65 CFUs, respectively, whereas on days 3 and 7, mean values in groups A-I, C-I, and B-II and C-II were 0 colony-forming units.

Conclusion: The present study concludes that multiple applications of 10% doxycycline gel and 1% chlorhexidine gel have high efficacy for disinfection of sandblasted acid-etched titanium implants infected with P. gingivalis and thus, both these gels can be considered as cost-effective treatment options for management of peri-implantitis.

Keywords: *Chlorhexidine, doxycycline, Porphyromonas gingivalis,sandblasted acid-etched, titanium implants.*

How to Cite: Dr. Subhasmita Bhol, Dr. Kukkadapu Likhitha, Dr. Sovamayee, Dr. Artabandhu Tripathy,Dr. Sheetal Kumar Sahoo, Dr Amrita Kumari, (2025) Disinfection potential of 1% CHX Gel Compared to 10% Doxycycline for Titanium Implants Infected with P. gingivalis, *Journal of Carcinogenesis*, *Vol.24, No.7s*, 803-808

1. INTRODUCTION

Oral health constitutes a vital part of the overall general health of a subject. Quality of life in an individual is largely governed by the ability to smile, efficient mastication, and a healthy dentition. With various advancements in implant dentistry, replacement of the missing teeth has raised a great interest in the edentulous subjects. Despite implants showing a high success rate in the clinical scenario, complications associated still pose various challenges that need to be considered. These complications can be divided in various groups, namely biological, technical, and mechanical. Biological complications with implants include peri- implantitis, which is an infectious disease

affecting peri-implant tissues.1

Previous existing literature data on microbial biofilm profile of peri-implantitis has reported that the most common bacteria from the red complex are seen in the sites affected by peri-implantitis and with P. gingivalis (Porphyromonas gingivalis). The management for peri-implantitis is widely divided into two groups as surgical and non-surgical management. Non-surgical methods of treating peri-implantitis are photodynamic therapy, Lasers, local drug delivery, and mechanical debridement. The surgical approaches for peri-implantitis treatment are regenerative and resective surgeries. The use of antibiotics can serve as a replacement option for places with difficult and limited access and for sites and subjects that are unsuitable to be managed surgically.2

Antibiotic therapy can be used combined with mechanical debridement, which has always been assessed to date, including 14% doxycycline, citric acid, minocycline, 25% metronidazole, 5% tetracycline HCL solution, 10% hydrogen peroxide, and/or 0.2% chlorhexidine. However, to date, there is no single antimicrobial agent which is been proven to have 100% efficacy in the decontamination of dental implants.3

The decontamination of the dental implant surface plays a vital role in the healing of the peri-implant defects to avoid the loss of the dental implant from the disease progression. Although peri-implantitis is caused by a complex microbiome, P. gingivalis is one of the major pathogens associated with peri-implantitis in affected implants. Among various antimicrobial agents, chlorhexidine has been studied extensively for managing peri-implantitis at different concentrations owing to its high substantivity. However, chlorhexidine is reported to be ineffective in low concentrations of 0.05% to 0.2% against Porphyromonas gingivalis when used as a single application. Similar results are reported for doxycycline, reporting short-term results for managing peri-implantitis. However, scarce literature data exist in literature concerning the comparison of chlorhexidine and doxycycline against P. gingivalis.4 Hence, this study was conducted with an aim to assess and compare the disinfection potential of 1% CHX Gel compared to 10% Doxycycline for sandblasted acid-etched Titanium Implants Infected with

P. gingivalis.

2. MATERIALS AND METHODS

The present study was conducted with an aim to assess and compare the disinfection potential of 1% CHX Gel compared to 10% Doxycycline for sandblasted acid-etched Titanium Implants Infected with P. gingivalis. The study subjects were from the Department of Pediatrics of the Institute. Verbal and written informed consent were taken from guardians/parents of all the subjects before study participation.

The study assessed 144 sandblasted acid-etched Titanium Implants that were divided into two groups, comprising 72 implants in each group. In Group I, implants were treated using 1% chlorhexidine gel, and in Group II, implant treatment was done with 10% doxycycline gel. Both groups were further divided into three subgroups, including 24 implants each, and were named as A- I, B-I, and C-I, and A-II, B-II, and C-II in groups I and II, respectively. The study utilized doxycycline gel from Sisco Research Laboratories Private Limited.

Poloxamer gel was prepared and stored following the standard protocols as discussed by Schmolka,5 1972, and Bansal M et al6 in 2018. 10% doxycycline and 1% chlorhexidine gels were stored at 4 degrees Celsius. The ATCC (American Type Culture Collection) 33277 strain of P. gingivalis was taken from HiMedia and was preserved at 4 degrees Celsius in lyophilized form, which was recovered following ATCC guidelines. Before inoculation, sterilization was done in a dry autoclave for 15 minutes at 121 degrees Celsius. Sterile cryovials of plastic with 2ml capacity were used to embed the implants. TG (Thioglycolate) at 1% concentration was used as culture media and was autoclaved.

Culture media was then cooled till it was semisolid, and then sterile implants were embedded in a manner allowing submerging of half of the implant in solidifying agar. After agar solidification, the exposed implant part was contaminated with a 0.5ml aliquot of P. gingivalis suspension having 1.2 x 108 bacterial cells. All vials were placed in an anaerobic chamber, followed by incubation at 37 degrees Celsius in a carbon dioxide incubator for 48 hours. The implants were considered at days 1, 3, and 7.

At day 1, all implants from Group II were treated with 10% doxycycline gel with a syringe fitted in a blunt cannula and allowed to stay in place for 5 minutes, and implants from Group I were treated using chlorhexidine gel and kept in place for 10 minutes.

After decontamination, implant surfaces from both groups were irrigated using sterile saline. Implants from Group I-A and Group II-A were kept in a sterile test tube containing TG (thioglycolate) broth. Each microtube was allowed 1 minute to allow bacterial detachment from the implant surface, followed by serial dilution of bacterial suspension. The diluted suspension was kept in blood agar for analysis of CFUs (colony-forming units). Inoculated blood agar was incubated at 37 degrees Celsius for 48 hours in a carbon dioxide incubator. Remnant implants from subgroups Group I-B and Group I-C, and from group II, as II-B and II-C, were returned to the incubator.

At day 3, both antimicrobial gels were applied on the implant surface from subgroups I-B, I-C, II-B, and II-C, followed a similar procedure as day 1. Implants from subgroups I-B and II-B were then transferred to sterile microtubes containing thioglycolate broth and were vortexed. The resultant diluted suspension was kept in blood agar medium, followed by incubation at 37 degrees Celsius in a carbon dioxide incubator for 48 hours. The implants from subgroups I-C and II-C were kept back in the incubator.

At day 7, implants from subgroups I-C and II-C were managed using antimicrobial gels following the protocol identical to days 1 and 3. Implants were then transferred to thioglycolate broth medium to attain a bacterial suspension. The diluted suspensions were then kept on blood agar medium, followed by incubation at 37 degrees Celsius in a carbon dioxide incubator for 48 hours. After 48 hours of incubation on days 1, 3, and 7, colonies of P. gingivalis were assessed on blood agar and were counted using a magnifying glass.

3. RESULTS

The present in-vitro study was conducted with an aim to assess and compare the disinfection potential of 1% CHX Gel compared to 10% Doxycycline for sandblasted acid-etched Titanium Implants Infected with P. gingivalis. The study assessed 144 sandblasted acid-etched Titanium Implants that were divided into two groups, comprising 72 implants in each group. In Group I, implants were treated using 1% chlorhexidine gel, and in Group II, implant treatment was done with 10% doxycycline gel. Both groups were further divided into three subgroups, including 24 implants each, and were named as A-I, B-I, and C-I, and A-II, B-II, and C-II in groups I and II, respectively.

It was seen that CFU counts of P. gingivalis in Group I titanium implants before and after 1% chlorhexidine gel in Group I, at baseline, mean count was 110,000,000±0.000 which decreased significantly to 0.00±0.000 at day 1 in Group I-A, 0.00±0.000 at day 3 in Group I-B, and 0.00±0.000 at day 7 in group I-C which was a statistically significant reduction with p=0.000 (Table 1). Similar results were seen for Group II implants, where CFU counts at baseline were highest with 110,000,000±0.000 CFUs, which significantly decreased to 3289.65±5449.32 at day 1 in subgroup II-A and further to 0.00±0.000 on day 3 II-B and to 0.00±0.000 at day 7 in group II-C, which was a highly significant reduction with p=0.000 (Table 2).

Time	Number (n)	Mean ± S. D	Minimum	Maximum	p-val
Baseline	72	110,000,000±0.000	110,000,000	110,000,000	0.000
Day-1 I-A	24	0.00±0.000	0	0.00	0.000
Day-3 I-B	24	0.00±0.000	0	0.00	0.000
Day-7 I-C	24	0.00±0.000	0	0.00	0.000

Table 1: CFU counts of P. gingivalis in Group I titanium implants before and after 1% chlorhexidine gel

Time	Number (n)	Mean ± S. D	Minimum	Maximum	p-val
Baseline	72	110,000,000±0.000	110,000,000	110,000,000	0.000
Day-1 II-A	24	3289.65±5449.32	0	18,000	0.000
Day-3 II-B	24	0.00±0.000	0	0.00	0.000

Table 2: CFU counts of P. gingivalis in Group II titanium implants before and after 10% doxycycline gel

The study results showed that for CFU counts of P. gingivalis for implants from subgroups I-A and II-A managed with 1% chlorhexidine and 10% doxycycline gel, in Group I-A, mean CFUs were 0.00±0.000 and were 3289.65±5449.346 in subgroups A of group II, which was significantly higher with p=0.000 (Table 3).

On comparing the CFU counts of P. gingivalis for implants from subgroups I-B and II-B managed with 1% chlorhexidine and 10% doxycycline gel, mean CFU counts for subgroup I-B at day 3 were 0.00±0.000, and were 0.00±0.000 for subgroup II-B at day. The difference in the two groups at day 3 was statistically non-significant with p=1.000 (Table 4).

Groups	Number (n)	Mean ± S. D	Median	Z	p-val
I-A (day-1)	24	0.00±0.000	0	-3.583	0.000
II-A (day-1)	24	3289.65±5449.346	0	-3.583	0.000

Table 3: CFU counts of P. gingivalis for implants from subgroups I-A and II-A managed with 1% chlorhexidine and 10% doxycycline gel

Groups	Number (n)	Mean ± S. D	Median	Z	p-val
I-B(day-3)	24	0.00±0.000	0	-0.446	1.000
II-B(day-3)	24	0.00±0.000	0	-0.446	1.000

Table 4: CFU counts of P. gingivalis for implants from subgroups I-B and II-B managed with 1% chlorhexidine and 10% doxycycline gel

It was also noted that on day 7 for CFU counts of P. gingivalis for implants from subgroups I-C and II-C managed with 1% chlorhexidine and 10% doxycycline gel, mean CFU counts for subgroup C from group I were 0.00±0.000 and mean CFU counts for subgroup C from Group II were 0.00±0.000. The difference in the two groups was statistically non-significant with p=1.000 (Table 5).

groups	Number (n)	Mean ± S. D	Median	Z	p-val
I-C(day-7)	24	0.00±0.000	0	0.000	1.000
II-C(day-7)	24	0.00±0.000	0	0.000	1.000

Table 5: CFU counts of P. gingivalis for implants from subgroups I-C and II-C managed with 1% chlorhexidine and 10% doxycycline gel

4. DISCUSSION

Implant surface decontamination by elimination of bacteria is a vital step in the management of peri-implant diseases, which can be attained via combined chemical and mechanical methods. Various methods assessed in different studies have failed to show a superior effect of any agent. The present study assessed the efficacy of chlorhexidine and doxycycline as single and multiple applications to disinfect implant surfaces contaminated with the strain of P. gingivalis. Despite various in-vitro studies depicting biofilms having varying microbial complexes, various studies have reported a strong correlation of peri-implantitis and bacteria from the red-complex.7 A study by Ghensi et al8 has reported that P. gingivalis is the most common pathogen associated with peri-implantitis and has a pivotal role in assessing the structure and composition of biofilm at sites with peri-implantitis. They can also facilitate the colonization and growth of other species as T. forsythia and T. denticola. Most commonly used implants are sandblasted acid-etched surfaces, which allow high adhesion of P. gingivalis.9 These findings suggest that P. gingivalis is a key colonizer of peri-implantitis, and there is a need for therapeutic agents that target P. gingivalis for surfaces having peri-implantitis.

The present study assessed 1% CHX, which is bactericidal, and 10% doxycycline, which is a bacteriostatic antibiotic.10 The present study utilized 10% doxycycline, which has successful and predictable results as reported by previous studies.11,12 One such study by Patianna et al13 in 2018 has reported efficacy of 10% doxycycline when applied for 3 minutes on the implant surface. Similarly, the efficacy of 1% chlorhexidine has been confirmed by previous studies.14,15 The study used chlorhexidine application for 10 minutes following the previous study of Sbricoli et al, 16 in 2018, who reported complete decontamination in 10 minutes.

Anti-infective agents used as a solution are excreted rapidly from the GCF (gingival crevicular fluid), which warrants a high concentration initially and repeated agent's application to attain sustained efficacy of antimicrobial agents.17 On the contrary, the formulations as gels increase the duration of contact of the agent to the surface and increase the efficacy. This was in line with Lollobrigida et al, who reported that antimicrobials applied as a gel have

superior effects than liquid agents, which supports the selection of gel in the present study.

The mean CFU counts of P. gingivalis assessed from the implant surface that underwent doxycycline gel were significantly lower when compared to baseline values. These results were consistent with the findings by Patianna et al, 13 who suggested a significant decrease in bacteria on surfaces treated with 14% doxycycline compared to controls. Also, decontamination rate was increased on day 3 compared to day 1 in present study which was in agreement with Trajano et al.18 Also, significant CFU reduction in chlorhexidine group on day 1, 3, and 7 was in line with Paolantonio et al15 who reported decrease in bacterial colonization in implants managed with 1% chlorhexidine compared to controls depicting highly efficacious antimicrobial activity of chlorhexidine.

In a study by Wheelis et al,19 the efficacy of various chemical agents was assessed on elemental composition and surface texture in two grades of titanium implants with two decontamination methods. The authors reported that 1% chlorhexidine results in implant surface discoloration with no corrosion. However, 50% doxycycline led to oxide layer removal, surface discoloration, and pitting. EDS (Energy dispersive spectroscopy) showed 0.06% to 0.85%titanium component in swab samples. Also, almost all assessed samples increased surface roughness in both titanium concentrations. The study also suggested that implant surface decontamination by immersion is suitable for more acidic chemicals. However, these results are farther than the scope of the present study and need further exploration, with authors reporting significant improvement in parameters on day 60.

As no study in existing literature has reported either a single or combined approach as successful for peri-implantitis treatment, the present study assessed the efficacy of antimicrobial agents against P. gingivalis and assessed strategies that could result in complete disinfection of the implant surfaces. The findings of the present study focus on the fact that the efficacy of antimicrobial agents is largely governed by application frequency, focusing on the need for dose tailoring and application time to specific antimicrobial and clinical scenarios. The efficacy of locally applied antimicrobial gel agents used in the study can be used as a preferable alternative to the systemic antimicrobial agents with minimal systemic side-effect risk and improved patient compliance. Hence, with application of the results of the present study to the in-vivo system, a standard chemical decontamination protocol can be achieved for implants, helping clinicians in the effective management of peri-implantitis.

5. CONCLUSION

With the various limitations of the mentioned study, the study concludes that doxycycline and chlorhexidine can help in achieving the complete decontamination of the implant surfaces that are P. gingivalis; however, the number of applications needed varies based on the agent being used. 1% chlorhexidine gel depicted immediate efficacy with no viable CFU being assessed after the first application, and inhibited further growth of bacteria on repeated application. On the contrary, 10% doxycycline gel led to a significant reduction of viable P. gingivalis CFU counts following a single application; however, complete elimination was attained on subsequent applications. However, there are a few limitations, as the study was done in an in-vitro setup, and further in-vivo studies are vital for validation of these findings in a clinical context. Additionally, it is vital to assess the effects of antimicrobials on various dental implant surface characteristics to get a standard protocol for treatment.

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