BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume21,Issue7

DOI:10.58240/1829006X-2025.21.7-5

ORIGINAL ARTICALE

IMPACT OF DETOXIFICATION SOLUTIONS ON THE ORAL IMPLANT SURFACE- AN INVITRO STUDY

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Received: *Jun 7.2025;****Accepted:****Jul 28, 2025;****Published:****Aug 5, 2025*

ABSTRACT

Background: Bacterial plaque has been associated with peri-implant conditions. It has been difficult to remove the bacteria and their metabolites, including lipopolysaccharides, after these biofilms have formed on the implant surface. Therefore, the purpose of this study was to evaluate the effectiveness of detoxification solutions on the surface of oral implants.

Materials and Methods: In the current investigation, a total of 45 implants were employed. Prior to biofilm growth, the sterile saliva was kept at -20°C until it was required to produce pellicle formation on the implant surfaces. For detoxification of the surface of the oral implant, all samples have been divided evenly into three groups, Group 1: Control, Group 2: Immersed in Chlorhexidine gluconate solution, Group 3: Immersed in citric acid solution. Each implant that was assigned remained 60 and 120 seconds immersed in its respective solution. Following preparation, implants were examined under a scanning electron microscope. The SPSS Statistics version 21.0 was used to analyse the data that was gathered. One-way analysis of variance and post-hoc testing were used to assess the impact of detoxification procedures. Results with a p-value of less than 0.05 were considered statistically significant.

Results: After implants immersed for 60 seconds duration, the maximum biofilms was removed in Citric acid solution group (1.08±0.01) followed by Chlorhexidine gluconate group (1.22±0.04) and control group (1.98±0.06). There was a statistically significant difference found between the different detoxification solutions. After implants immersed for 120 seconds duration, the maximum biofilms was removed in Citric acid solution group (1.04±0.06) followed by Chlorhexidine gluconate group (1.14±0.10) and control group (1.90±0.02). But there was no significant difference found between the different detoxification solutions.

Conclusion: The present study concluded that the maximum detoxification found in Citric acid solution group compared to Chlorhexidine gluconate group and control group.

Keywords*:* Chlorhexidine gluconate, citric acid, detoxification, implant, peri-implantit

INTRODUCTION

Dental implants have proven to be a safe and dependable treatment option for individuals who are partially or completely edentulous, with great long-term survival rates. However, there are issues with dental implants, and peri-implant conditions such peri-implant mucositis and peri-implantitis are common.[1]

There are several causes of implant failures. Bruxism, periodontal disease impacting neighbouring teeth, and poor bone quality are clearly linked to implant failure. Compared to older patients, younger patients experience increased implant failure, presumably as a result of their higher masticatory effort. Calcium malabsorption has been linked to medications used to manage stomach acidity, which can lead to dental implant failure. Clearly, smoking and poor oral hygiene can lead to dental implant failure. Smokers' risks of failing are increased when they disregard their dental care.[2]

Peri-implantitis is a pathological disease that is characterised by increasing loss of supporting bone and inflammation in the peri-implant connective tissue. Clinical signs of inflammation, including bleeding on probing with/without suppuration, increased probing depth over time compared to earlier measurements, and progressive radiographic bone loss, which typically leaves part of the implant surface exposed and makes it more susceptible to possible bacterial contamination, are what define this disease, which has an estimated prevalence of 22 to 28%.[3]

Surface roughness, chemical composition, hydrophobicity, surface electrical charge, and energy all affect the biofilm's colonization, structure, and composition on implant surfaces. It was suggested that dental implants' micro- and nano-topography be changed to improve bone-to-implant contact. However, when implant threads get exposed to the oral cavity, biofilm builds up more quickly on rougher surfaces, making these places more challenging to clean.[4]

However, moderately rough implants exhibit greater reosseointegration rates than machined implants. Given that decontaminating rough implant surfaces is difficult when treating peri-implant diseases, it is crucial to create an environment that is conducive to re-osseointegration by eliminating the entire load of contaminants. This is because bacterial residues hinder re-osseointegration and cause peri-implantitis to recur. Implant surfaces can be decontaminated and/or detoxified using chemical and mechanical techniques. There is no clarity in the literature about the optimal technique for disinfecting implant surfaces. Hence the present study was conducted to evaluate the effectiveness of detoxification solutions on the surface of oral implants.

MATERIALS AND METHODS

Saliva preparation and development of biofilm:

The present study was conducted in the department of oral surgery. In the current investigation, a total of 45 implants were used.2.5 mmol/L DL-Dithiothreitol was added to an unstimulated saliva sample and stirred continuously for 10 minutes in order to decrease the aggregation of salivary proteins. Following a 10-minute centrifugation at 4°C the supernatant was diluted 1:1 with phosphate-buffered saline (PBS). When no bacterial growth was seen on supplemented blood agar plates after 72 hours at 37°C, the saliva was deemed sterile. Before biofilm growth, the sterile saliva was kept at -20°C until it was needed to produce pellicle formation on the implant surfaces.

A modified brain heart infusion medium was used to cultivate a pure colony of bacteria anaerobically for 24 to 48 hours at 37°C. For pellicle formation, the mounted implants were placed in a sterile 24-well cell culture plate and submerged in saliva for four hours at 37°C. After that, the implants were moved to a fresh, clean 24-well cell culture plate. 1.5 mL of the bacterial suspension was added to each well, and the wells were then incubated for 72 hours at 37°C in an anaerobic conditions.

Detoxification of oral implant surface:

All samples in were equally divided into three groups as follows,

Group 1: Control:

15 contaminated implants were immersed in saline for 60 and 120 seconds.

Group 2: Immersed in Chlorhexidine gluconate solution:

Using 0.2% chlorhexidine gluconate mouthwash, 15 contaminated implants were immersed for 60 and 120 seconds, respectively. Saline and distilled water were then used to rinse the implants.

Group 3: Immersed in citric acid solution:

15 contaminated implants were immersed in

a solution of prepared citric acid. 30 grammes of pure citric acid crystals were dissolved in 100 millilitres of distilled water to create a citric acid solution, then implants were submerged for 60 and 120 seconds, respectively. After that, distilled water and saline were used to rinse the implants.

Effectiveness of the detoxification solutions:

After the implants were washed three times with 2 mL PBS for 10 s, a fixative solution containing 4% paraformaldehyde and 2.5% glutaraldehyde was administered for four hours at 4°C in order to prepare them for SEM. Using a series of graded ethanol solutions (30, 50, 70, 80, 90, and 100%), the samples were dehydrated after a 10-second washing with PBS and sterile water. The gold was applied and the implants were dried at the critical time. Using a backscattered electron detector and a 25 kV
image resolution, the processed samples were viewed using a SEM (JEOL, Tokyo, Japan).

Statistical Analysis:

The SPSS Statistics version 21.0 was used to analyse the data that was gathered. One-way analysis of variance and post-hoc testing with Bonferroni correction were used to assess the impact of detoxification procedures. Normality was evaluated using the data distribution and Shapiro-Wilk goodness-of-fit tests. Results with a p-value of less than 0.05 were deemed statistically significant.

RESULTS

Table 1 shows the efficacy of different detoxification solutions on the oral implant surface at 60 seconds duration. The maximum biofilms was removed in Citric acid solution group (1.08±0.01) followed by Chlorhexidine gluconate group (1.22±0.04) and control group (1.98±0.06). There was a statistically significant difference found between the different detoxification solutions.

Table 1. Comparative evaluation of the efficacy of different detoxification solutions on the oral implant surface at 60 seconds duration

Solution groups	n	Mean±SD	F value	P value
Group 1: Control	**15**	1.98±0.06	10.162	0.001
Group 2: Chlorhexidine gluconate	**15**	1.22±0.04		
Group 3: Citric acid	**15**	1.08±0.01		

Table 2 depicts the efficacy of different detoxification solutions on the oral implant surface at 120 seconds duration. The maximum biofilms was removed in Citric acid solution group (1.04±0.06) followed by Chlorhexidine gluconate group (1.14±0.10) and control group (1.90±0.02). But there was no significant difference found between the different detoxification solutions.

Table 3 reveals the comparison of mean difference among the efficacy of three different detoxification solutions. There was a statistically significant difference found between Control v/s Chlorhexidine gluconate group and Control v/s Citric acid group. But there was no significant difference found between Chlorhexidine gluconate v/s Citric acid group.

Table 2. Comparative evaluation of the efficacy of different detoxification solutions on the oral implant surface at 120 seconds duration

Solution groups	n	Mean±SD	F value	P value
Group 1: Control	**15**	1.90±0.02	6.108	0.064
Group 2: Chlorhexidine gluconate	**15**	1.14±0.10		
Group 3: Citric acid	**15**	1.04±0.06		

Table 3. Comparison of mean difference among the efficacy of three different detoxification solutions

Solution groups	Compared with	Mean Difference	P value
Control	**Chlorhexidine gluconate**	0.76	0.001
	Citric acid	0.90	0.001
Chlorhexidine gluconate	**Control**	-0.76	0.001
	Citric acid	0.14	0.842
Citric acid	**Control**	-0.90	0.001
	Chlorhexidine gluconate	-0.14	0.842

DISCUSSION

The main goal of peri-implantitis therapy is to prevent the inflammatory process and perhaps promote re-osseointegration, yielding stable, long-term outcomes. It is important to remove the biofilm if we believe that bacteria cause and worsen periimplantitis.[6] Regardless of the cleaning method, nonsurgical therapy seldom eradicates the condition, and recurrence is a definite possibility. Additional surgical therapy is therefore recommended when nonsurgical treatment fails to yield good outcomes.[7]

The most frequently tested antibacterial agents in recent years have been hydrogen peroxide (H2O2), citric acid (CA), and chlorhexidine (CHX). CA has shown promise against biofilms of both single and multiple species on titanium surfaces.[8] It frequently falls short of saline rinses in terms of effectiveness, though, and it has never been tested against mature biofilms. CHX has demonstrated bactericidal activity against both immature and established biofilms, although it has no inherent cleaning capabilities.[9]

In the present study citric acid solution group was superior to Chlorhexidine gluconate group and control group. This is similar to the study conducted by Dennison DK et al.[10] In vitro, citric acid has also been demonstrated to decrease bacterial endotoxins when administered for one or two minutes. The endotoxin was reduced by up to 85.8% following a one-minute treatment for machined surfaces, 27% for titanium plasma sprayed, and 86.8% for titanium implants coated with hydroxyapatite. Gosau M. et al.[11] found that after immersing smooth titanium discs in a 40% CA solution for one minute, citric acid was unable to inactivate bacterial biofilms that had developed on the discs when taken intraorally by humans. This could be because of the properties of the glycocalix-protected biofilms or because the immersion research did not experience any mechanical disintegration.

In the present research, the 2% chlorhexidine group eliminated the most biofilm in comparison to the control group. At each stage of implant therapy, the bacterial load can be decreased by using an antiseptic mouthwash containing chlorohexidine. Abraham et al.[12] state that Rinse after surgery until the incision line heals and any additional infections are under control. Hand and intraoral/extraoral scrubs are used as surface antiseptics before implant surgery. because it just takes a few seconds to destroy a significant amount of the bacterial flora. According to Fiorillo[13] Chlorhexidine can be used inside the fixture or abutment of a dental implant connection for conservative or endodontic therapy, to prevent alveolitis, or to reduce peri-implant peri-implantitis. Thus, there is much discussion over the benefits of chlorhexidine. A research by Sajjan et al.[14] states that chlorohexidine can be used as an irrigant for root canals, an antiplaque agent to prevent caries by inhibiting S. mutans, and a preventive measure against secondary infections in apthous ulcers and alveolar osteitis. They showed promising outcomes as implant-associated biofilms.

It has been demonstrated that the clinical use of CHX necessitates durations longer than 30 seconds or one minute. It has been demonstrated that CHX causes oxidative stress, intracellular Ca2+ rise, and disruption of mitochondrial activity, which results in apoptosis and cell necrosis.[15] CHX has also been demonstrated to suppress collagen production and cell division.[16] The decrease in bacterial numbers may be explained by these adverse consequences.

One of the study's limitation is that the biofilm produced cannot be compared to biofilms observed in diseased peri-implant locations, which exhibit deeper pockets containing bacteria of different quantities and quality. Their lack of solid clinical results, cytotoxicity, surface modification, and unrealistic settings all raise questions about their therapeutic usefulness. Incorporating careful concentration, exposure control, and mechanical debridement is crucial. Therefore, more clinical study has to be carried out in order to confirm the outcome.

CONCLUSION

Within the limitation the present study concluded that themaximum detoxification found in Citric acid solution group compared to Chlorhexidine gluconate group and control group.

DECLARATION

Funding
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest:
The authors declare no conflict of interest.

Ethical approval:
Not applicable.

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