Sustained Release of Chlorhexidine from Modified Titanium Surfaces (In-Vitro Study)

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Abstract: Background and Aim: Adjunctive antiseptic agent chlorhexidine is often recommended for decontamination in the treatment of peri-implant infections. However, action of chlorhexidine on the titanium surface in the peri-implant environment needs further research. The purpose of present study was to assess chlorhexidine interaction with titanium implant surfaces to estimate its antiplaque efficacy. The purpose of present study was to assess interaction of chlorhexidine with titanium surfaces to estimate its antiplaque potential in the peri-implant environment. Material and Methods: Four modified titanium surfaces were prepared from grade 4 commercially available pure titanium. No surface treatment- control (machined surface, MA), Acid mix of 10% HNO3 and 5% HF (HNF), Hydroxy apatite coated - Resorbable blast media (hydroxyapatite particles cleaned with nitric acid) (HAC), Sandblasting and acid etching (SBAE). Each sample was incubated with phosphate-buffered saline (PBS) or whole saliva for 2 hours. After 1 minute exposure to 0.2 % chlorhexidine gluconate solution, Spectrophotometer was used to measure chlorhexidine release on days 1, 2, and 5. Results: Chlorhexidine exposed titanium surfaces exhibited chlorhexidine release for short duration of time. Chlorhexidine levels dropped rapidly within 3 days time. SBAE and HAC released more chlorhexidine than HNF and MA, specifically in saliva-coated group. Conclusion: This study suggests that titanium surface modifications significantly influence chlorhexidine uptake and release. In the saliva-filled oral cavity, SBAE and HAC shows increased chlorhexidine uptake capacity. The slow chlorhexidine release rate suggests substantivity, which provides a long-term antiplaque effect. [Parmar M Natl J Integr Res Med, 2019; 10(5):75-81]

Key Words: Chlorhexidine Digluconate; Titanium, Saliva

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Introduction: When exposed to the oral environment the titanium transmucosal implant surfaces allow bacterial plaque and calculus accumulation similar to the tooth surface.[1] Bacterial plaque on implant surface and on healing abutments can cause infectious complications and peri-implant bone loss.[2] It can lead to peri-implant mucositis and peri-implantitis.[3]

Therefore, Mechanical plaque control measures, supplemented with adjunctive antiseptic agents are recommended to prevent and reduce bacterial accumulation of plaque on the titanium surfaces.[4] Several anti-plaque chemical agents are commercially available, including citric acid, H2O2, and chlorhexidine (CHX). Chlorhexidine digluconate, widely used as an antiplaque agent,[5] Chlorhexidine is used in the form of a rinse, gel, or intrapocket irrigation to treat peri-implantitis. Chlorhexidine has bactericidal properties and it can penetrate the plaque biofilm.[6] It binds to salivary glycol-proteins and bacteria, thus inhibit plaque accumulation.[7]

When chlorhexidine is used as a local antimicrobial agent to prevent and treat peri-implant diseases, its efficacy on clinical and microbiological parameters is unknown. Several studies showed no clinical or microbiologic benefits after irrigation of peri-implant shallow pockets or rinsing with chlorhexidine solution.[8]Whereas some studies showed significant reduction in plaque index and modified gingival index after subgingival irrigation with chlorhexidine in periimplant pockets.[9]

Chlorhexidine has unique property of ''substantivity,'' i.e., the ability to adsorb to hard and soft oral surfaces and subsequently desorb in a biologically active form. This creates a reservoir of chlorhexidine.[7, 10] However, studies show that chlorhexidine bound to the tooth surface helps achieve antibacterial effect rather than the chlorhexidine desorbed from other oral surfaces.[11] In a natural tooth surface environment, chlorhexidine efficacy is attributed to the interaction of chlorhexidine molecule with hydroxyapatite, enamel, root dentin, and soft tissues.[12] Limited data is available on the interaction of chlorhexidine persistence on the titanium implant surface.[13] The purpose of this in vitro study was to evaluate the ability of modified

titanium surfaces to release chlorhexidine after chlorhexidine exposure. The protocol was approved by an institutional ethical committee. The study was conducted from April to July 2018.

Materials and Methods: Ethical approval was taken from hospital's research ethics committee and written informed consent was taken from the study participants.

Material Preparation : A total of 40 titanium implant specimens (3.5 mm in diameter and 15 mm length) were fabricated from grade 4 commercially available pure titanium and randomly divided into four different surface treatment groups.

1. No surface treatment control (Machined surface, MA)
2. Treated with a mixed acid of 10% HNO3 and 5% HF (HNF)
3. Sandblasting and acid etching sandblasted with 250 – 500 µm sized-aluminum oxide (Al2O3) and acid etched with hydrochloric acid. (SBAE)
4. Resorbable blast media, (resorbable blast media comprised of 100-µm-sized hydroxyapatite particles and cleaned with nitric acid) (HAC)

After surface treatments, the specimens were ultrasonically cleaned with a soap solution for 4 hours, washed, rinsed in distilled water, and air-dried. All samples were packed, sealed, and sterilized with ethylene oxide gas.

Saliva Preparation:Unstimulated whole saliva was collected from healthy volunteer with no acute caries or periodontal lesions. To avoid donor-specific variations in salivary samples, one healthy donor was used throughout the study. Saliva samples were obtained between 9:00 A.M. and 10:00 A.M to minimize the effects of diurnal variability in salivary composition. 22, 23 Saliva (stimulated by chewing paraffin) was collected (20 ml) during 30 minutes at the same hour of the day, before breakfast.

The collected saliva was centrifuged at 3,500 rpm for 10 minutes to remove any cellular debris and bacteria and the supernatant was filtered through a 0.45-mm filter to yield sterile saliva. The saliva samples were stored at -20oc before use.

Saliva Treatment : Samples from each group were randomly divided into a saliva-coated group and a non-coated control group. For the coated group, each implant specimen was incubated separately in 1.5 ml of the sterile saliva at 37°C for 2 hours with gentle shaking. After incubation, samples were gently dried on sterile filter paper for 30 minutes to remove excess saliva and prepared for chlorhexidine experiments. The samples in the non-coated group underwent the same protocol, except that sterile phosphate-buffered saline (PBS, ph = 7.2) was used instead of saliva.

Chlorhexidine Treatment: Ten specimens (5 saliva-coated and 5 non-coated control) from each group were conditioned with 0.2% chlorhexidine digluconate for 1 minute. After immersion, the chlorhexidine treated specimens were removed and gently dried by blotting on sterile filter paper. Each specimen was then placed in 1 ml of sterile distilled water and incubated for 24 hours with gentle shaking at room temperature.

After the first incubation, the same protocol – rinsing, blot-drying, and incubation – was repeated until the next chlorhexidine measurement points, 2 and 5 days after chlorhexidine treatment. Upon completion of each incubation period, the 1 ml-incubating solutions were collected to measure chlorhexidine release.

Analysis of Chlorhexidine Release : A single-beam diode array spectrophotometer with quartz quvette was used to quantify chlorhexidine levels in the tested chlorhexidine solution based on its ultraviolet light absorbance 22, 23. The spectral profile of chlorhexidine solutions was first tested in the range from 200 to 700 nm. Two regions of peak absorption were found: 230 to 233 nm and 254 to 256 nm. Because maximal absorbance was best presented at 230 nm, the samples were measured in that wave length.

The concentration of chlorhexidine was determined by comparison with the spectrophotometric absorbance of the standard chlorhexidine solution at 230 nm using an Ultraviolet-Visible Spectrophotometer. Measurements of chlorhexidine release from the incubating solutions were performed using spectrometry on days 1, 2, and 5. Spectrophotometer was used to quantify chlorhexidine levels in the tested aqueous chlorhexidine solution based on its ultraviolet light absorbance. The spectral profile of

chlorhexidine aqueous solutions was first tested in the range from 200 to 700 nm. Two regions of peak absorption were found: 230 to 233 nm. Because maximal absorbance was best presented at 230 nm, the samples were measured in that wavelength. (Figure 1). Chlorhexidine release from titanium surfaces (spectrophotometer readings, Figure 2)

Figure 1: Spectrophotometer

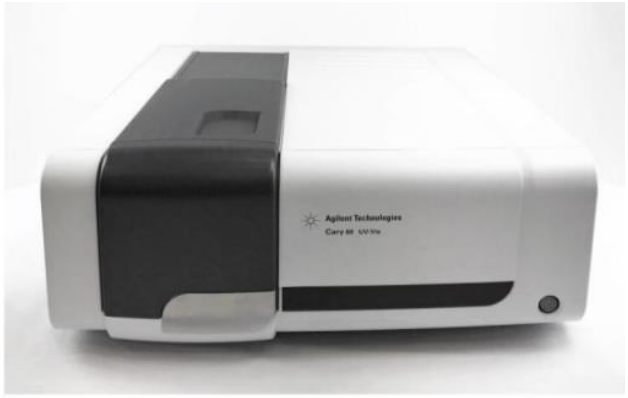
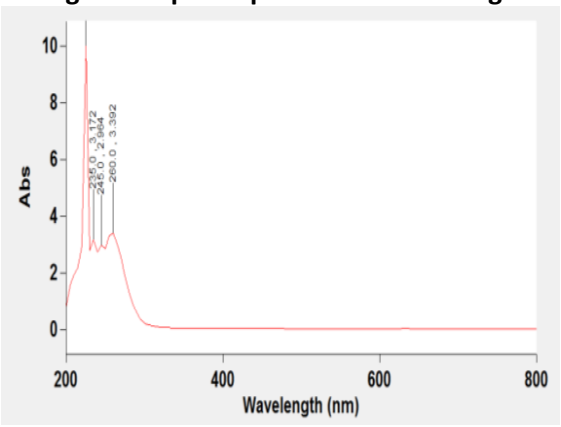


Figure 2: Spectrophotometer Readings



Statistical analysis: The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2007) and then exported to data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics included computation of percentages, means and standard deviations. Multiple comparisons were performed by two-way analysis of variance (ANNOVA) tests using the Bonferroni correction For all tests, confidence level and level of significance were set at 95% and 5% respectively.

Results : The chlorhexidine release profile was almost similar among all different titanium surface groups, regardless of the presence of saliva-coating. Chlorhexidine exposed titanium surfaces exhibited chlorhexidine release for short duration of time. Chlorhexidine levels dropped rapidly within 3 days time. However, there was significant difference in the quantitative extent of chlorhexidine release among the different surface groups. SBAE and HAC released more chlorhexidine than HNF and MA (HAC, SBAE > MA, HNF, $P < 0.005$).

The presence of saliva-coating had a significant effect on the amount of chlorhexidine release. ($P < 0.005$) Saliva-coated samples released about 2 times more chlorhexidine than non-coated disks. In particular, the non-coated disks treated with MA and HNF released close to the baseline level of chlorhexidine throughout the experimental period.

Chlorhexidine release in MA between PBS and Saliva: During day 1 mean values were 0.60 and 1.31 respectively (in PBS and Saliva) which then reduces to 0.07 and 0.1 (PBS and Saliva) respectively on day 2 and 0.001 in both groups on day 5. Difference between these values on different days was significant statistically both in PBS and salivary coating. ($p \leq 0.05$) there was also significant difference between day 1 and day 2 both in PBS and salivary coating. ($p > 0.05$)

Chlorhexidine release in HNF between PBS and Saliva: There was major difference in mean values of PBS and saliva coating on day 1 (0.60 and 1.22) which then reduced to similar values on day 2 (0.1069 and 0.1039) and on day 5 PBS coating had lesser mean values compare to saliva and difference between these values were significant statistically. ($p \leq 0.05$).

Chlorhexidine release in SBAE between PBS and Saliva: Similarly in above findings day 1 had highest mean values in both coating and day 5 had least values both in PBS and Saliva. Statistically significant difference was observed between day 1, day 2 and day 5 both in PBS and Saliva ($p \leq 0.05$). During intergroup comparison all results and findings were statistically significant between all the days in both coatings. ($p \leq 0.05$)

Chlorhexidine release in HAC between PBS and Saliva: During day 1 mean values were 0.88 and 1.88 respectively (in PBS and Saliva) which then reduces to 0.14 and 0.19 respectively (in PBS and Saliva) on day 2 and 0.02 and 0.07 in both groups on day 5. Difference between these values on different days was significant statistically both in PBS and salivary coating. ($p \leq 0.05$) During intergroup comparison all results and findings

were statistically significant between all the days in both coating. (p≤0.05)

Table 1 explains all group wise distribution on Day 1. During study it was observed that HAC surface had highest mean values in PBS coating while SBAE had highest mean values in Salivary coating. Difference between all surfaces during day 1 was statistically significant on both PBS and Salivary coatings. (p≤0.05) during intergroup comparison non-significant difference was observed between MA and HNF surface on day 1 (p>0.05) on both coatings.

Table 1: All group wise distribution during day 1

Groups	Coating	CHX release		P value
		Mean	SD	0.005*
MA	PBS	0.6077	0.0778	
	Saliva	1.3141	0.1810	
HNF	PBS	0.6053	0.08973	
	Saliva	1.2288	0.2827	
SBAE	PBS	0.6835	0.07635	
	Saliva	1.9900	0.1355	
HAC	PBS	0.8899	0.09254	
	Saliva	1.8817	0.0291	

* indicates statistically significance at p≤0.05
Test applied one way ANNOVA

Table 2 describes all group wise distribution on Day 2. SBAE surface had highest mean values both in PBS and Saliva (0.15 and 0.20 respectively). Statistically significant difference was observed between all four surfaces on both coatings on day 2. (p≤0.05) during intergroup comparison all surface had significant difference except HAC and SBAE had nonsignificant difference. (p>0.05)

Table 2: All group wise distribution during day 2

Groups	Coating	CHX release		P value
		Mean	SD	0. 002*
MA	PBS	0.0766	0.0810	
	Saliva	0.1098	0.0115	
HNF	PBS	0.1069	0.02160	
	Saliva	0.1039	0.0270	
SBAE	PBS	0.1574	0.01738	
	Saliva	0.2097	0.0407	
HAC	PBS	0.1466	0.0729	
	Saliva	0.1984	0.0094	

* indicates statistically significance at p≤0.05,
Test applied one way ANNOVA

Table 3 explains all group wise distribution on Day 5. During this study it was observed that MA surface had least mean values both in PBS and Saliva coating. Difference between all surfaces during day 5 was statistically significant on both PBS and Salivary coatings (p≤0.05). During intergroup comparison non-significant difference was observed between MA and HNF surface on day 5 (p>0.05) on both coatings.

Table 3: All group wise distribution during day 5

Groups	Coating	CHX release		P value
		Mean	SD	0.02*
MA	PBS	0.0108	0.0071	
	Saliva	0.0171	0.0025	
HNF	PBS	0.0108	0.066	
	Saliva	0.0207	0.0041	
SBAE	PBS	0.0211	0.01738	
	Saliva	0.0425	0.00098	
HAC	PBS	0.0277	0.0099	
	Saliva	0.0706	0.0051	

* indicates statistically significance at p≤0.05
Test applied one way ANNOVA

Discussion: Peri-implantitis is an inflammatory condition of the tissues surrounding an implant, leading to bone loss.14 The prevalence of peri-implantitis varies by diagnostic criteria, implant site, and maintenance procedures14,15, which ranges from 12 to 43%.16 This inflammatory process is reversible during the initial stage.17 But, it may progress to bone loss, osseointegration loss leading to failure of the implant, if left untreated .18

To increase the surface area implant surfaces are made rough which helps in good osseointegration. But it attracts more oral bacteria which develop a biofilm. Unlike tooth surface, roughness makes effective mechanical debridement difficult.19This suggests that rough surfaces show greater risk of peri-implantitis compared to smooth surfaces.20

Chlorhexidine can penetrate the plaque biofilm. Chlorhexidine is known to be the most effective and widely used antiplaque agent.5 However; no data is available in the literature on the interaction between titanium surface properties and chlorhexidine adsorption. The effect of chlorhexidine in the treatment of peri-implantitis requires further research.

Our study indicate that regardless of surface type, chlorhexidine exposed titanium surfaces exhibited chlorhexidine release for short duration of time, though levels quickly dropped within 3 days after exposure. After chlorhexidine exposure, the initial burst of chlorhexidine release might be associated with washout of chlorhexidine molecules that were retained on the surface or in the pores of the surfaces during chlorhexidine application.21 This finding show that various titanium surfaces have ability to uptake chlorhexidine introduced from external chlorhexidine sources.

Our results show that titanium surface modifications significantly affect the amount of chlorhexidine release after exposure. In particular, SBAE and HAC released more chlorhexidine than HNF and MA (HAC, SBAE > MA, HNF, $P < 0.005$). Several studies have reported that rough titanium surface has larger surface area and more binding sites, so it can adsorb more chlorhexidine.13, 22, 23 The results of our study were consistent with these findings. SBAE and HAC, which had rougher surface, showed higher chlorhexidine release than MA. However, surface roughness is not the sole factor responsible for chlorhexidine release. Surface texture, specifically narrower and smaller depressions, may shelter chlorhexidine from environmental fluctuations, represented by the cleaning and washing procedures in our study. In addition, compared to broader and larger ones, the narrower and smaller depressions of SBAE and HAC may provide a greater surface area for interaction with chlorhexidine molecules, leading to increased chlorhexidine binding site. This shows that surface texture is a more important factor for chlorhexidine retention than surface roughness. 22, 23

According to our study results, saliva-coating had significant role in enhanced chlorhexidine release from various titanium surfaces. The quantity of chlorhexidine released from the saliva-coated samples was approximately twice than that from the non-saliva-coated samples. This is attributed to the fact that the dicationic chlorhexidine molecule forms an electrostatic linkage with the acidic protein groups of the salivary pellicle, thus chlorhexidine molecule binds to salivary glycoprotein in the titanium pellicle.7, 12

Chlorhexidine treated SBAE and HAC may exert an immediate broad bactericidal effect after chlorhexidine exposure. In addition, sustained selective antibacterial effect may also be anticipated. The quantity of chlorhexidine adsorbed on titanium pellicle decides the antibacterial activity to be either bacteriostatic or bactericidal. Our study results support the ability of modified titanium surfaces to retain chlorhexidine.

Some studies also shows that intraoral mucosa and the tooth surfaces also act as reservoirs of chlorhexidine molecule.11 But there is difference in physicochemical properties and surface energies of enamel and titanium surfaces. The titanium pellicle contains more high-molecular weight proline-rich acidic glycoprotein than the enamel pellicle, which can serve as binding receptors for dicationic chlorhexidine molecule by electrostatic interactions.24 Hence, SBAE or HAC-type implants along with regular topical application of chlorhexidine to maintain its levels in saliva filled oral cavity may be recommended for patients at high risk of peri-implantitis.

Widely used mouth-rinses have chlorhexidine concentration of 0.1 – 0.2% and gel forms have 0.5 – 1.0% which have proven to reduce bacterial load and improve clinical parameters in the peri-implant environment.25 Therefore, the efficacy of different chlorhexidine concentrations with different exposure time to determine the optimal therapeutic level for the treatment of peri-implant infections requires further research. Furthermore, this is an in vitro study similar to most of the other researches on chlorhexidine release. As the in-vitro experimental environment may differ from intraoral environment considering factors like saliva composition, systemic conditions etc. Moreover, chlorhexidine has side effects of formation of extrinsic stains, bitter taste, cytotoxicity against fibroblast.26, 27 Therefore, interpretation of this study needs to be considered with caution.

Conclusion : This study suggests that titanium surface modifications have significant influence on chlorhexidine release. SBAE and HAC titanium surface provide effective chlorhexidine uptake capacity in the saliva-filled oral cavity. The slow chlorhexidine release rate suggests substantivity i.e. persistence of this agent at the titanium-pellicle surface, which provide long-term plaque control. Therefore, for patients at high risk of peri-implantitis regular exposure of chlorhexidine with the use of SBAE and HAC type implants may

be recommended. Considering that enamel surface acts as a chlorhexidine reservoir, further in situ studies using the removable intraoral appliances, which has enamel and titanium slabs, will be needed to simulate the oral environment and enhance clinical significance.

Hence, in future studies, further research is needed on the efficacy of various chlorhexidine concentrations with different exposure time and its effect on different surface types to determine the optimal therapeutic level for the treatment of peri-implant infections.

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