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Research Article

Effect of Citric Acid and Chlorhexidine on the Implant Surface-an *Invitro* Experiment

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Abstract

Objectives: To find out and compare the effect of citric acid and chlorhexidine on the surface roughness and elemental composition of dental implants and to find out the effect of duration and concentration of citric acid and chlorhexidine treatment on the surface of dental implants.

Material and Methods: 135 specimens were prepared by sectioning 15 dental implants and abutments. These were embedded in polyvinylsiloxane putty blocks such that the sectioned surface was exposed. The specimens were subjected to the various surface treatments viz; immersion in 30%, 40%, 50% and 60% citric acid and 0.2% chlorhexidine for different durations viz; 40 sec, 60 sec and 80 sec. Surface roughness and elemental composition of the specimens were analysed using profilometer, scanning electron microscope and energy dispersive spectroscopy respectively, before and after immersion in different solutions. Data obtained was statistically analysed using factorial ANOVA.

Results: The highest mean surface roughness on implant specimens was obtained with 50% citric acid and the least roughness was observed with 30% citric acid. On the abutment specimens, the highest mean surface roughness was observed with a concentration of 40% citric acid and the least roughness was with 30% citric acid. Chlorhexidine showed lower roughness both in implant and abutment specimens. The elemental analysis for implants as well as abutments showed decrease in elemental titanium with increase in surface roughness. The greatest concentration of elemental titanium was present at 30% citric acid for 40 sec and 0.2% chlorhexidine at 40 sec for implants, and 30% citric acid for 60 sec and 0.2% chlorhexidine at 40 sec for abutments.

Conclusion: Surface treatment of dental implants and abutments with citric acid and chlorhexidine increased the surface roughness. The surface roughness of implants and abutments increased along with an increase in the duration of immersion in Citric acid and Chlorhexidine. Increase in surface roughness of implants and abutments caused a decrease in elemental titanium concentration. Titanium concentration is maintained when implants and abutments are treated with 30% citric acid. The clinically acceptable concentration of citric acid is 30% for 40 sec and 0.2% chlorhexidine for 40 sec to be used in surface conditioning decontamination of failing implants.

Keywords: Implant; Abutment; Citric Acid; Chlorhexidine; Surface Roughness; Elemental Composition

Introduction

In the recent times, dental implants have become the most preferred treatment option for complete and partial edentulism. Progressive departure from the conventional treatment to dental implants has happened because of the improved predictability presently enjoyed by dental implant treatment. Implant prosthesis offers longevity, improved function and bone preservation. Available statistics prove that failures encountered by dental implants are less than 10% over a period of ten years [1,2]. Conventional fixed and removable prosthesis could never claim such an impres-

sive statistics. Implants do face failures in spite of the documented advantages of biologic preservation of tissues and the improved functional efficiency.

Implant failures can be attributed to multiple factors. Poor quality of bone, periodontal disease affecting the adjacent teeth and bruxism are evidently related to implant failure. In younger patients, implant failure is more when compared to older individuals, possibly because of the higher masticatory force exerted by youngsters. Medicaments used in the treatment of gastric acidity have been related to malabsorption of calcium and thereby causing failure of dental implants. Poor oral hygiene and smoking can obviously cause failure to dental implants. Smokers neglect oral hygiene and the failure chances are doubled [3].

Peri-implant infections manifest as redness of mucosa around the implants, increased bleeding on probing, increased pocket depth, exposure of implant threads, mobility of implants and at times suppuration might also be present. There are two distinct entities included in this class viz. peri-implant mucositis and peri-implantitis. The former is a reversible inflammatory response affecting the peri-implant soft tissues. The later is an inflammatory process which ends up in the loss of implant supporting osseous structures [4,5]. The pathogenesis starts with colonisation of microbes on the implant surface. Most of the peri-implant infections are caused by gram negative anaerobic bacteria. The pathogenic bacteria release toxins which are capable of causing immune responses and which eventually causes loss of implant supporting bone [6-8].

The treatment of peri-implantitis aims at regaining the bone attachment around dental implants by modifying the implant surface. The primary requisite of treatment is the reduction of pathogenic bacteria through decontamination of the implant surface. First the affected site is surgically exposed, followed by debridement of the exposed area. Many physical and chemical methods are used for decontamination. Physical methods include lasers, photo dynamic therapy and air driven abrasives. Chemicals are popular because of the ease of application. For this purpose, 30% citric acid, chlorhexidine gluconate, hydrogen peroxide, and tetracycline hydrochloride are used [9,10]. Once the affected site is thoroughly debrided and implant surface is decontaminated, a graft material, either autograft or synthetic graft is placed over the exposed implant threads and covered with a membrane. Many authors have used Citric acid for detoxification of the implant surface. But extensive studies have not been carried out on the effect of citric acid on the surface of dental implants. Chlorhexidine, a commonly used antiseptic mouthwash, is also used for similar purpose but not well documented. The present study was carried out to find out the effects of citric acid and chlorhexidine on the dental implant surfaces with the following objectives

- To find out and compare the effect of citric acid and chlorhexidine on the surface roughness and elemental composition of dental implants.
- To find out the effect of duration and concentration of citric acid and chlorhexidine treatment on the surface of dental implants.

Methodology

The present study was conducted to determine the effect of different concentrations and duration of immersions of two commonly used debriding solutions, viz: citric acid and chlorhexidine gluconate mouthwash on the surface of titanium dental implant. The summary of methodology is given in figure 1.

15 dental implants (ADIN dental implant systems, Israel) of 16mm length and 5 mm diameter and corresponding abutments were selected (Figure 2). The surface characteristics of both the implants and abutments were evaluated using scanning electron microscope (SEM). In order to accommodate in the SEM, both the implants and the abutments were sectioned horizontally limiting the height of the specimens to 5mm. From the implant fixture three pieces were obtained and from the abutment two pieces (Figure 3). A total of 135 specimens were prepared.

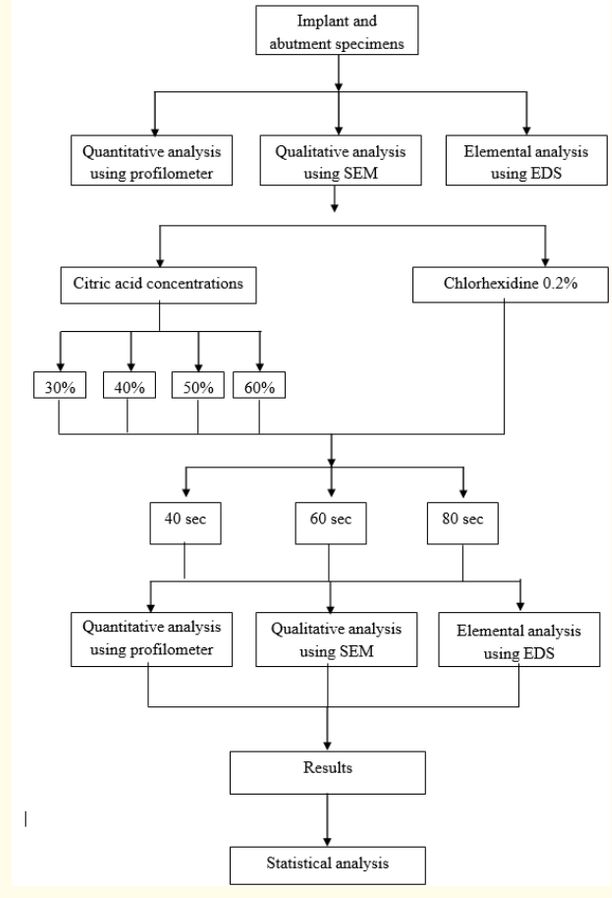

Figure 1: Flow chart on methodology

Figure 2: ADIN dental implant with abutment.

Figure 3: Sectioned implant and abutment.

Preparation of specimens

Each specimen was embedded in a putty block measuring 30 x 30 x 30mm (Figure 4). Light body polyvinyl siloxane impression material was injected around the implant specimen surface to avoid fluid seepage through the interface (Figure 5). Around the putty block a wax trough was made to store different solutions (Figure

Figure 4: Specimen transferred on the putty.

Figure 5: Light body applied around the specimen.

Figure 6: Wax box built around the specimen.

Figure 7: Immersion in citric acid solution.

Figure 8: Immersion chloehexidine.

6-8). The sectioned implants were divided into three groups based on the different surface conditioning methods used, namely, distilled water, citric acid and chlorhexidine gluconate mouthwash.

Preparation of citric acid solution

To prepare 30% solution of citric acid, 30gms of pure citric acid crystals were dissolved in 100ml of distilled water. Similarly to prepare 40%, 50% and 60% solution of citric acid, 40g, 50g and 60g of pure citric acid crystals were dissolved in 100ml of distilled water respectively (Figure 9).

Figure 9: 0.2% chlorehexidine mouth wash and citric acid crystals.

Immersion of specimens

Immersion in distilled water

The first group of specimens were immersed in distilled water for 40 seconds, 60 seconds and 80 seconds respectively. This served as the control group.

Immersion in citric acid

The second group of specimens were further divided into four depending on the concentrations of citric acid used which were 30%, 40%, 50% and 60% respectively. The sectioned implants were immersed in the different concentrations of citric acid for 40 seconds, 60 seconds and 80 seconds respectively (Figure 7). For different concentrations and time intervals, separate specimens were used. The sectioned implants were then rinsed with saline and distilled water.

Immersion in chlorhexidine gluconate mouthwash

The sectioned implants were immersed in chlorhexidine gluconate mouthwash 0.2% for 40 seconds, 60 seconds and 80 seconds respectively (Figure 8). The sectioned implants were then rinsed with saline and distilled water.

Surface roughness evaluation

Quantitative analysis using profilometer

Surface roughness of the specimens were analysed quantitatively (Ra) using profilometer (Zeta instruments) before and after immersion in distilled water, citric acid and chlorhexidine gluconate mouthwash (Figure 10).

Figure 10: Optical profilometer.

Qualitative analysis using scanning electron microscope (SEM)

Surface characteristics of the specimens were analysed qualitatively using SEM (Zeiss) before and after immersion in distilled water, citric acid and chlorhexidine gluconate mouthwash (Figure 11).

Figure 11: Scanning electron microscope with energy dispersive microscopic.

Elemental analysis using energy dispersive spectroscope (EDS)

All the specimens were analysed for surface elements using EDS (Zeiss) before and after immersion in distilled water, citric acid and chlorhexidine gluconate mouthwash (Figure 11).

Statistical analysis

The results obtained were subjected to factorial ANOVA to detect statistically significant difference.

Results

In the present study, two factors influenced Surface Roughness (Ra) viz. Immersion medium and Immersion Time. Six different types of immersion media were used - Distilled Water, Citric Acid 30%, Citric Acid 40%, Citric Acid 50%, Citric Acid 60% and Chlorhexidine 0.2%. Immersion time was of three different types - 40 sec, 60 sec and 80 sec (Table 1).

Factor	Levels
Immersion medium	Distilled Water, Citric Acid 30%, Citric Acid 40%, Citric Acid 50%, Citric Acid 60%, Chlorhexidine 0.2%
Immersion Time	40 sec, 60 sec, 80 sec

Table 1: The factors and their levels are tabulated below.

Null hypotheses

- **$H_{0(a)}$:** There is no significant difference between the different types of immersion medium.
- **$H_{0(b)}$:** There is no significant difference between the different immersion times.
- **$H_{0(c)}$:** The interaction (joint effect) of immersion medium and immersion time is not significant.

Alternate hypotheses

- **$H_{1(a)}$:** There is a significant difference between the different types of immersion medium.
- **$H_{1(b)}$:** There is a significant difference between the different immersion times.
- **$H_{1(c)}$:** The interaction (joint effect) of immersion medium and immersion time is significant.

Level of significance: α = 0.05.

- **Decision Criterion:** The p-values were compared with the level of significance. If P < 0.05, the null hypothesis was rejected and accepted the alternate hypothesis. If P > 0.05, the null hypothesis was accepted.
- **Statistical technique used:** Factorial ANOVA
- **Computations:** The tables give the various computations and P-values.

Surface roughness of implant specimens

The difference in mean surface roughness (Ra) of implant specimens, recorded with different immersion media was found to be statistically significant (P < 0.001). Similarly, the difference in mean surface roughness (Ra) recorded with different immersion times was found to be statistically significant (P < 0.001). The interaction (joint effect) of immersion medium and immersion time on surface roughness(Ra) was also found to be statistically significant (P < 0.001) (Table 2,3).

Immersion medium	Immersion Time	Mean	Stddev	SE of Mean	Median	Min	Max
Distilled Water	40 sec	1.67	4.08	1.67	0.0	0	10
	60 sec	1.67	4.08	1.67	0.0	0	10
	80 sec	3.33	5.16	2.11	0.0	0	10
Citric Acid 30%	40 sec	66.67	25.82	10.54	65.0	30	110
	60 sec	75.00	13.78	5.63	80.0	50	90
	80 sec	45.00	32.71	13.35	35.0	10	90
Citric Acid 40%	40 sec	65.00	42.31	17.27	55.0	20	130
	60 sec	713.33	271.78	110.96	750.0	200	950
	80 sec	223.33	166.57	68.00	195.0	90	540
Citric Acid 50%	40 sec	121.67	98.47	40.20	95.0	50	310
	60 sec	90.00	76.42	31.20	65.0	40	240
	80 sec	1606.67	815.83	333.06	1615.0	260	2810
Citric Acid 60%	40 sec	101.67	49.56	20.23	90.0	50	160
	60 sec	93.33	63.14	25.78	90.0	20	170
	80 sec	85.00	61.56	25.13	55.0	40	200
Chlorhexidine 0.2%	40 sec	45.00	18.71	7.64	45.0	20	70
	60 sec	171.67	160.05	65.34	125.0	10	470
	80 sec	126.67	101.32	41.37	80.0	30	260

Table 2: Mean Surface Roughness (Ra)of implant specimens recorded in different immersion media at differentimmersion times: (nm).

Source	Df	Sum of Squares (SS)	Mean SS	F	P-Value
Immersion medium	5	4673229.630	934645.926	20.197	<0.001*
Immersion Time	2	1432012.963	716006.481	15.472	<0.001*
Immersion medium x Immersion Time	10	9005209.259	900520.926	19.460	<0.001*
Error	90	4164900.000	46276.667	---	---
Total	107	19275351.852	---	---	---

Table 3: Factorial ANOVA (Implant specimens). *Denotes significant difference.

In order to find out among which pair of immersion media there existed a significant difference, multiple comparisons were carried out using Bonferroni test. The results are given in Table 4: The difference in mean surface roughness was found to be statistically significant between Distilled water and Citric Acid 40% (P < 0.001), Distilled water and Citric Acid 50% (P < 0.001), Citric Acid 30% and Citric Acid 40% (P < 0.01), Citric Acid 30% and Citric Acid 50% (P < 0.001), Citric Acid 40% and Citric Acid 50% (P < 0.01), Citric Acid 40% and Citric Acid 60% (P < 0.05), Citric Acid 40% and Chlorhexidine 0.2% (P < 0.05), Citric Acid 50% and Citric Acid 60% (P < 0.001)as well as Citric Acid 50% and Chlorhexidine 0.2% (P < 0.001). No significant difference was observed between the other pair of groups (P > 0.05) (Table 4).

(I) Immersionmedium	(J) Immersionmedium	Mean Difference(I-J)	P-Value	95% CI for Mean Diff	
				Lower Bound	Upper Bound
Distilled Water	Citric Acid 30%	-60.000	1.000	-276.236	156.236
	Citric Acid 40%	-331.667	<0.001*	-547.903	-115.430
	Citric Acid 50%	-603.889	<0.001*	-820.125	-387.653
	Citric Acid 60%	-91.111	1.000	-307.347	125.125
	Chlorhexidine 0.2%	-112.222	1.000	-328.459	104.014
Citric Acid30%	Citric Acid 40%	-271.667	0.004*	-487.903	-55.430
	Citric Acid 50%	-543.889	<0.001*	-760.125	-327.653
	Citric Acid 60%	-31.111	1.000	-247.347	185.125
	Chlorhexidine 0.2%	-52.222	1.000	-268.459	164.014
Citric Acid40%	Citric Acid 50%	-272.222	0.004*	-488.459	-55.986
	Citric Acid 60%	240.556	0.017*	24.319	456.792
	Chlorhexidine 0.2%	219.444	0.044*	3.208	435.681
Citric Acid50%	Citric Acid 60%	512.778	<0.001*	296.541	729.014
	Chlorhexidine 0.2%	491.667	<0.001*	275.430	707.903
Citric Acid 60%	Chlorhexidine 0.2%	-21.111	1.000	-237.347	195.125

Table 4: Multiple comparisons of immersion medium (Implant specimens). *Denotes significant difference.

In order to find out among which pair of immersion time intervals there existed a significant difference, multiple comparisons were carried out using Bonferroni test and the results are given in table 5. The difference in mean surface roughness (Ra) was found to be statistically significant between 40 sec and 80 sec (P < 0.001) as well as between 60 sec and 80 sec (P < 0.001). No significant difference was observed between 40 sec and 60 sec (P > 0.05).

Among the immersion medium, higher mean surface roughness was recorded in Citric Acid 50%followed by Citric Acid 40%, Chlorhexidine 0.2%, Citric Acid 60%, Citric Acid 30% and Distilled Water respectively. The difference in mean surface roughness among the immersion medium was found to be statistically significant (P < 0.001).

Among the three immersion times, higher mean surface roughness was recorded at 80 sec followed by 60 sec and 40 sec respectively. The difference in mean surface roughness among thedifferent immersion times was found to be statistically significant (P < 0.001) (Figure 12).

At all the time intervals, distilled water recorded the lowest mean surface roughness compared to the other chemicals. The highest mean surface roughness was recorded in Citric Acid 50% at 80 sec. At 60 sec Citric Acid 40% and Chlorhexidine 0.2% recorded higher mean surface roughness compared to the other immersion medium respectively. At 80 sec, Citric Acid 40%, Chlorhexidine 0.2%, Citric Acid 60% and Citric Acid 30% recorded higher surface roughness respectively after Citric Acid 50%.

Figure 12: Main effects plot: (Shows the mean surface roughness recorded at different levels of each factor)-Implant specimens.

The interaction (joint effect) of immersion medium and immersion time on surface roughness (Ra) was found to be statistically significant (P < 0.001) (Figure 13).

Figure 13: Interaction plots: (Shows the mean surface roughness recorded at different levels of each factor against each level of the other factors)-Implant specimens.

Surface roughness of abutment specimens

The difference in mean surface roughness (Ra) implant abutments recorded with different immersion media was found to be statistically significant (P < 0.001). The difference in mean surface roughness (Ra) recorded with different immersion times was found to be statistically significant (P < 0.001). The interaction (joint effect) of immersion medium and immersion time on surface roughness(Ra) was also found to be statistically significant (P < 0.001) (Table 6,7).

Immersion medium	Immersion Time	Mean	Stddev	SE of Mean	Median	Min	Max
Distilled Water	40 sec	3.33	5.16	2.11	0.0	0	10
	60 sec	3.33	5.16	2.11	0.0	0	10
	80 sec	1.67	4.08	1.67	0.0	0	10
Citric Acid 30%	40 sec	43.33	28.05	11.45	45.0	10	90
	60 sec	23.33	33.27	13.58	10.0	0	90
	80 sec	40.00	12.65	5.16	35.0	30	60
Citric Acid 40%	40 sec	153.33	138.37	56.49	120.0	30	420
	60 sec	315.00	155.53	63.50	255.0	230	630
	80 sec	800.00	259.92	106.11	770.0	440	1210
Citric Acid 50%	40 sec	213.33	49.26	20.11	220.0	130	260
	60 sec	146.67	63.46	25.91	160.0	40	210
	80 sec	138.33	93.68	38.25	120.0	40	250
Citric Acid 60%	40 sec	68.33	7.53	3.07	70.0	60	80
	60 sec	131.67	90.65	37.01	130.0	10	230
	80 sec	335.00	287.11	117.21	245.0	80	800
Chlorhexidine0.2%	40 sec	213.33	269.42	109.99	115.0	20	730
	60 sec	181.67	193.23	78.88	110.0	0	540
	80 sec	268.33	77.82	31.77	290.0	150	350

Table 6: Mean Surface Roughness (Ra) of Implant abutments recorded with different immersion media atdifferent immersion times**:** (nm)**.**

In order to find out among which pair of immersion medium there existed significant difference, multiple comparisons were carried out using Bonferroni test. The results are given in table 8,9.

The difference in mean surface roughness was found to be statistically significant between Distilled water and Citric Acid 40% (P < 0.001), Distilled Water and Citric Acid 50% (P < 0.01), Distilled Water and Citric Acid 60% (P < 0.01), Distilled Water and Chlorhexidine 0.2% (P < 0.001), Citric Acid 30% and Citric Acid 40% (P < 0.001), Citric Acid 30% and Citric Acid 60% (P < 0.05), Citric Acid 30% and Chlorhexidine 0.2% (P < 0.01), Citric Acid 40% and Citric Acid 50% (P < 0.001), Citric Acid 40% and Citric Acid

Source	df	Sum of Squares(SS)	Mean SS	F	P-Value
Immersion medium	5	2027244.444	405448.889	21.790	<0.001*
Immersion Time	2	470505.556	235252.778	12.643	<0.001*
Immersion medium x Immersion Time	10	1166250.000	116625.000	6.268	<0.001*
Error	90	1674666.667	18607.407	---	---
Total	107	5338666.667	---	---	---

Table 7: Factorial ANOVA-abutment specimens. *Denotes significant difference.

(I) Immersion medium	(J) Immersionmedium	Mean Difference (I-J)	P-Value	95% CI for Mean Diff	
				LowerBound	Upper Bound
Distilled Water	Citric Acid 30%	-32.778	1.000	-169.895	104.339
	Citric Acid 40%	-420.000	<0.001*	-557.117	-282.883
	Citric Acid 50%	-163.333	0.008*	-300.450	-26.217
	Citric Acid 60%	-175.556	0.003*	-312.672	-38.439
	Chlorhexidine 0.2%	-218.333	<0.001*	-355.450	-81.217
Citric Acid 30%	Citric Acid 40%	-387.222	<0.001*	-524.339	-250.105
	Citric Acid 50%	-130.556	0.076	-267.672	6.561
	Citric Acid 60%	-142.778	0.034*	-279.895	-5.661
	Chlorhexidine 0.2%	-185.556	0.001*	-322.672	-48.439
Citric Acid 40%	Citric Acid 50%	256.667	<0.001*	119.550	393.783
	Citric Acid 60%	244.444	<0.001*	107.328	381.561
	Chlorhexidine 0.2%	201.667	<0.001*	64.550	338.783
Citric Acid 50%	Citric Acid 60%	-12.222	1.000	-149.339	124.895
	Chlorhexidine0.2%	-55.000	1.000	-192.117	82.117
Citric Acid 60%	Chlorhexidine 0.2%	-42.778	1.000	-179.895	94.339

Table 8: Multiple comparisons of immersion medium - abutment specimens.*denotes significant difference.

(I) Immersion Time	(J) Immersion Time	Mean Difference (I-J)	P-Value	95% CI for Mean Diff	
				LowerBound	UpperBound
40 sec	60 sec	-17.778	1.000	-96.214	60.658
	80 sec	-148.056	<0.001*	-226.492	-69.619
60 sec	40 sec	17.778	1.000	-60.658	96.214
	80 sec	-130.278	<0.001*	-208.714	-51.842
80 sec	40 sec	148.056	<0.001*	69.619	226.492
	60 sec	130.278	<0.001*	51.842	208.714

Table 9: Multiple comparisons of immersion time - abutment specimens. *Denotes significant difference.

60% ($P < 0.001$) as well as Citric Acid 40% and Chlorhexidine 0.2% ($P < 0.001$). No significant difference was observed between the other pair ofgroups (Table 8). In order to find out among which pair of immersion time intervals there existed a significant difference, we carried out multiple comparisons using Bonferroni test and the results are given in table 9.

The difference in mean surface roughness (Ra) was found to be statistically significant between 40 sec and 80 sec ($P < 0.001$) as well as between 60 sec and 80 sec ($P < 0.001$). No significant difference was observed between 40 sec and 60 sec ($P > 0.05$). (Table 9).

Among the immersion medium, higher mean surface roughness was recorded in Citric Acid 40%followed by Chlorhexidine 0.2%, Citric Acid 60%, Citric Acid 50%, Citric Acid 30% and Distilled Water respectively. The difference in mean surface roughness among the immersion medium was found to be statistically significant ($P < 0.001$). Among the three immersion times, higher mean surface roughness was recorded at 80 sec followed by 60 sec and 40 sec respectively. The difference in mean surface roughness among the different immersion times was found to be statistically significant ($P < 0.001$).

At all the time intervals, distilled water recorded the lowest mean surface roughness compared to the other chemicals. The next lowest mean surface roughness at all the time intervals was recorded in Citric Acid 30%. The highest mean surface roughness was recorded in Citric Acid 40% at 80 sec. At 60 sec also, Citric Acid 40% recorded the highest mean surface roughness. The next highest surface roughness at 80 sec was recorded in Citric Acid 60% followed by Chlorhexidine 0.2% and Citric Acid 50% respectively. At 60sec, the next highest mean surface roughness was recorded in Chlorhexidine 0.2% followed by Citric Acid 50% and Citric Acid 60% respectively. At 40 sec, the highest mean surface roughness was recorded in Citric Acid 50% and Chlorhexidine 0.2% followed by Citric Acid 40% and Citric Acid 60% respectively. The interaction (joint effect) of chemical and immersion time on surface roughness (Ra) was found to be statistically significant ($P < 0.001$) (Figure 14-17).

Elemental composition

Table 10 and 11 gives the elemental composition of both implant and abutment specimens with different concentrations of

Figure 14: Main effects plot: (Shows the mean surface roughness recorded at different levels of each factor).

Figure 15: Interactions plot: (Shows the mean surface roughness recorded at different levels of each factor against each level of other factors).

Figure 16: Surface of implant specimen treated with Citric acid.

Figure 17: Surface roughness of implant specimen after treating with Chlorehexidine 0.2%.

Citric acid, Chlorehexidine and different immersion times. There is considerable fluctuation in Titanium, Aluminium and Oxygen presence which can be related to the concentration of the decontaminating agents and the duration of immersion (Figure 18).

Discussion

Inflammation of the peri-implant tissue is considered as a common complication with dental implant treatment. According to epidemiologic data, peri-implant mucositis affects 80% of the subjects and 50% of the dental implants. Whereas periimplantitis affects 28 to 56% of the individuals and 12 to 43% of the implants [11]. The identifiable aetiologic factors could be microbiologic, systemic or occlusal factors or a combination of all these factors.

Elements	Control	Citric acid 30%			Citric acid 40%			Citric acid 50 %			Citric acid 60 %			Chlorhexidine 0.2%		
		40 sec	60 sec	80 sec	40 sec	60 sec	80 sec	40 sec	60 sec	80 sec	40 sec	60 sec	80 sec	40 sec	60 sec	80 sec
Ti	87.56	80.55	69.37	85.07	80.55	45.04	74.65	60.64	68.11	37.89	60.75	64.32	68.11	85.07	54.28	56.18
Al	5.86	5.12	5.7	5.33	5.12	2.48	4.69	3.88	4.75	1.97	4.69	4.36	4.75	5.33	3.28	3.31
V	0	3.32	0	0	3.32	0	4.59	4.17	2.91	2.66	0	1.48	2.91	0	0	0
C	6.58	11	9.35	9.6	11	3.01	16.08	4.22	3.69	4.88	3.45	3.53	3.69	9.60	4.24	3.73
O	0	0	15.58	0	0	39.14	0	25.31	20.54	38.04	26.39	26.31	20.54	0	32.02	28.57
Fe	0	0	0	0	0	5.37	0	0	0	8.86	3.18	0	0	0	3.57	5.11
P	0	0	0	0	0	0	0	1.78	0	4.85	1.54	0	0	0	2.60	3.11
Si	0	0	0	0	0	0	0	0	0	0.85	0	0	0	0	0	0

Table 10: Elemental analysis of implant specimens (wt. %).

Elemental titanium levels close to control were present after treatment with 30% citric acid and 0.2% chlorhexidine for 40 sec.

Elements	Control	Citric acid 30%			Citric acid 40%			Citric acid 50 %			Citric acid 60 %			Chlorhexidine0.2%		
		40 sec	60 sec	80 sec	40 sec	60 sec	80 sec	40 sec	60 sec	80 sec	30 sec	60 sec	80 sec	40 sec	60 sec	80 sec
Ti	94.58	90.62	93.85	90.90	90.07	87.22	81.06	89.12	90.12	90.42	90.56	87.62	81.59	89.01	89.46	88.42
Al	5.42	5.79	6.15	6.36	6.57	6.77	6.01	7.28	6.53	6.35	6.13	5.50	5.10	5.76	5.87	5.39
V	0	3.59	0	2.74	3.36	6.02	4.17	3.60	3.35	3.23	3.31	3.30	3.29	5.23	4.67	6.19
C	0	0	0	0	0	0	8.76	0	0	0	0	3.58	10.02	0	0	0

Table 11: Elemental analysis of abutment specimens (wt. %).

Elemental titanium levels close to control were present after treatment with 30% citric acid and 0.2% chlorhexidine for 60 sec.

Figure 18: Elemental analysis.

Peri-implant infections are initiated by microbial colonization - mostly gram negative anaerobic bacteria - on the implant surface and subsequently the bacteria proliferate and release toxins which are capable of causing massive immune response and eventually leading to degradation of the implant supporting bone [8,12]. This is analogous to the periodontal condition clinically manifested as gingival recession and bone loss eventually causing exposure of the root surface. When such a condition occurs, the routinely followed periodontal procedure is root planing followed by citric acid treatment of the denuded root surface and covering it with gingival grafts. Newman., *et al.* have recommended the use of citric acid along with gingival grafts for the coverage of denuded root surface [13]. Topical application of citric acid on the root surface accelerated healing and formed new cementum. Citric acid is used to remove tissue debris from the root surface and expose the underlying dentinal tubules and initiates the formation of new cementum over it.

Treatment of peri-implantitis too involves similar procedures. It begins with surgical debridement of devitalized peri-implant tissues followed by decontamination of the exposed implant surface. There are different methods to accomplish implant surface decontamination, like mechanical cleaning or by chemical treatment using citric acid, hydrogen peroxide, chlorhexidine gluconate or ethylene diamine tetra acetic acid (EDTA). Once the implant surface is cleaned a suitable graft material is placed over it and covered with membrane and allowed to heal. This form of treatment is widely accepted with implants because fairly good results are achieved [14]. However, the rationale behind this treatment is not explored adequately. The chemical treatment must be modifying the implant surface but the extent to which the surface characteristics are altered is not fully evaluated. That is the context in which the present study was taken up. The study aimed at determining the effect of citric acid and chlorhexidine on the titanium implant surface.

In an extensive review conducted by Patil C., *et al.* have found out that citric acid is the most effective chemotherapeutic agent to decontaminate the implant surface. However, they have stated that no singular agent is capable of producing hundred percent removal of the debris [15]. While decontaminating, citric acid can dissolve the oxide layer of the titanium if the acid is vigorously rubbed against the implant surface. This may alter the electrochemical behaviour of titanium leading to release of chemicals detrimental to the surrounding tissues.

Many authors have evaluated the surface topography of commercially pure titanium discs which were subjected to surface treatments similar to those done for decontamination of implants [16]. In contrast to that the present study used specimens prepared from actual dental implants and abutments (ADIN Dental Implant Systems Ltd, Israel). This was done with an intention to make the experiments more realistic. Abutments have been included in the experiments because abutments are also frequently exposed to chemicals like chlorhexidine because it is a commonly prescribed mouthwash. Hence it is relevant to find the changes in surface roughness and elemental composition of implants as well as abutments when they are subjected to chemical treatment.

Surface roughness

Implant surface characteristics are categorized mainly into three depending on the measurements of the surface features: 1. Macro roughness (millimeters to tens of microns) 2. Micro roughness (1-10 μm) and 3. Nano roughness (1 and 100 nm). Macro roughness contributes to mechanical stability of the implant especially in the long range. Micro roughness ensures superior bone implant contact. Nanoscale topography enhances adhesion of osteoblastic cells and the adsorption of proteins. Through this, the rate of osseointegration improves [17].

In the present study, surface roughness was measured using optical profilometer(nm). When surface roughness was analysed in the experimental groups of implant specimens, it was found that the highest mean roughness was obtained with 50% citric acid at a time interval of 80 seconds. 40% Citric acid occupies the second

higher position and it was obtained at 60 seconds. The third higher position was obtained with Chlorehexidine at 60 seconds. The least roughness was observed with 30% citric acid. (Figure 12, 13) (Table 4, 5). On the abutments, the highest mean surface roughness was observed with a concentration of 40% citric acid both at 60 and 80 seconds. Second higher position was observed with Chlorehexidine at 60 seconds. The least roughness was with 30% citric acid. (Table 6-8) (Figure 14, 15).

An increase in surface roughness always favours osseotintegration [18]. It was observed that treatment of implant as well as abutment surfaces with citric acid and chlorhexidine causes an increase in the surface roughness. Treatment with citric acid and chlorhexidine provides a favourable surface that promotes bone deposition through the enhanced roughness.

Elemental composition

Commercially pure titanium is available in four grades, which is based on the oxygen, carbon and iron contained in it. Compositional changes can make substantial differences in the physical properties of the metal. Most dental implants are made from grade 4 cpTi as it is stronger than the other grades. Abutments are made from titanium alloy (Ti6Al4V). Titanium and its alloys are resistant to corrosion because of the formation of an insoluble and continuous titanium oxide layer on the surface which begins to form within nanoseconds and reaches a thickness of 20-100 Å in 1 sec. It is very adherent to the parent titanium, protects the metal from other impurities and it is impenetrable to oxygen. TiO_2 helps in various chemical interactions which influence biological processes at the implant interface. The oxide film permits a compatible layer of biomolecules to adhere over the implant surface. The low rate of dissolution and chemical inertness of titanium dissolution products allow bone to osseointegrate with titanium [18].

Mouhyi., *et al.* [19] have done an elemental analysis with Xray Induced Photoelectron Spectroscopy and found that unused implants had a concentration of 16.6% titanium, 55.1% oxygen, 2.7% nitrogen and 25.5% carbon. X-ray induced photoelectron spectroscopy (XPS) of failed implants showed only traces of titanium (0.1%), carbon (75%), oxygen (18%) and nitrogen (4%). After cleaning with citric acid, the concentration of titanium was 2.9%, oxygen - 24.4%, carbon - 65.5% and nitrogen-7.2%. These findings indicate that cleaning with citric acid helps to remove surface decontamination to expose underlying titanium. It was concluded that decontamination of failed implant surfaces is best done with citric acid for 30 sec but compared to unused surfaces the degree of cleaning was still unsatisfactory.

In the present study surface elemental composition was found out using energy dispersive spectroscopy (EDS). When the elemental composition was analysed in the experimental groups of implants, it was found that with 30% citric acid, the elemental titanium present was 85.07%. With 50% citric acid it came down to 37.89%. The elemental titanium concentration in the control group was 87.56% (Table 10). It was also observed that with decrease in elemental titanium there was a corresponding increase in oxygen concentration.

In the experimental groups of abutments, highest elemental titanium was 93.85% which was seen with 30% citric acid and the least elemental titanium was 87.22% which was obtained with 40% citric acid. In the control group the elemental titanium was present in a concentration of 94.58% (Table 11).

Elemental titanium is important for osseointegration to occur. In this study it was observed that higher elemental titanium concentrations, close to that of control, was maintained on treatment with 30% citric acid, for the implants. But the reason for increase in oxygen concentration with decrease in elemental titanium remains unexplained and further research is needed.

Duration of exposure to chemical

Commonly followed duration of exposure of failed implants to citric acid has been 30 sec, and it has been found that a 30 sec exposure time is beneficial to treat infected implant surfaces [14,20,21]. However, Cordeiro., *et al.* have demonstrated that citric acid (10%) application for 4 minutes eliminated both *in vitro* and *in situ* biofilm formed on machined and SLA surfaces [22].In the present study the duration of exposure to citric acid and chlorhexidine was limited to 40, 60 and 80 sec. It was found that there was a significant increase in surface roughness with the increase in exposure time, for both implants and abutments (Table 2,3,5,7,9). So, it can be concluded that with increase in duration, the surface roughness of implants and abutments will increase.

Concentration of chemicals

Citric acid is used in a concentration of 30 - 40% to treat infected implant surfaces and the concentration of chlorhexidine used in mouthwashes is 0.2% [8,10,12,14,16]. But the effect of increasing concentration of citric acid on implant surfaces is not fully understood. In this study various concentrations of citric acid were used, viz, 30%, 40%, 50% and 60%.–Fifty percent concentration of citric acid showed greatest increase in surface roughness with implant specimens whereas highest increase of roughness was observed in abutment specimens with forty percent citric acid. Increase in surface roughness was observed along with the increase in exposure time of citric acid. (Figure 12,14). But with increased surface roughness a corresponding decrease in elemental titanium was also noticed (Table 10 and Table 11). Whereas on treatment with 30% citric acid, there was increase in surface roughness without greatly diminishing the elemental titanium levels. So, it can be concluded that use of 30% citric acid caused increase in surface roughness without depleting titanium levels.

The aim to decontaminate with either citric acid or chlorhexidine is to remove the surface debris, expose the underlying titanium to surrounding bone and create a roughness which will help in apposition of the bone cells. But it is a challenging task because of the complex microbiological composition and structure of the bio film, difficult access to the implant surface which has very intricate surface geometry. A roughness beyond 2000 nm can result in plaque formation and resultant peri-implantitis [17]. Although higher roughness values were obtained with 50% citric acid for 80 sec on implants (1606.67 nm) and 40% citric acid for 80 sec on abutments (800 nm), there was a greater decrease in the elemental titanium concentrations. In this experiment 30% concentration of citric acid for 40 sec and 0.2% concentration of chlorhexidine for 40 sec was found to be adequate to help in detoxification without significantly altering the implant surface especially the elemental titanium.

Conclusions

The following conclusions were drawn from the study

- Treatment with citric acid and chlorhexidine causes an increase in surface roughness of both implant and abutment specimens.
- The surface roughness of implant and abutment specimens increased with the increase in duration of immersion in citric acid and chlorhexidine.
- Increase in citric acid concentration makes the implant and abutment surface rough, but the change in surface roughness is not directly proportional to the increase in concentration.
- Increase in surface roughness of implants and abutments causes a decrease in elemental titanium. Titanium concentration is maintained when implants and abutments are treated with 30% citric acid. When treated with citric acid concentration above 30%, elemental titanium concentration decreases.
- Use of 30% citric acid for 40 sec and 0.2% chlorhexidine for 40 sec is recommended for use in surface conditioning of failing implants as it increases the surface roughness without decreasing elemental titanium levels.

Bibliography

1. Busenlechner D., *et al.* "Long-term implant success at the Academy for Oral Implantology: 8-year follow-up and risk factor analysis". *Journal of Periodontal and Implant Science* 44 (2014): 102-108.

2. Yang., *et al.* "The survival rates and risk factors of implants in the early stage: a retrospective study". *BMC Oral Health* 21 (2021): 293.

3. Chrcanovic BR., *et al.* "Analysis of risk factors for cluster behavior of dental implant failures". *Clinical Implant Dentistry and Related Research* 19 (2017): 632-642.

4. Renvert S., *et al.* "Non-surgical treatment of peri-implant mucositis and peri-implantitis: a literature review". *Journal of Clinical Periodontology* 35 (2008): 305-315.

5. Zitzmann NU and Berglundh T. "Definition and prevalence of periimplant diseases". *Journal of Clinical Periodontology* 35 (2008): 286-291.

6. Kotsovilis S., *et al.* "Therapy of periimplantitis:a systematic review". *Journal of Clinical Periodontology* 35 (2008): 621-629.

7. Vilarrasa J., *et al.* "*In vitro* evaluation of a multispecies oral biofilm over antibacterial coated titanium surfaces". *Journal of Biomedical Materials Research Part B* 29 (2018): 164.

8. Misch CE., *et al.* "Implant success, survival, and failure: the International Congress of Oral Implantologists (ICOI) Pisa Consensus Conference". *Implant Dentistry* 17 (2008): 5-15.

9. Patil C., *et al.* "Effectiveness of Different Chemotherapeutic Agents for Decontamination of InfectedDental Implant Surface: A Systematic Review". *Antibiotics* 11 (2022): 593.

10. Verdeguer P., *et al.* "Citric Acid in the Passivation of Titanium Dental Implants: Corrosion Resistance and Bactericide Behavior". *Materials* 15 (2022): 545.

11. Elena figuero., *et al.* "Management of peri-implant mucositis and peri-implantitis". *Periodontology 2000* 66 (2014): 255-273.

12. Leonhardt A., *et al.* "Microbial findings at failing implants". *Clinical Oral Implants Research* (1999).

13. Newman MG., *et al.* "Clinical Periodontology". 9th edition, Pennsylvania, Elsevier (2003): 811-936.

14. Misch C. "Contemporary Implant Dentistry". 3rd edition, St.Louis, Mosby (2008): 1077-1081.

15. Patil C., *et al.* "Effectiveness of Different Chemotherapeutic Agents for Decontamination of Infected Dental Implant Surface: A Systematic Review". *Antibiotics* 11 (2022): 593.

16. Yuki Ichioka., *et al.* "*In vitro* evaluation of chemical decontamination of titanium discs". *Scientific Reports* 11 (2021): 22753.

17. Anil S., *et al.* "Dental implant surface enhancement and osseointegration". *Implant Dentistry* (2011): 88-89.

18. Turzo K. "Surface aspects of titanium dental implants". Biotechnology - Molecular Studies and Novel Applicationsfor Improved Quality of Human Life, InTech (2012).

19. Mouhyi J., *et al.* "An XPS and SEM evaluation of six chemical and physical techniques for cleaning ofcontaminated titanium implants". *Clinical Oral Implants Research* 9 (1998): 185-194.

20. Mouhyi J., *et al.* "Re-establishment of the atomic composition and the oxide structure of contaminated titanium surfaces by means of carbon dioxide laser and hydrogen peroxide: An in vitro study". *Clinical Implant Dentistry and Related Research* 2.4 (2000): 190-202.

21. Cordeiro JM., *et al.* "Optimizing citric acid protocol to control implant-related infections: An *in vitro* and *in situ* study". *Journal of Periodontal Research* (2021): 1-11.