Comparative Evaluation of Antifungal Effect of Titanium, Zirconium and Aluminium Nanoparticles Coated Titanium Plates Against *C. albicans*

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ABSTRACT

Introduction: The topographical modifications may vary from millimeter wide grooves to nano size structures. Recently growing nano technology is rapidly advancing surface engineering in implant dentistry. This advancement has resulted in difference in surface properties including the morphology, chemistry, crystal structure and mechanical properties of the implant.

Aim: To evaluate the anticandidal effect of titanium, zirconium and aluminium nanoparticles against *C. albicans* at 24 hours, 72 hours and one week time interval.

Materials and Methods: According to ISO/TR 11175:1993, the samples were prepared with the dimension of 20mm diameter and 1mm thickness in grade IV titanium. A total of 40 samples were made and the samples were divided into four groups. The samples without coating were Group-A (control), samples coated with titanium nano particles were Group-B, samples coated with zirconium nano particles were Group-C and samples coated with aluminium nano particles were Group-D. The samples were cleaned by sonicating in acetone and subsequently in water three times for 15 min. Then they were treated with TiO_2, ZrO_2 and Al_2O_3 nanoparticles. The discs were sterilized under uv radiation and placed in SDA for *C.albicans*. The colonies were counted in 24, 72 hours and one week intervals.

Results: The values were statistically analyzed using one-way ANOVA and Tukey HSD Test. Significance p-value was < .001, which showed that significant difference in C.F.U among the groups in titanium coated samples at 24 hours, 72 hours and one week time intervals.

Conclusion: TiO_2 nanoparticles coated titanium plates showed significant anticandidal effect compared to ZrO_2 and Al_2O_3 nanoparticles at 24, 72 hours and one week time interval.

INTRODUCTION

Conventional methods of tooth replacements are slowly and steadily being replaced by newer modes of tooth replacements like implants. Thus the introduction of dental implants has changed the face of dentistry over the last 50 years [1]. Dental implants should be considered as an option for replacing missing teeth and they often provide more predictable results than conventional treatments like RPD, FPD and CD [2,3].

The use of titanium and its alloys as an implantation material: In 1969 Branemark, an orthopedic surgeon solidly embedded titanium in the bone. He called this phenomenon as "Osseointegration". He hypothesized that titanium might be capable of withstanding occlusal forces and published long term success of titanium as an artificial implantation material [4].

Inspite of the high success rate, occurrence of implant failures have also been reported. The aetiology of implant failures can be broadly classified into three categories- infection impaired healing and occlusal overloading. Peri-implantitis is the most common cause for implant failures. Peri-implantitis is referred to as an inflammatory reaction with the loss of supporting bone in the soft tissues surrounding the implants [5,6]. Numerous microbes and bacteria are involved in the pathogenesis of peri-implantitis. Thus to improve the antimicrobial potential of the implants, titanium alloys were used followed by mechanical blasting, acid etching, bioactive coating, anodized LASER modified surfaces [7].

HA nano coated dental implants could induce a chemical bond with bone and achieve biological fixation. Zirconium and aluminium nano composites are well known for their high flexural strength and biocompatibility and are classified as a bioinert material [8].
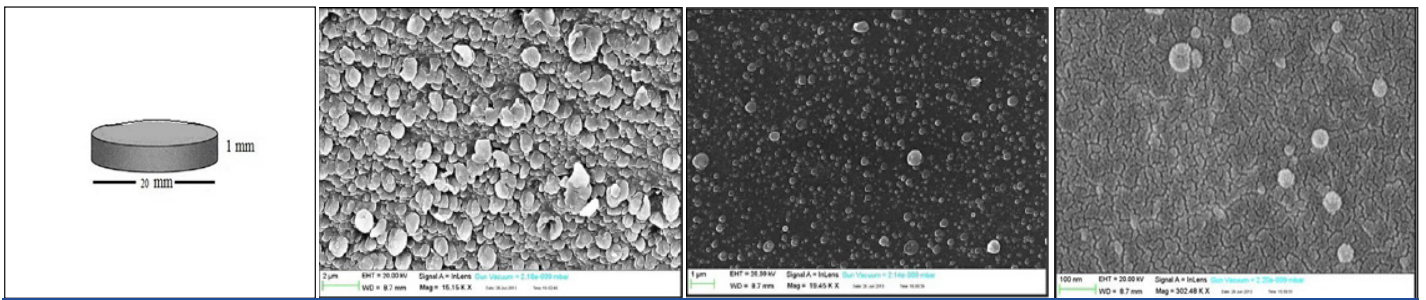
Titanium nano particles are said to be the most biocompatible material with more osteogenic potential and offers an effective avenue for the development of implant surfaces with better bone generative and regenerative potential [9]. The centers for disease control recommended 37°C for 0.5,1,6,12,48,72 and 96 hours incubation or more (until the colonies were seen on the plates) to determine the numbers of CFU/mL. Hence this study was done to evaluate the anticandidal effect of titanium, zirconium and aluminium nanoparticles against *C. albicans* at 24 at hours,72 hours and one week time interval [10].
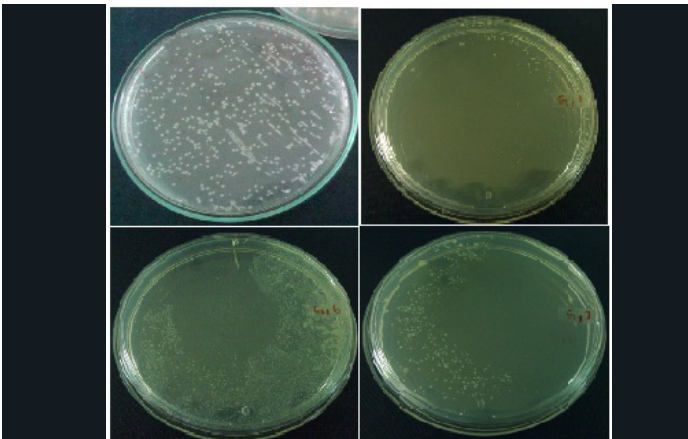
MATERIALS AND METHODS

According to ISO/TR 11175:1993, the samples were prepared with the dimension of 20mm diameter and 1mm thickness. Commercially available pure grade IV titanium (Orotec titec) was made into sheets of 1mm thickness. The master press was used to prepare the individual discs measuring 20mm in diameter and 1mm in thickness [Table/Fig-1]. A total of 40 samples were prepared by using the master press.

The samples were decontaminated by treating it in acetone and subsequently in water. The decontamination procedure was repeated 3 times for 15 minutes. The samples were then coated with titanium (TiO_2) (Alfa Aesar, Bredent), zirconium (ZrO_2) (Aldrich) and aluminium (Al_2O_3) (SRL chem) nanoparticles by a method called Pulsed laser deposition (PLD) [Table/Fig-2-4].

Initially titanium, zirconium and aluminium nanoparticles were made into pallots to act as targets. The nanoparticles were made in the form of discs about 16mm in diameter and four mm in thickness by compressing the nanoparticles in the pallotizer at 300N for five

[Table/Fig-1]: Line diagram of master die. [Table/Fig-2]: SEM of Titanium nanoparticle coating. [Table/Fig-3]: SEM of Zirconium nanoparticle coating. [Table/Fig-4]: SEM of Aluminium nanoparticle coating.

[Table/Fig-5]: Growths of *C.albicans* at 24,72 hours and 1 week interval.

Groups	Mean	Std. Dev	Min	Max
Group A	283.60	10.844	269	300
Group B	72.00	7.165	64	86
Group C	365.55	50.097	301	461
Group D	444.33	32.265	389	476
Total	289.40	142.120	64	476

[Table/Fig-6]: Mean and SD of CFU of group A,B,C& D for *C.albicans* in 24 hours.

	Sum of Squares	df	Mean Square	f-value	p-value
Between Groups	752782.473	3	250927.491	258.502	<0.001
Within Groups	34945.127	36	970.698		
Total	787727.600	39			

[Table/Fig-7]: One-way ANOVA for group A,B,C & D on *C. albicans* in 24 hours.

Pairs		Mean Difference	p-value
Control	TiO$_2$ nanoparticle	211.60	<0.001
	ZrO$_2$ nanoparticle	-81.95	<0.001
	Al$_2$O$_3$ nanoparticle	-160.73	<0.001
TiO$_2$ nanoparticle	ZrO$_2$ nanoparticle	-293.55	<0.001
	Al$_2$O$_3$ nanoparticle	-372.33	<0.001
ZrO$_2$ nanoparticle	Al$_2$O$_3$ nanoparticle	-78.79	<0.001

[Table/Fig-8]: Tukey's HSD test between groups for *C. albicans* at 24 hours.

minutes. Then the pallots were retrieved carefully from the pallotizer and sintered at a temperature of 1600°C in a box furnace (VB ceramics pvt ltd) for 8 hours and was allowed to cool down to room temperature. The sintered pallots were then retrieved from the furnace after 24 hours.

The samples without any surface coating were considered as Group A (control), samples coated with titanium nano particles were Group B, samples coated with zirconium nano particles were Group C and samples coated with aluminium nano particles were Group D. Sabouraud Dextrose Agar (SDA) was used as the culture media for *C.albicans*. SDA is a combination of 5% of enzymatic digest of

Groups	Mean	Std. Dev	Min	Max
Group A	332.70	6.865	321	341
Group B	97.20	9.390	83	111
Group C	402.36	78.139	311	560
Group D	558.00	23.611	512	589
Total	343.68	169.973	83	589

[Table/Fig-9]: Mean and SD of CFU for group A,B,C & D on *C. albicans* in 72 hours.

	Sum of Squares	df	Mean Square	f-value	p-value
Between Groups	1060008.530	3	353336.177	190.608	<0.001
Within Groups	66734.245	36	1853.729		
Total	1126742.775	39			

[Table/Fig-10]: One-way ANOVA for group A,B,C& D on *C. albicans* in 72 hours.

Pairs		Mean Difference	p-value
Control	TiO$_2$ nanoparticle	235.50	<0.001
	ZrO$_2$ nanoparticle	-69.66	0.004
	Al$_2$O$_3$ nanoparticle	-225.30	<0.001
TiO$_2$ nanoparticle	ZrO$_2$ nanoparticle	-305.16	<0.001
	Al$_2$O$_3$ nanoparticle	-460.80	<0.001
ZrO$_2$ nanoparticle	Al$_2$O$_3$ nanoparticle	-155.64	<0.001

[Table/Fig-11]: Tukey's HSD test between the Groups for *C. albicans* in 72 hours.

Casein and animal tissue, 40% of dextrose and 15% of agar. The colonies were counted under digital colony count tester (Toshiba) in 24 hours, 72 hours and 1 week interval [Table/Fig-5].

RESULTS

One way Analysis of variance (ANOVA) was used for comparison within the groups and Tukey's HSD Post Hoc test for multiple group comparison [Table/Fig-6-9]. Showed the mean colony forming units of Group A, B, C and D against *C.Albicans* in 24 hours were 283.60, 72, 365.55 and 444.33. The Significance p-value was <.001, which showed significant difference in C.F.U among the groups. The statistical analysis showed the p-value <0.001, hence it was statistically significant between group A & B and insignificant for group C &D. [Table/Fig-10-12] showed the mean colony forming units of Group A,B,C and D against *C. albicans* in 72 hours were 332.70, 97.20, 402.36 and 558. The significance p-value was <.001 that showed significant difference in C.F.U among the groups. Multiple group comparison showed the p-value is <0.001, hence it was statistically significant between group A & B and insignificant for group C&D. [Table/Fig-13-15] showed the mean colony forming units of Group A, B, C and D against *C.Albicans* at one week time interval were 359.60,93.70,388.09 and 557.11. The significance p-value was <.001 that showed significant difference in C.F.U among the groups. Multiple group comparison showed the p-value <0.001, hence it was statistically significant between group A & B and insignificant for group C &D.

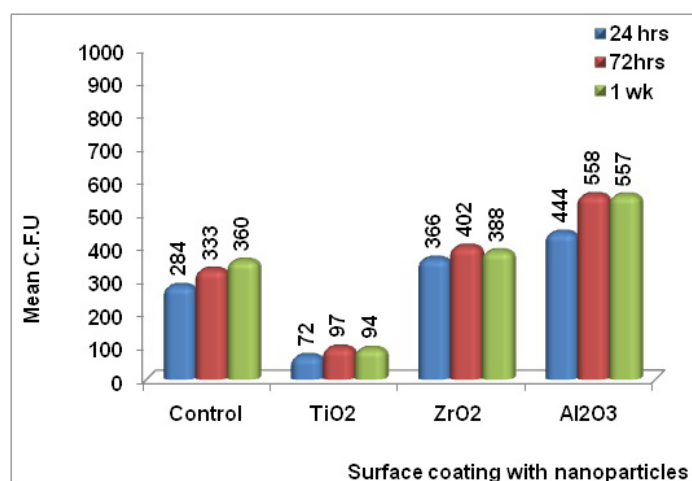| Groups	Mean	Std. Dev	Min	Max
Group A	359.60	29.029	278	374
Group B	93.70	9.286	79	104
Group C	388.09	64.074	311	560
Group D	557.11	33.569	512	618
Total	345.40	169.268	79	618

[Table/Fig-12]: Mean and SD of CFU for group A,B,C& D on *C. albicans* in 1 week.

	Sum of Squares	df	Mean Square	F-Value	P-Value
Between Groups	1058987.302	3	352995.767	217.487	<0.001
Within Groups	58430.298	36	1623.064		
Total	1117417.600	39			

[Table/Fig-13]: One-way ANOVA for group A,B,C & D on *C. albicans* in 1 week.

Pairs		Mean Difference	P-Value
Control	TiO$_2$ nanoparticle	265.90	<0.001
	ZrO$_2$ nanoparticle	-28.49	0.381
	Al$_2$O$_3$ nanoparticle	-197.51	<0.001
TiO$_2$ nanoparticle	ZrO$_2$ nanoparticle	-294.39	<0.001
	Al$_2$O$_3$ nanoparticle	-463.41	<0.001
ZrO$_2$ nanoparticle	Al$_2$O$_3$ nanoparticle	-169.02	<0.001

[Table/Fig-14]: Tukey's HSD test between the Groups for *C. albicans* in 1 week.

[Table/Fig-15]: Comparison of the anti-candidal effect of TiO$_2$, ZrO$_2$, Al$_2$O$_3$ nanoparticle coated titanium plates at 24 hours, 72 hours and 1 week time interval.

[Table/Fig-15] showed the Bar diagram of the values obtained for colony formation in Group A, B, C and D against *C. albicans* at 24 hours, 72 hours and one week time interval.

DISCUSSION

The goal of modern dentistry is to restore aesthetics to natural form, function, comfort, speech and health regardless of the atrophy, disease or injury of the stomatognathic system. As a result of continued research in materials and techniques, the predictable success is now a reality for the rehabilitation of many challenging situations. The documented high survival rate of osseointegrated root form dental implants has led to their acceptance as a realistic alternative treatment in modern dentistry [1-3].

Titanium and its alloys have been used as the gold standard material in implant dentistry because of the osseointegration phenomenon [4]. Inspite of the high success rate, implant failures have also been reported [11] [Table/Fig-16]. The reasons for implant failures are infection impaired healing and occlusal overloading. The previous studies result showed that peri-implantitis was the most common cause for implant failures [5,6]. Peri-implantitis is an inflammatory reaction with the loss of supporting bone and the soft tissues surrounding the implants [5].

Factors	Comment
Implant	Previous failure Surface roughness Surface purity and sterility Fit discrepancies Intra-oral exposure time
Mechanical	Premature loading
Overloading (Late failure)	Traumatic occlusion due to inadequate restorations
Patient (local factors)	Oral hygiene Gingivitis Bone quantity/quality Adjacent infection/inflammation (periimplantitis) Presence of natural teeth Periodontal status of natural teeth Impaction of foreign bodies (including debris from surgical procedure) in the implant pocket Soft tissue viability
Patient (systemic factors)	Vascular integrity Smoking Alcoholism Predisposition to infection, e.g. age, obesity, steroid therapy, malnutrition, metabolic disease (diabetes) Systemic illness Chemotherapy/radiotherapy Hypersensitivity to implant components
Surgical technique/ environment	Surgical trauma Overheating (use of handpiece) Perioperative bacterial contamination, e.g. via saliva, perioral skin, instruments, gloves, operating room air or air expired by patient

[Table/Fig-16]: Factors related to the failure of dental implants.

The most commonly involved microbes in peri-implantitis are *Streptococcus mutans*, *Staphylococcus aureus*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Candida albicans*, *Actinomycetum concomitans*, *prevotella intermedia* and *fusobacterium* [12,13]. Many studies are there related to bacteria. But no other studies are there related to *Candida albicans*.

In the previous studies, vector ultrasound system and carbon fiber curettes were used to treat peri-implantitis. There were no significant differences between the two techniques to treat peri-implantitis [14]. Earlier Er: YAG laser was used to treat perimplantitis but it could only stop bleeding on probing [15]. Also, the treatments with local antibiotics improved clinical parameters such as pocket depth and bleeding [16].

For the success of the implants, antimicrobial property of the implant surface is mandatory so that the initial attachment and colonization of the micro organisms can be prevented. When the initial attachment is prevented it obviously prevents the proliferation of the organisms. It had been shown in literature that coating of nanoparticles like titanium, zirconium and aluminium nanoparticles over titanium surfaces have likely to change not only the physical properties but also increased the osteogenic potential and the antimicrobial efficacy of the titanium implants [9,17].

Pulsed laser deposition is an advanced and trusted method of coating when compared to sputtering, plasma spraying, Chemical Vapour Deposition (CVD) and Powder Vapour Deposition (PVD) because a relatively thin, homogenous and uniform coating can be obtained through PLD. The particles will be evenly distributed along the entire surface area of the samples when the coating is done through PLD [18,19].

In this study, statistical analysis was done using one-way analysis of variance (ANOVA) among the groups and multiple group comparisons were done using Tukey's HSD Post Hoc test for the antimicrobial effects of the titanium, zirconium and aluminium nanoparticles against *C. albicans* in 24 hours, 72 hours & one week

time interval. The calculated p-value is < 0.001, hence the results were significant. The antifungal effect of titanium nanoparticles coated implant was significant when compared to zirconium and aluminium nanoparticles coated implant against *C. albicans* at 24 hours, 72 hours and one week time interval.

SDA is used for culturing pathogenic & commensal fungi and yeasts. The high dextrose concentration and acidic pH of the media permits selectivity of fungi. According to RP Allaker, the antimicrobial efficacy of titanium nanoparticle was due to the ion release by the particles. When the Biotrophic Interfacial Complex (BIC) was invaded by the microbes, the titanium nanoparticles started releasing positively charged titanium ions. These positively charged ions were attracted towards the microbes which carry negative charge. When the ions bind to the cell wall, they slowly started dissolving the cell wall and destroyed the cellular components of the organism [20]. So titanium nanoparticles have been proven to have a potent bactericidal as well as fungicidal potential.

LIMITATION

The limitation of the study is that the uniformity of the nanoparticle is unpredictable.

Clinical significance: The necessity of preventing periimplantitis is mandatory for success of implants. Coating the implant surface with titanium nanoparticles might be an efficient method to prevent the fungal adhesion, thereby preventing periimplantitis.

CONCLUSION

Within the limitations of the study, following conclusions were made. The antifungal effect of titanium nanoparticles coated implants against *C. albicans* was significant at 24 hours, 72 hours and one week time interval, it was significant at 24 hours and insignificant at 72 hours and one week time interval for zirconium nanoparticle coating and insignificant for aluminium nanoparticles coating at all intervals.

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