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Original Research Article

Evaluation of antimicrobial properties of conventional poly methyl methacrylate denture base resin materials containing silver doped titanium dioxide nanoparticles against cariogenic bacteria and *candida albicans*

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ABSTRACT

Aim and Background: The main aim of the study was to find the antimicrobial properties of a poly methyl methacrylate resin (PMMA) which is modified with silver-doped titanium dioxide nanoparticles. This research study was done to prove that the incorporation of silver-doped TiO2 nanoparticles improves the antimicrobial and antifungal properties.

Materials and Methods: The poly methyl methacrylate resin (PMMA) powder was modified using 0 wt%(control) 1wt% and 3wt% silver doped TiO2 nanoparticles Test specimens were prepared of dimensions 5 mm × 5 mm × 2 mm. The antimicrobial properties of the specimens were evaluated by calculating CFU/ml. The obtained values were analyzed by one-way analysis of variance (ANOVA), Kruskal Wallis test, and post-hoc Tukey's test at a significance level of 5%.

Results: The incorporation of silver-doped titanium dioxide nanoparticles into denture base resin improved antimicrobial activity. Compared to 1% and 3% nanoparticle doped, 3% has given better antimicrobial activity calculated using CFU/ml. It is reduced to one-fourth the microbial growth compared to the control group.

Conclusion: The addition of silver-doped titanium dioxide nanoparticles to denture base resin is an effective method for antimicrobial and antifungal action.

1. Introduction

Poly methyl methacrylate (PMMA) acrylic resin is most commonly used material for the fabrication of complete and partial removable dentures and intraoral maxillofacial prostheses. PMMA material has some of the favorable properties like ease of processing, accurate fit, chemical stable in the oral environment, low cost, and light weight that have made it a suitable material for denture base fabrication. Despite these desirable properties, PMMA denture base resin is susceptible to the colonization of microorganisms in the oral environment.[1]

One of the most common complications of wearing complete dentures is denture stomatitis or atrophic chronic candidiasis. With continuous use, the tissue side of the denture and the space created between the tissue surface and the mucous tissue of the patient gradually becomes prone to the growth and colonization of various microorganisms.[2]

This increased the quest for a material that is active in inhibiting these oral microflorae. Most denture cleansers which are available are not effective in reducing plaque accumulation. It may not be affordable and prohibitive in cost especially for elderly and handicapped denture wearers. Hence, there is a need to develop a single, economical, and effective method to achieve denture hygiene.[3]

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Titanium dioxide nanoparticles (TiO2-NPs) are well-known for its photocatalytic activity. It has been widely utilized as self-cleaning antimicrobial materials in a wide variety of applications including food packaging, coating of medical devices as well as environmental cleaning of wastewater, and air purification.[4]

TiO2-NPs is exposed to light with energy equal to or greater than its band gap energy, an electron gets excited and moves from its valance band to a conduction band leaving behind a positive hole. It consequently results in the formation of an electron-positive hole pair (e-/h+). The photocatalytic activity of the TiO2-NPs is derived from the ability of the positive holes to oxidize water molecules producing hydroxyl radicals (HO.) and the ability of the conduction band electrons to reduce oxygen-producing superoxide ions (O2).[5]

However, the wide range technological use of TiO2 in photocatalysis is to some extent limited by requiring UV light irradiation for photocatalytic activation. Since UV light accounts for only a small fraction (5%) of solar energy compared to visible light (~ 50%) their uses in everyday applications are limited. We have to shift the optical response from UV light to visible light for better practical application. Different methods have been followed increase the efficiency of TiO2 nano particles under visible light. Some of these works were based on TiO2 particles modification with metals such as silver,[6] zinc[7] and copper.[8] Previous studies reported that silver addition enhances the photocatalytic efficiency of titanium dioxide.[8]

Silver shows a broad spectrum of antibacterial activity and it is active against gram-negative and gram-positive bacteria. The Ag acts by three different mechanisms, it releases toxic metal ions inhibiting the production of adenosine triphosphate (ATP) and deoxyribonucleic acid replication which are required for cellular survival. The second one is the generation of ROS that generates oxidative stress and cellular death, and the third one is damage to the cell membrane due to direct contact with nanoparticles.

Silver nano particles in large quantities can be toxic. In the attempt to take advantage of both materials' properties, numerous methods have been reported in the literature.[9] Therefore, this study aimed to investigate the antimicrobial properties of poly methyl methacrylate denture base resin materials containing silver-doped titanium dioxide nanoparticles against cariogenic bacteria and *candida albicans*. The null hypothesis is that the addition of silver-doped titanium dioxide nanoparticles into denture base polymer does not affect its antimicrobial properties.

2. Materials and Methods

Materials used for synthesis of silver doped titanium dioxide nanoparticles were Titanium (IV) oxide, (anatase nano powder 99.7% Sigma-Aldrich) and Silver nitrate (AgNO3, 99.9% Sigma-Aldrich), and ethanol (99.5% pure). The above solutions were prepared using deionized water.

2.1. TiO2 nanoparticles silver addition-Wet impregnation (Ex situ)

TiO2 particles with 1% wt. silver content was prepared by the wet impregnation method. A solution of silver nitrate 1.9 mM in ethanol was prepared and 1 g of TiO2 nanoparticles (previously synthesized) was added to the solution. The resulting solution was constantly stirred for 6 h at room temperature and aged for 24 h. Finally, the solution was dried in an oven furnace at 800^0c, overnight and calcined at 450 ^{0}C for 5 h.[9]

2.2. Preparation of samples with nanoparticle

Mold spaces for specimens were prepared using wax pieces (5 mm*5 mm* 2 mm thickness) in a denture flask. Then 0.1 g and 0.3g of nanoparticles were weighed and mixed with 8.55 g of polymer (DPI Heat Cure, India) to this 1.7 ml of methyl methacrylate monomer was added. It was packed in the mold space obtained after dewaxing. After curing, the samples were trimmed, sandpapered, and polished.[3]

2.3. Microbial analysis

Ninety rectangular sample PMMA with silver doped TiO2 nanoparticle concentrations of 0 wt.% (control), 1 wt.% (minimum), and 3 wt. % (maximum) were used for the microbial adhesion analysis.

2.4. Antibacterial test

Group A- 15 PMMA resin samples with silver doped TiO2 nanoparticle concentrations of 0 wt. % (control).

Group B- 15 PMMA resin samples silver doped TiO2 nanoparticle concentrations of 1 wt. % (minimum).

Group C- 15 PMMA resin samples with silver doped TiO2 nanoparticle concentrations of 3 wt. % (maximum).

2.5. Antifungal test

Group 1- 15 PMMA resin samples with silver doped TiO2 nanoparticle concentrations of 0 wt.% (control).

Group 2- 15 PMMA resin samples with silver doped TiO2 nanoparticle concentrations of 1 wt.% (minimum).

Group 3- 15 PMMA resin samples with silver doped TiO2 nanoparticle concentrations of 3 wt.% (maximum).

2.6. Microbial strains and growth conditions

Candida albicans MTCC 227 and Streptococcus mutans MTCC 890 cultures were stored and preserved at Dextrose technologies Pvt. Ltd laboratory, Bangalore. S. mutans was grown in Brain-Heart Infusion (BHI) broth at 37 °C. *Candida albicans* strain was cultured on the Sabouraud dextrose broth (SDB).

Table 1: a: Showing morphometic data of all specimen of stapes classified according to sex (Matrix value given in mm)

S.No.	Organism	Nanoparticle concentration	OD@660 nm	Plate count	CFU/ml
1	Streptococcus mutans	Control- 0%	0.476	236	2.36 x 103
2			0.482	292	2.92 x 103
3			0.467	288	2.88 x 103
4			0.483	300	3 x 103
5			0.465	252	2.52 x 103
6			0.474	272	2.72 x 103
7			0.424	224	2.24 x 103
8			0.486	228	2.28 x 103
9			0.465	299	2.99 x 103
10			0.476	308	3.08 x 103
11			0.487	248	2.48 x 103
12			0.476	224	2.24 x 103
13			0.472	356	3.56 x 103
14			0.486	268	2.68 x 103
15			0.459	260	2.60 x 103
S.No.	**Organism**	**Nanoparticle concentration**	**OD@660 nm**	**Plate count**	**CFU/ml**
1	Streptococcus mutans	1%	0.285	156	1.56 x 103
2			0.265	180	1.8 x 103
3			0.276	173	1.73 x 103
4			0.249	174	1.74 x 103
5			0.268	130	1.3 x 103
6			0.269	123	1.23 x 103
7			0.278	164	1.64 x 103
8			0.298	178	1.78 x 103
9			0.283	195	1.95 x 103
10			0.268	133	1.33 x 103
11			0.276	149	1.49 x 103
12			0.284	163	1.63 x 103
13			0.276	179	1.79 x 103
14			0.287	157	1.57 x 103
15			0.267	141	1.41 x 103
S.No.	**Organism**	**Nanoparticle concentration**	**OD@660 nm**	**Plate count**	**CFU/ml**
1	Streptococcus mutans	3%	0.285	110	1.1x 103
2			0.175	98	0.98x 103
3			0.285	115	1.15x 103
4			0.254	120	1.20x 103
5			0.156	75	0.75x 103
6			0.242	89	0.89x 103
7			0.175	85	0.85x 103
8			0.178	88	0.88x 103
9			0.159	79	0.79x 103
10			0.178	88	0.88x 103
11			0.165	98	0.98x 103
12			0.259	102	1.02x 103
13			0.248	100	1x 103
14			0.145	99	0.99x 103
15			0.273	104	1.04x 103

Continued on next page

Table 1 continued

S.No.	Organism	Nanoparticle concentration	OD@660 nm	Plate count	CFU/ml
1			0.498	289	2.89x 103
2			0.479	256	2.56 x 103
3			0.483	273	2.73 x 103
4			0.474	253	2.53 x 103
5			0.462	254	2.54 x 103
6			0.499	290	2.9 x 103
7			0.676	302	3.02 x 103
8	*Candida albicans*	Control- 0%	0.487	280	2.8 x 103
9			0.486	265	2.65 x 103
10			0.487	278	2.78 x 103
11			0.474	297	2.97 x 103
12			0.467	288	2.88 x 103
13			0.489	240	2.4 x 103
14			0.479	233	2.33 x 103
15			0.498	257	2.57 x 103
S.No.	**Organism**	**Nanoparticle concentration**	**OD@660 nm**	**Plate count**	**CFU/ml**
1			0.286	178	1.78 x 103
2			0.275	167	1.67 x 103
3			0.263	153	1.53 x 103
4			0.268	198	1.98 x 103
5			0.267	156	1.56 x 103
6			0.267	200	2 x 103
7			0.258	186	1.86 x 103
8	*Candida albicans*	1%	0.273	174	1.74 x 103
9			0.264	169	1.69 x 103
10			0.254	158	1.58 x 103
11			0.267	199	1.99 x 103
12			0.264	136	1.36 x 103
13			0289	139	1.39 x 103
14			0.272	140	1.40 x 103
15			0.275	144	1.44 x 103
S.No.	**Organism**	**Nanoparticle concentration**	**OD@660 nm**	**Plate count**	**CFU/ml**
1			0.265	110	1.1 x 103
2			0.271	102	1.02 x 103
3			0.163	98	0.98 x 103
4			0.175	79	0.79 x 103
5			0.164	88	0.88 x 103
6			0.101	100	1 x 103
7			0.278	121	1.21 x 103
8	*Candida albicans*	3%	0.265	85	0.85 x 103
9			0.176	94	0.94 x 103
10			0.169	79	0.79 x 103
11			0.189	96	9.6 x 103
12			0.103	102	1.02 x 103
13			0.160	88	0.88 x 103
14			0.165	93	0.93 x 103
15			0.159	75	0.75 x 103

2.7. Methodology-microbiological parameter

After incubation, test specimens were washed with sterile water and immersed in sterile water containing plate. Plates containing test specimens were exposed to visible light for 2 hours both above and below the discs using 60-Watt Philips incandescent bulb from the distance of 15 cm. At the end of the light exposure, test specimen were placed in media and cell adhered to test specimens were detached by shaking at 220 rpm for 15 min. Then test specimens were inoculated in respective media and incubated at respective growth conditions. After incubation CFU/ml were determined.

2.8. Bacterial enumeration

Streptococcus mutans and *Candida albicans* were spread on BHIA and SDA respectively.Figures 1 and 2

2.9. Results of streptococcus mutans

Table 1 gives the mean, standard deviation and 95% confidence interval for mean of Streptococcus mutans.

It is seen in the above table the mean values are decreasing as the concentration is increased.

This research study was to prove that increasing the concentration of silver doped TiO_2 nanoparticlecan improve the antimicrobial activity i.e. 3% silver doped nanoparticle gives better antimicrobial activity compared to 1%.

The statistical tool One way ANOVA was used at 5% level of significance to test this research hypothesis and the results of the same along with Tukey's Post hoc test was tabulated Table 2.

Since all the P values are less than 0.05 it is evident that there is significant difference in the antimicrobial activity due to different concentration level. Also, the post hoc test results show that the difference between 0% and 1% concentration level as well as difference between 1% and 3% are also significantly different for the two parameters OD@660 nm and plate count.Table 3

2.10. Results of candida albicans

It is seen in the above table also that the mean values are decreasing as the concentration is increased. Table 4

The null hypothesis stating is there is no significant difference in the antimicrobial activity due to different concentration level is tested at 5% level against the research hypothesis that Increasing the concentration of nanoparticle doped TiO_2 can improve the antimicrobial activity using the statistical tool one way Analysis of variance. Tukey's post hoc test for intergroup comparison was also used.

2.11. Results of ANOVA for candida albicans

Since all the P values are less than 0.05 it is evident that there is significant difference in the antimicrobial activity due to different concentration level. Also, the post hoc test results show that the difference between 0% and 1% concentration level as well as difference between 1% and 3% are also significantly different for the two parameters OD@660 nm and plate count.Table 5

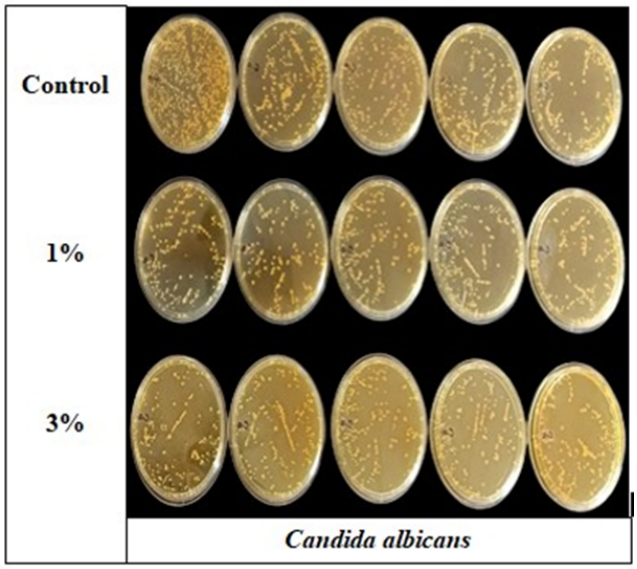

Figure 1: *Candida albicans* was spread on SDA respectively.

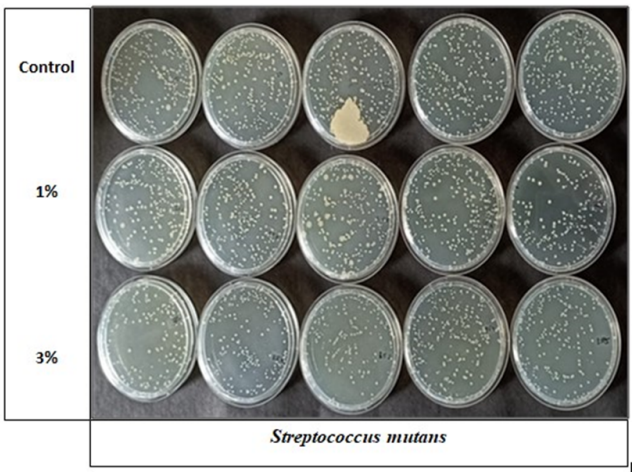

Figure 2: Streptococcus mutans was spread on BHIA.

3. Results and Discussion

Poly methyl methacrylate denture base resins are prone to microbial adhesion, resulting in stomatitis, which influences the palatal mucosa and is commonly recognized as a contagious disease among denture users. Usually, oral hygiene, as well as denture cleansing, are employed to avoid stomatitis, but for hospitalized and geriatric patients, denture cleansing might be compromised as a result of reduced motor dexterity, cognitive impairment, and memory

Table 2:

	Nanoparticle concentration	N	Mean	Std. Deviation	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
OD@660 nm	0%	15	.4719	.0158	.4631	.4806
	1%	15	.2753	.0117	.2688	.2817
	3%	15	.2118	.0523	.1829	.2407
	0%	15	270.33	37.28	249.69	290.98
Plate count	1%	15	159.67	21.05	148.01	171.32
	3%	15	96.67	12.78	89.59	103.75

Table 3: Results of ANOVA for Streptococcus mutans

Group	0%		1%		3%		F Value	P value	Intergroup comparison (Tukey's Posthoc test)
	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev			
OD@660 nm	.4719	.0158	.2753	.0117	.2118	.05226	265.488	0.000**	0% vs 1% (p=0.000**) 1% vs 3 %(p=0.000**) 0% vs 3% (p=0.000**)
Plate count	270.33	37.28	159.6667	21.05	96.67	12.78	174.25	0.000**	0% vs 1% (p=0.000**) 1% vs 3 %(p=0.000**) 0% vs 3% (p=0.000**)

Table 4:

	Nanoparticle concentration	N	Mean	Std. Deviation	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
OD@660 nm	0%	15	.4959	.0510	.4676	.5241
	1%	15	.2695	.0094	.2643	.2747
	3%	15	.1869	.0571	.1552	.2185
	0%	15	270.33	21.07	258.67	282.00
Plate count	1%	15	166.47	22.37	154.08	178.85
	3%	15	94.00	12.35	87.16	100.84

Table 5:

Group	0%		1%		3%		F Value	P value	Intergroup comparison
	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev			
OD@660 nm	.4959	.0510	.2695	.0094	.1869	.0571	193.519	0.000**	0% vs 1% (p=0.000**) 1% vs 3 %(p=0.000**) 0% vs 3% (p=0.000**)
Plate count	270.33	21.07	166.47	22.37	94.00	12.35	322.345	0.000**	0% vs 1% (p=0.000**) 1% vs 3 %(p=0.000**) 0% vs 3% (p=0.000**)

loss. Previous studies have shown that mechanically employed cleaning methods are inadequate in preventing microorganism adherence on denture bases.

Different attempts have been made to overcome this drawback of denture base resins. The incorporation of biocide additives like silver zeolites, silver nanoparticles (AgNPs) and titania nanoparticles into the polymer matrix is an approach to developing a denture base acrylic resin with antimicrobial potential. [1]

In this study silver doped titanium dioxide nanoparticle was incorporated into denture base acrylic resin to improve its antibacterial properties. Titanium dioxide (TiO_2) is a semiconductor material that exhibits antibacterial activity due to its photocatalytic properties under ultraviolet light. On the other hand, silver also exhibits strong antibacterial activity towards a wide range of microorganisms and TiO_2 with silver addition exhibits more efficient photocatalytic properties than unmodified TiO_2. The influence of the modification method of TiO_2 and incorporation in denture base resin on its bactericidal properties has not been studied. Accordingly, the aim of this work was to evaluate the effect of silver-doped TiO_2 nanoparticles on cariogenic bacteria and candida albican at different concentrations.

For synthesis of TiO2 nanotubes, we employed the anatase phase of TiO2 nanoparticles. The crystalline

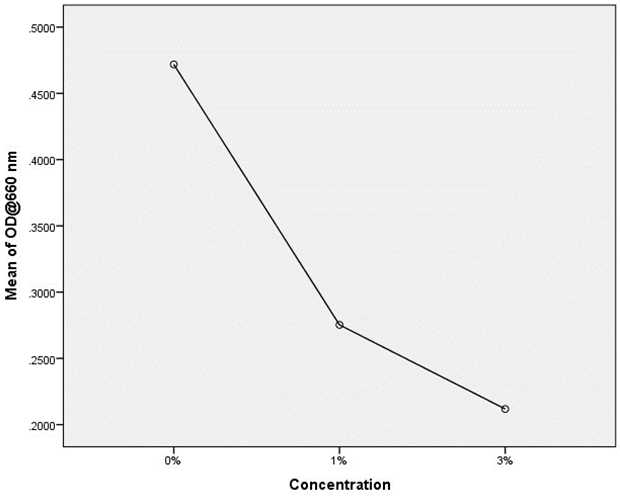

Figure 3: Mean plot for OD@660nm of Streptococcus mutans

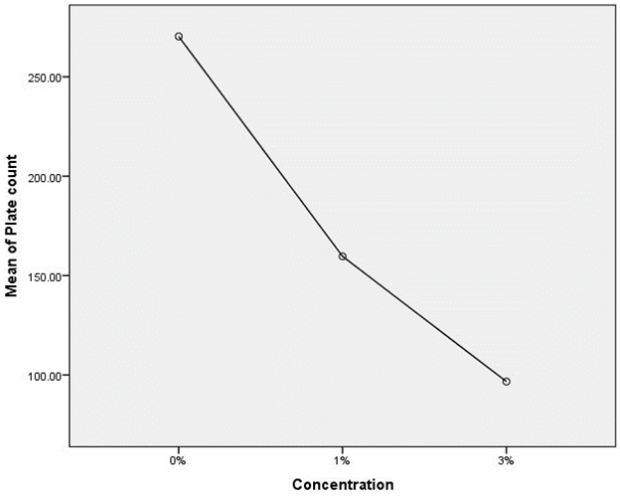

Figure 4: Mean plot for plate count of Streptococcus mutans

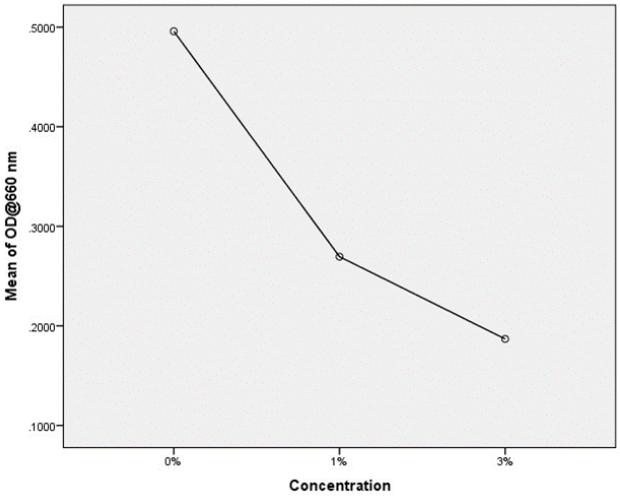

Figure 5: Mean plot for OD@660nm of *Candida albicans*

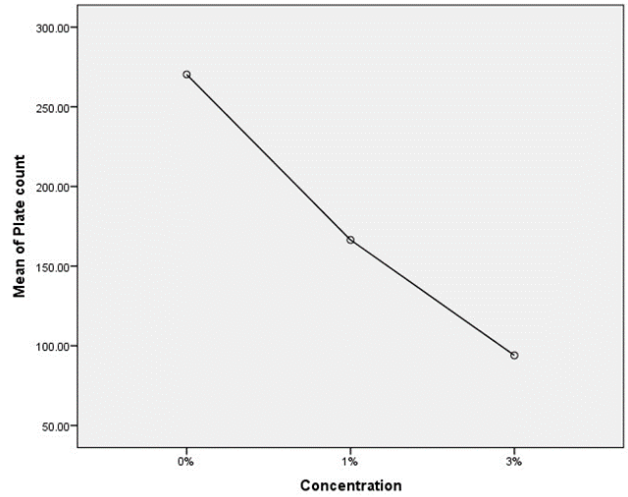

Figure 6: Mean plot for Plate count of *Candida albicans*

structure of the material has an important role in its antimicrobial properties. In this regard, different studies determined improved antimicrobial properties for the anatase and rutile crystalline phase of titania.[10] Li et al. determined the highest antibacterial activity for the anatase nanotubes among three crystalline phases of titania including anatase, rutile, and amorphous.[11] The significant difference between the (Ag-doped TiO2-VL) and the control group groups may be explained by the silver's ability to extend the absorption spectrum of the TiO2 into the visible light range. This explanation is confirmed by the results of the UV-VL spectroscopy which showed that the Ag-doped TiO2-NPs had a wider absorption peak that extended more into the VL spectrum compared to the TiO_2-NPs. This effect of silver doping is believed to be attained by narrowing the band gap of TiO_2. The band gap is defined as the energy difference between the valence band (from which the electron escapes upon photo-excitation) and the conduction band (that receives the excited electron).[12] Narrowing the band gap of TiO_2, by the action of silver, means that lower amount of energy (e.g., visible light energy) would be sufficient for exciting the electrons and for triggering the photocatalytic reaction with its subsequent antibacterial and antifungal effect. This research study proved that incorporation of silver doped TiO_2 nanoparticlecan improve the antimicrobial activity i.e., 3% silver doped nanoparticle gives better antimicrobial activity compared to 1%. These mechanisms ultimately prevent plaque formation on denture surfaces. Silver doped TiO2 when used with PMMA would contribute to have better denture hygiene by using cheaply available solar energy/light. However, there was slight change in the color of the acrylic samples which can be considered as limitation of the study which is nullified by health benefits.[13–15]

4. Conclusion

Based on our results, it can be concluded that addition of silver doped titanium dioxide nanoparticles can greatly improve its antimicrobial properties. Hence this can be considered as a novel method for fabrication of acrylic resin base dental materials with inbuilt antimicrobial action. The results obtained gives definitive scope of study by in vivo methods and also to check the inhibitory activity of silver doped TiO2 nanoparticles against other oral colonizers. However, further studies have to be done to evaluate the mechanical properties, consistent presence of silver doped TiO2 nanoparticles over a period of time when used by patients.

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6. Conflict of Interest

None.

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