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Research Article

A Study on the Effect of Photocatalyst Incorporated Tissue Conditioners in Controlling the Growth of Oral Microorganisms Viz. *Streptococcus mutans, Staphylococcus aureus* and *Candida albicans*

Kiran John[1], K Chandrasekharan Nair[2]*, Vahini Reddy[3] and Chiranjeevi Reddy[4]

[1]*Consultant Prosthodontist, Clove Dental, Bengaluru, India*

[2]*Professor Emeritus, Department of Prosthodontics, Sri Sankara Dental College, Akathumuri, Thiruvananthapuram, Kerala, India*

[3]*Former Professor of Prosthodontics, AECS Maaruti College of Dental Sciences, Bangalore, India*

[4]*Professor of Prosthodontics, AECS Maaruti College of Dental Sciences, Bangalore, India*

***Corresponding Author:** K Chandrasekharan Nair, Professor Emeritus, Department of Prosthodontics, Sri Sankara Dental College, Akathumuri, Thiruvananthapuram, Kerala, India.

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Abstract

Objectives: To find out the antimicrobial and antifungal effects of tissue conditioners containing a photocatalyst (titanium dioxide).

Materials and Methods: TiO2 (20 wt%) was added to the tissue conditioner powder and added to the liquid in the desired concentration for the preparation of the tissue conditioner specimens. These specimens were divided into four groups.

Gr.1 – Tissue conditioner only (control), Gr.2 – Tissue conditioner + Photocatalyst + UV irradiation,

Gr.3 – Tissue conditioner + Photocatalyst, Gr.4 – Tissue conditioner + UV irradiation

Three strains of microorganisms were used to test the growth against the four groups of tissue conditioner specimens. The microorganisms used were: 1. Streptococcus mutans, 2. Staphylococcus aureus and 3. Candida albicans. After inoculation and incubation, the colony forming units were counted to evaluate the antimicrobial activity. Data was statistically analysed with the Kruskal Wallis test.

Results: Higher mean growth of Streptococcus Mutans was recorded in Group-3 followed by Group-1. Group-4 and Group-2 showed lowest growth. Higher mean growth of Staphylococcus Aureus was recorded in Group-1 followed by Group-3. Group-4 and Group-2 showed lower growth of the microorganisms. Higher mean growth of Candida Albicans was recorded in Group-1 followed by Group-3, Group-4 and Group-2 respectively. The difference in mean growth of Candida Albicans among the groups was found to be statistically significant.

Conclusions: Tissue conditioners do not possess antimicrobial potential. Tissue conditioners that incorporate Titanium dioxide and when subjected to UV radiation gains very potent antimicrobial capacity. Tissue conditioner which was subjected to ultra violet radiation also gains good antimicrobial activity. Titanium dioxide did not possess antimicrobial potential.

Keywords: Tissue Conditioner; Resilient Liner; Photocatalyst; Titanium Dioxide; UV Irradiation

Introduction

Tissue conditioners are useful adjuncts to complete dentures and obturators in improving mucosal conditions and retention. These materials degenerate with time and are susceptible to colonization by microorganisms due to changes in the surface roughness. Proliferation of microorganisms is quite common with resilient liners especially the acrylic-based ones. Microorganisms present in elderly persons have pathologic potential and frequently cause denture stomatitis. Many methods have been tried to prevent the colonization by microorganisms such as incorporation of therapeutic agents like nystatin, cotrimoxazole, fluconazole etc. These drugs are therapeutically effective for a controlled period of time and hence require frequent replacement. Most drugs have an undesirable taste also. Titanium dioxide (TiO2) a photocatalyst is a recent entry to this field. TiO2 is a stable nontoxic photocatalyst and upon UV irradiation, it acquires potent antimicrobial activity due to the release of hydroxyl radicals (OH-). These radicals oxidize organic substances into water and carbon dioxide. Outer membrane of the microorganisms gets damaged due to the release of the radicals. This makes disinfecting the prosthesis against a number of organisms very easy. The beneficiaries are elderly persons who have diminished immunity and also for institutionalised patients. The advantage that TiO2 has over the conventional methods employed in disinfection is that in photocatalytic cleaning, no mechanical force or drugs are used and it is cost effective also. This material has a broad antimicrobial spectrum against gram positive and gram-negative bacteria, fungi and viruses. Extensive studies however, have not been carried out to evaluate the photocatalytic effect against the microorganisms commonly present in the oral cavity. This study was undertaken to evaluate the antimicrobial activity of UV irradiated Titanium dioxide photocatalyst, incorporated in tissue conditioners, against Streptococcus mutans, Staphylococcus aureus and Candida albicans which are mainly responsible for causing denture stomatitis [1-7].

Methodology

Preparation of the specimens

Tissue conditioner (G C Soft liner, Tokyo, Japan) specimens measuring 16x16x16mm with a well having 5mm diameter and 8mm depth were made (Figure 1). A metal die with similar measurements was made and duplicating it, wax patterns were prepared. By investing the wax pattern in dental stone, two-piece moulds were prepared. Tissue conditioner was packed into the mould to prepare the specimens (Figure 2-5).

Figure 1: GC Soft liner

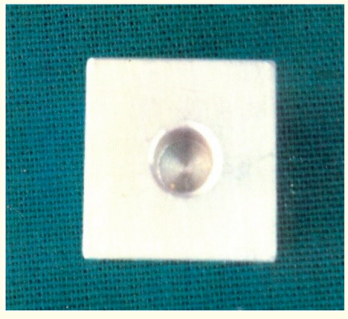

Figure 2: Metal die.

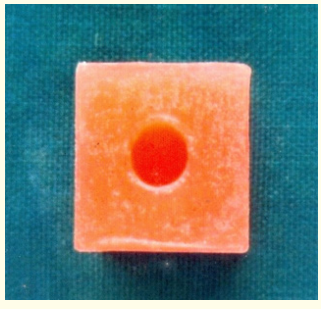

Figure 3: Wax pattern made out of metal die.

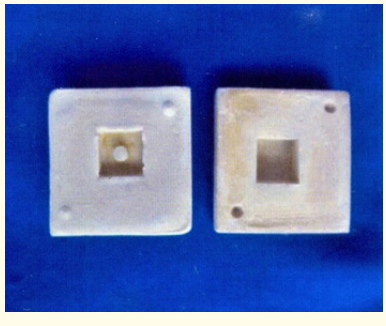

Figure 4: Dental stone mould prepared by investing the wax pattern.

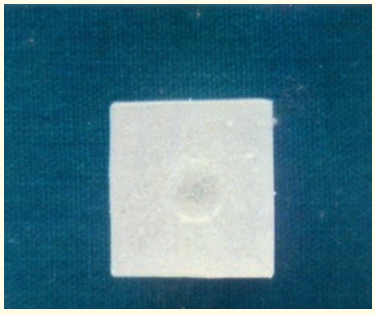

Figure 5: Specimen made in tissue conditioner.

Titanium Dioxide Photocatalyst (anatase type) was obtained from M/s. Thomas Baker Chemical, Mumbai, Maharashtra (Figure 6). Three strains of microorganisms which are commonly found in human oral cavity viz. 1. Streptococcus mutans (UA159), 2. Staphylococcus aureus (FDA209P), 3. Candida albicans (ATCC10231) were procured from Nawal Analytical Laboratories, Hosur, Karnataka.

Figure 6: Titanium dioxide weighed in digital balance.

Tissue conditioner specimens (control group, Group - 1)

Powder and liquid components of the tissue conditioner were mixed according to the manufacturer's recommendation i.e., 2.2g of powder was mixed with 1.8 ml of liquid (Figure 5). This was then poured into the prepared stone moulds to form the specimens which were allowed to set under humid conditions. This served as the control group. 300 µl (micro litre) suspensions of Streptococcus mutans, Staphylococcus aureus and Candida albicans were obtained from their respective growth media and was inoculated into the wells of the specimens (Figure 9). Each specimen received only one strain of the microorganism. The specimens inoculated with Streptococcus mutans and Staphylococcus aureus were incubated for forty eight hours and the specimens with Candida albicans were incubated for seventy two hours.

Figure 7: UV radiation chamber.

Figure 8: Specimens irradiated in UV chamber.

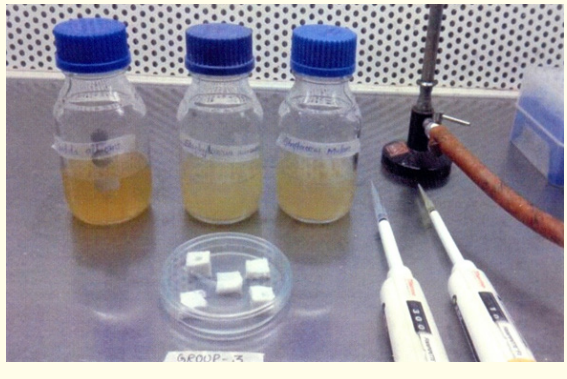

Figure 9: Microorganisms innoculated into the specimens.

Tissue conditioner specimens with photocatalyst and subjected to UV radiation (Group - 2)

1.92 g of photocatalyst powder (Figure 8) was incorporated in 7.68 g of tissue conditioner powder (photocatalyst weight adjusted to 20 wt%) (Figure 10). This was mixed using a mortar and pestle, and the mixture was added to 8 ml of tissue conditioner liquid. This was then poured into the prepared moulds to form the specimens which were allowed to set under humid conditions. Streptococcus mutans, staphylococcus aureus and candida albicans were added onto the specimens and incubated similar to those of control group. This was then subjected to UV radiation for four hours.

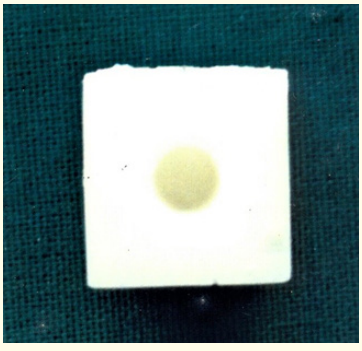

Figure 10: Specimen with photocatalyst.

Tissue conditioner specimens with photocatalyst (Group - 3)

Specimens in this group were prepared similar to those of group - 2. Streptococcus mutans, Staphylococcus aureus and Candida albicans were added onto these specimens and incubated as done with the control group.

Tissue conditioner specimens subjected to UV radiation (Group - 4)

Group - 4 specimens were also prepared similar to those of Group - 1 specimens. After incubation these specimens were subjected to UV radiation.

Group 2 and Group 4 specimens were irradiated with a UV lamp of 16 watts with a wavelength of 352 nm for four hours. The irradiation was done from a distance of 20 cm for Streptococcus mutans and Staphylococcus aureus. For Candida albicans it was done at a distance of 10 cm. UV chamber had a dimension of 450 x 300 x 250mm (Figure 7-8).

For the counting of the colony forming units, first 0.01ml of the microbial suspension was pipetted out from the specimens and incubated on specific growth agar media viz. Brain heart infusion agar for Streptococcus mutans and Tryptone yeast agar for Staphylococcus aureus and then incubated for 48 hours. For Candida, the incubation period was 72 hours on Sabourads agar. After the incubation period, the colony forming units were counted using a Colony Counting Pen (Cole Parmer, India) (Figure 11).

Figure 11: Colony counter pen.

Statistical analysis

The results obtained were subjected to one-way ANOVA (Figure 12).

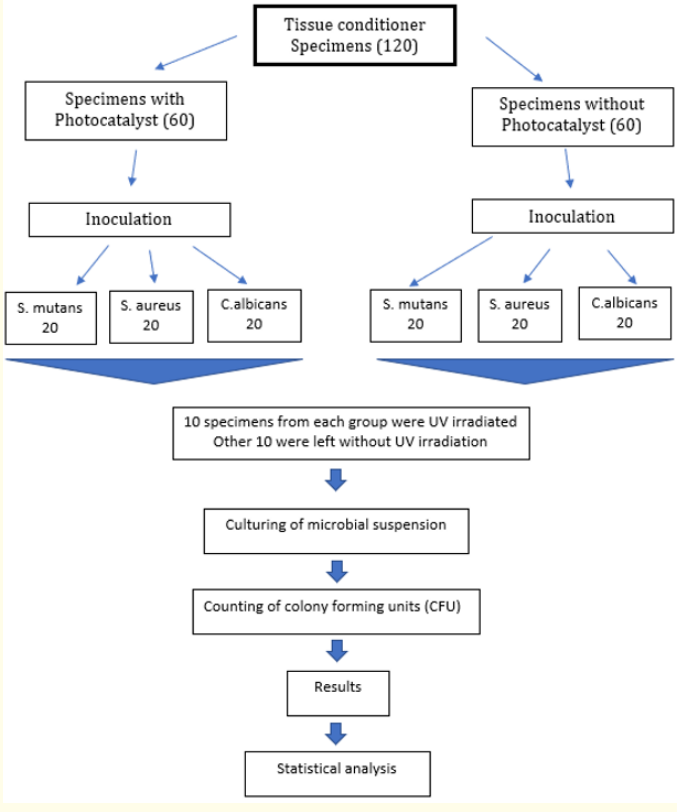

Figure 11: Flow Chart on Methodology.

Results

- **Null Hypothesis:** There is no significant difference in the mean organism growth (CFU/ml) between the groups 1,2,3 and 4
- **Alternate Hypothesis:** There is a significant difference in the mean organism growth (CFU/ml) between the groups 1,2,3 and 4
- **Level of Significance:** $\alpha = 0.05$
- **Statistical test used:** Kruskal-Wallis test
- **Decision Criterion:** *p*-Values were compared to calculate the level of significance. If *p* value was < 0.05, the null hypothesis was rejected and the alternate hypothesis was accepted. If *p* value was ≥0.05, the null hypothesis was accepted. If there is a significant difference, we carried out multiple comparisons using Mann-Whitney test.

Four different types of tissue conditioner specimens were prepared and they were grouped as follows:

Group 1: Tissue conditioner only (control)

Group 2: Tissue conditioner + Photocatalyst + UV irradiation

Group 3: Tissue conditioner + Photocatalyst

Group 4: Tissue conditioner + UV irradiation

Three strains of microorganisms were used to test the growth against the four groups of tissue conditioner specimens. The microorganisms used were:

- *Streptococcus mutans*,
- *Staphylococcus aureus* and
- *Candida albicans*

Higher mean growth of Streptococcus Mutans was recorded in Group-3 followed by Group-1. Group-4 and Group-2 showed lowest growth (Table 1). The difference in mean growth of Streptococcus Mutans among the groups was found to be statistically significant (P < 0.001).

The difference in mean growth of Streptococcus mutans (Table 2) was found to be statistically significant between Group-1 and Group -2 (P < 0 .001), Group-1 and Group-4 (P < 0.001), Group-2 and Group-3 (P < 0.001), Group-2 and Group-4 (P < 0.01) as well as between Group-3 and Group-4 (P < 0.001). No statistically significant difference was observed between Group-1 and Group-3 (P > 0.05).

Group	Mean	Std Dev	SE of Mean	Min	Max	Kruskal-Wallis Chi Sq	p Value
Tissue Conditioner (Gr.1)	1304.70	82.66	26.14	1215	1415		
Tissue Conditioner + Photocatalyst	22.90	2.73	0.86	18	26		
+ UV Irradiation (Gr.2)						32.408	< 0.001 *
Tissue Conditioner+ Photocatalyst (Gr. 3)	1317.10	98.18	31.05	1200	1508		
Tissue Conditioner + UV	27.20	1.93	0.61	26	32		
Irradiation (Gr.4)							

Table 1: Comparison of the growth (CFU/ml) of *Streptococcus Mutans.*

*Denotes significant difference.

(I) Group	(J) Group	Mean Difference (1-J)	Z	p-Value
	Tissue Conditioner + Photocatalyst +	128l.800	-3.785	< 0.001 *
	UV Irradiation			
Tissue Conditioner	Tissue Conditioner + Photocatalyst	-12.400	-0.265	0.791
	Tissue Conditioner + UV	1277.500	-3.838	< 0.001*
	Irradiation			
Tissue	Tissue Conditioner + Photocatalyst	-1294.200	-3.785	< 0.001 *
Conditioner + Photocatalyst	Tissue Conditioner + UV	-4.300	-3.445	0.001 *
+ UV Irradiation	Irradiation			
Tissue	Tissue Conditioner + UV	1289.900	-3.838	< 0.001 *
Conditioner+Photocata1yst	Irradiation			

Table 2: In order to find out among which pair of groups there existed a significant difference with respect to the mean growth of *Streptococcus Mutans*, we carried out multiple comparisons using Mann- Whitney test and the results are given below.

*Denotes significant difference.

Higher mean growth of Staphylococcus Aureus was recorded in Group-1 followed by Group-3. Group-4 and Group-2 showed lower growth of microorganisms. The difference in mean growth of Staphylococcus aureus among the groups was found to be statistically significant (P < 0.001). (Table 3).

The difference in mean growth of Staphylococcus Aureus was found to be statistically significant between Group-1 and Group-2, Group-1 and Group-4, Group-2 and Group-3, Group-2 and Group-4 as well as Group-3 and Group-4 (P < 0.001). No statistically significant difference was observed between Group-1 and Group-3 (P > 0.05). (Table 4)

Group	Mean	Std Dev	SE of Mean	Min	Max	Kruskal-Wallis Chi-Sq	p-Value
Tissue Conditioner	1505.70	76.63	24.23	1400	1620		
Tissue Conditioner + Photocatalyst	23.90	3.00	0.95	20	28		
+ UV Irradiation						31.373	<0.001*
Tissue Conditioner + Photocatalyst	1500.20	75.49	23.87	1405	1615		
Tissue Conditioner + UV	30.20	4.57	1.44	22	36		
Irradiation							

Table 3: Comparison of the growth (CFU/ml) *of Staphylococcus Aureus.*

*Denotes significant difference.

(I) Group	(J) Group	Mean Difference - (I -J)	Z	p-Value
	Tissue Conditioner + Photocatalyst +	1481.800	-3.787	< 0.001 *
	UV Irradiation			
Tissue Conditioner	Tissue Conditioner + Photocatalyst	5.500	-0.607	0.544
	Tissue Conditioner + UV	1475.500	-3.798	< 0.001 *
	Irradiation			
Tissue	Tissue Conditioner + Photocatalyst	-1476.300	-3.788	< 0.001 *
Conditioner + Photocatalyst	Tissue Conditioner + UV	-6.300	-2.753	0.006*
+ UV Irradiation	Irradiation			
Tissue	Tissue Conditioner + UV	1470.000	-3.800	< 0.001 *
Conditioner + Photocatalyst	Irradiation			

Table 4: In order to find out among which pair of groups there existed a significant difference with respect to the mean growth of *Staphylococcus Aureus,* multiple comparisons were carried out using Mann- Whiteny test and the results are given below.

*Denotes significant difference.

Higher mean growth of Candida Albicans was recorded in Group-1 followed by Group-3, Group-4 and Group-2 respectively. The difference in mean growth of Candida Albicans among the groups was found to be statistically significant (P < 0.001). (Table 5).

The difference in mean growth of Candida Albicans was found to be statistically significant between Group-1 and Group-2, Group-1 and Group-4, Group-2 and Group-3, Group-2 and Group-4 as well as Group-3 and Group-4 (p 0.001). No statistically significant difference was observed between Group-1 and Group-3. (Table 6)

Results can be Summarised as Follows

- Maximum antimicrobial effect was observed with Group-2 specimens that contained titanium dioxide and were subjected to UV irradiation. These specimens had maximum effectiveness against Streptococcus mutans (23cfu/ml) followed by Staphylococcus aureus (24cfu/ml) and Candida albicans (49cfu/ml). Photocatalyst with UV irradiation produced effective antimicrobial activity.

Group	Mean	Std Dev	SE of Mean	Min	Max	Kruskal-Wallis Chi-Sq	p-Value
Tissue Conditioner	1389.20	98.54	31.16	1205	1542		
Tissue Conditioner + Photocatalyst	48.70	2.67	0.84	46	54		
+ UV Irradiation						31.074	< 0.001 *
Tissue Conditioner + Photocatalyst	1380.50	92.51	29.25	1215	1515		
Tissue Conditioner + UV	52.60	3.17	1.00	48	58		
Irradiation							

Table 5: Comparison of the growth (CFU/ml) of *Candida Albicans.*

*Denotes significant difference.

(I) Group	(J) Group	Mean Difference (I-J)	Z	p-Value
	Tissue Conditioner + Photocatalyst +	1340.500	-3.792	<0.001*
	UV Irradiation			
Tissue Conditioner	Tissue Conditioner + Photocatalyst	8.700	-0.493	0.622
	Tissue Conditioner + UV	1336.600	-3.787	<0.001*
	Irradiation			
Tissue	Tissue Conditioner + Photocatalyst	-1331.800	-3.792	<0.001*
Conditioner + Photocatalyst +	Tissue Conditioner + UV	-3.900	-2.558	0.011 *
UV Irradiation	Irradiation			
Tissue	Tissue Conditioner + UV	1327.900	-3.787	<0.001*
Conditioner + Photocatalyst	Irradiation			

Table 6*:* In order to find out among which pair of groups there existed a significant difference with respect to the mean growth of *Candida Albicans*, multiple comparisons were carried out using Mann-Whiteny test and the results are given below.

*Denotes significant difference.

- The next highest antimicrobial activity was seen in Group-4 specimens which were subjected to UV irradiation but did not contain TiO2. Maximum effectiveness was against Streptococcus mutans (27cfu/ml) followed by Staphylococcus aureus (30cfu/ml) and Candida albicans (52cfu/ml). UV irradiation has effective antimicrobial activity.
- In the order of effectiveness, Group-3 (tissue conditioner with photocatalyst but not subjected to UV irradiation) followed Group-4 specimens. Higher effectiveness was against Streptococcus mutans (1317cfu/ml), followed by Candida albicans (1380cfu/ml) and Staphylococcus aureus (1500cfu/ml). These values showed that the photocatalyst inherently did not possess useful antimicrobial activity unless activated by UV irradiation.
- The control group (Group-1) which contained neither photocatalyst (TiO2) nor were subjected to UV irradiation, the CFU values were as follows: for Streptococcus mutans it was 1334cfu/ml Staphylococcus aureus it was 1505cfu/ml, for Candida albicans 1390cfu/ml. These values showed that the tissue conditioners did not possess any antimicrobial activity.
- Highest antimicrobial effect was observed in samples which contained TiO_2 and were subjected to UV radiation (Group-2 - 22cfu/ml). The next in the order of effectiveness was Group-4 samples that were subjected to UV irradiation but did not contain TiO_2. The differences between these groups were statistically significant. These two groups had significant difference against all other groups.

Discussion

Oral cavity of a healthy individual harbours a wide variety of microorganisms. In fact, oral environment is conducive to the growth of the microorganisms but they don't create any alarming pathological situations until the individual's immunity goes down. By nature, oral cavity consists of irregularities which are not easily cleansable. Poor maintenance of oral hygiene, the body warmth and the multiple food and beverages consumed create a favourable atmosphere for the growth of microorganisms. Many of the conventional prosthetic devices cover a large area of the mucosa and it provides a shelter for the microorganisms to grow and at the slightest diminishing of immunity, stomatitis is initiated. Systemic diseases also increase the vulnerability of the situation. It has been estimated that Streptococcus mutans, Staphylococcus aureus and Candida albicans are closely related to denture initiated stomatitis [8]. To counteract the growth of microorganisms, specific drug therapy is indicated. In candida infections, drugs like Nystatin are applied topically but for many individuals, it is not a pleasant experience [9]. Though highly polished acrylic dentures, deter the accumulation of microorganisms, a resilient liner/tissue conditioner may not offer such a resistance. Many of the resilient liners do not have stable structure and composition which do not favour the use for a longer duration. The liners easily allow colonization of microorganisms because of the rough surfaces and the porous structure. Microorganisms can easily penetrate into the resilient liners. Bacterial and fungal growth in resilient liners is counteracted by employing a sodium hypochlorite or hydrogen peroxide dip. Hypochlorite is capable of producing burns to the mucosa whereas hydrogen peroxide can unaesthetically bleach and denature the resilient liners. It is in this context; newer method of microbial inhibition using Titanium dioxide was thought of.

Titanium dioxide, particularly in the anatase form, is a photocatalyst. These compounds, when stimulated by mild ultra violet radiation (320-400 nm) acquire oxidative and disinfectant potential [10]. On UV irradiation of Titanium dioxide (TiO_2) an electron is transferred from the valence band to the conduction band by the absorption of a photon. This results in the generation of electrons and holes in TiO_2. These holes react with water molecules and results in the formation of hydroxyl radical (OH-). The radicals can oxidize organic substances into water and carbon dioxide. The cell wall of bacteria is thin and slack [11]. The first step in the photocatalytic attack on microorganisms is the decomposition of the bacterial or fungal cell wall.

In the present study, specimens were prepared by incorporating TiO_2 particles measuring 3.78µm in diameter in the concentration of 20 wt% to the tissue conditioner powder. These specimens were then inoculated with Candida albicans, Streptococcus mutans and Staphylococcus aureus and selected groups of specimens were exposed to UV radiation for a period of four hours. After irradiation, 0.01 ml of the microbial solution from each specimen was pipetted out and incubated on specific growth agar media for each organism, followed by counting of the colony forming units to evaluate the antimicrobial activity. Gupta., *et al.* quoted that TiO_2 nanoparticles provide a pronounced antibacterial effect against the pathogens Escherichia coli, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus mutans and Enterococcus faecalis for up to two hours post UV radiation. In the specimens that contained only the TiO_2 and which were not subjected to UV radiation, no pronounced antibacterial effect was observed [12]. Similar findings were seen in the present study. UV irradiation alone also have significant antimicrobial and antifungal effect (Table 2,4,6).

Conclusions

The following conclusions were drawn from the present study

- Tissue conditioners, as such do not possess antimicrobial potential.
- Tissue conditioners that incorporate Titanium dioxide (TiO_2) and subjected to UV radiation gains very potent antimicrobial activity.
- Tissue conditioner which was subjected to ultraviolet radiation also gains good antimicrobial activity.
- In the present study, Titanium dioxide as such did not exhibit any significant antimicrobial potential.

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