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The Effect of Tellurium Oxide Micro Particles Incorporation into PMMA on *Candida Albicans* Adherence

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ABSTRACT

Background: For centuries, naturally accessible items have been widely used in the treatment of human disease. The current study aimed to scientifically determine the antifungal effectiveness of tellurium oxide against Candida Albicans adherence to polymethelmethacrylate (PMMA) material.
Methods: The antifungal ability of tellurium oxide evaluated against Candida Albicans adherence by incorporation of TEO into PMMA monomer in concentration (1%, 3%, 5%, 7% by wt.). Twenty five samples were prepared and divided into five groups (control group and four experimental groups), five samples for each group. The sterile PMMA samples were placed in sterilized tubes containing Sabouraud dextrose broth, in which a small quantity of the isolated yeast was suspended, and incubated at room temperature for 1 hour. The specimens were then removed, rinsed with phosphate buffered saline, dried, fixed with methanol, stained with crystal violet, and viewed under an inverted light microscope.
Results: The adherence test results revealed a significant decrease in the number of Candida cells adherent to PMMA after adding 3% and 5% Tellurium oxide compared to samples from the control group, 1% and 7% experimental group. In conclusions, the results of current study encourage use TEO as antimicrobial material in different ways.
Conclusion: Incorporating the Tellurium oxide powder into heat cure acrylic material can succeed in producing a heat cure acrylic material with antifungal activity against Candida micro-organisms.

Key words: Tellurium oxide, Micro-particles, Polymethelmethacrylate material, *Candida albicans* adherence

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INTRODUCTION

Poly methyl methacrylate (PMMA) resin is found to have a favourable combination of properties that accounts for its popularity of use. The properties that have contributed most to the success of this material as a denture base material are its excellent appearance, simple processing, and easy repair. Although adequate is in satisfying esthetic demands [1]. Removable denture acrylic resin bases made of heat polymerized poly (methyl methacrylate) (PMMA) may function as a reservoir for microorganisms, contributing to re-infection in denture wearers [2-5].

The most prevalent fungal infection in denture wearers is known to be denture-related stomatitis, affecting 11–67% of denture wearers and affecting females more than males [6,7]. Denture stomatitis can be caused by a number of systemic factors such as malnutrition (iron, folic acid), diabetes, physical debilitation, immune suppression, and local factors such as trauma caused by the prosthesis, fungal and bacterial infections, xerostomia, and lack of hygiene or denture cleaning [8-10].

Factors affecting the adherence of yeasts to surfaces and its ability to form biofilms include the substrate surface (type, chemical nature and surface properties), the presence of salivary proteins and serum, the presence of other adherent microorganisms, strain variability, consumption of carbohydrate-rich diet, and culture condition [11-13].

Surface properties (roughness, hardness, and wettability) of oral prostheses (including dentures) contribute to the adherence, bonding and colonization of *Candida Albicans* [14,15]. Tellurium is a member of the chalcogen family and appears in nature in four chemical oxidation states [16]. Tellurium has been considered for many years as a non-essential and toxic element, and its salts have been historically used as antimicrobial agents [17]. However from above mentioned statement tellurium oxide incorporation into PMMA will be considered and investigated as anti-adherent agent against *C. albicans*.

MATERIAL AND METHOD

The study were conducted to select the most appropriate and effective concentration of Telluirum oxide by testing *C. albicans* adherence on the heat cure acrylic material's surface after TEO addition. Five samples were taken for

each test and four concentrations of TEO material were used 1%, 3%, 5% and 7%.

Specimen's preparation

Plastic patterns (Disk shaped), 10mm in diameter, and 2mm in thickness used to prepare PMMA specimens [18]. These plastic disks were imbedded in in the lower portion of dental flask which already had dental stone (freshly mixed according to manufacturer's instructions W/P ratio: (25ml/100g). The excess of stone material was removed and smoothened [19]. When the stone was completely set, the stone surface and plastic patterns were covered with thin layer of separating medium and left to dry, then the upper part of the dental flask was placed over the lower part and filled completely with dental stone (with vibration to eliminate any incorporated air bubbles) and covered with its lid [19]. When the second layer of the stone was completely set, the flask was opened and plastic disks were removed leaving a space in the stone.

Proportioning and mixing of heat cure acrylic (PMMA)

According to the manufacturer's instructions (P/L ratio 3g: 1 ml), the amount of PMMA powder and liquid were determined and mixed in dry, clean glass jar and covered with a lid. The upper and lower halves of the dental flask were coated with separating medium to prevent the adherence of PMMA with the stone. After the mixture reaching to the dough stage, kneaded with hands and adapted to the lower portion of the dental flask then packing procedure conducted.

Incorporation of Telluirum oxide micro-particles

Telluirum oxide micro-particles was first weighted in clean, dry glass container and the PMMA monomer added to it and mixed with (mini electric hand mixer) probe sonication apparatus to break them into individual nano-particles by vibration at 120W and 60 KHz for 3 min [20,21]. The resulting Telluirum oxide-PMMA monomer suspension mixed immediately with PMMA powder as soon as possible to prevent particles aggregation. The weight of added Telluirum micro-particles should be subtracted from the PMMA powder weight to keep the same manufacturer's P/L ratio. The amount of Telluirum micro -particles and PMMA powder were weighed by using electronic balance (with accuracy of 0.0000) and the monomer measured by medical syringe (Table 1).

Table 1: The amount of Telluirum micro -particles, PMMA powder and PMMA monomer mixing ratios

The selected percentage	Amount of TEO micro-particles (g)	Amount of polymer powder (g)	Amount of monomer (ml)
Control	0	5.5	3
3%	0.165	5.335	3
5%	0.275	5.225	3

Packing

When the polymer reach to the dough stage, it was kneaded by the hands and placed on the previously prepared mold and covered by sheet of polyethylene, then the upper portion was covered with the lid and continuous pressure of 100Kg/cm2 applied to it by the hydraulic press to ensure equal distribution of soft lining material inside the mold and for expelling the excess material.

After that the flask was removed from the press and opened, the polyethylene sheet was removed as well as the excess material by wax knife and the stone surface coated again with separating medium and allowed to dry.

Finally, both pieces of the flask were brought in and secured in a good manner and retained to the press machine and left under Pressure of 100Kg/cm2 for 5 min, then clamping was done to be ready for curing [20].

Curing of acrylic resin

Concerning curing of acrylic samples, the short curing cycle was used. The curing process was performed by placing the clamped flasks into thermostatically controlled water bath at room temperature then the temperature raised gradually up to 70 °C at 30 min and left for 30 min at this temperature in the water bath then the temperature is raised gradually to 100°C at 30 min and left for 30 min at this temperature [22]. After the curing was completed, the flask left from the water path and allowed to cool gradually at the bench until reach room temperature then the flask opened and the acrylic samples removed carefully from the molds.

Finishing and polishing

All acrylic samples used for microbiological and mechanical tests were finished and polished except that for surface roughness test. After deflasking of samples all the flashes were trimmed away from the margins using finishing burs followed by verification of the dimensions with a digital vernier. Then polishing procedure accomplished by urge wheel using pumice with water at polishing speed of 1500 rpm to prevent excessive heat which distort the samples, until glossy surface was achieved.

To reach the state of standardization, the acrylic samples were stored in distilled water and incubated at 37°C for 48 h prior to the test procedure [23-25].

Isolation of *C. albicans*:

C. albicans was taken from the oral cavity of twenty patients come to the clinic of prosthodontics in college of dentistry seeking for treatment for their problem which was denture stomatitis with oral thrush (26). The oral lesion scrubbed gently by sterilized cotton swab then cultured in sabouraud dextrose agar medium [27,28] then placed in incubator at 37°C for 48 hrs. [29].

Identification of C*. albicans*

- Morphological examination: A smooth, creamy, and pasty Candida colonies appear in sabouraud dextrose agar medium [30].
- Microscopical examination: A small portion was taken from single isolated colony and emulsified in a drop of normal saline on the glass slide to prepare suspension that was spread, allowed to dry at room temperature, and fixed by passing the slide several times over the flame of Bunsen burner.The glass slide stained according to Gram's method [31].
- Germ tube formation: From a single colony, One lope inoculum of yeast cells was taken and suspended in tubes (contained 0.5 ml of serum), then these tubes incubated at temperature of 37 ° C for 3 hr.
- Then a drop of suspension was placed on a glass slide and this slide was examined under Light microscope to see the presence of germ tubes [32].
- Biochemical Identification: VITEK 2 is a fully automated device with a sensitive fluorescence-based technology that performs microorganism identification.
- Prior to testing, a suspension of cultured yeast was prepared in sterile saline at a turbidity of 2.0 McFarland standard, as determined by using a Densi Chek instrument, the VITEK ID-YST card was automatically filled with the suspension, sealed, and incubated at 35.5 °C for 18 hrs in the VITEK 2 instrument and optical density reading was taken automatically every 15 min. The final results were compared with the database, and the identification of the unknown microorganism was obtained (33). A final identification of excellent , very good , good , acceptable , low discrimination in the laboratory report was considered to be correct.

Evaluating the effect of Telluirum oxide/PMMA specimens on adherence of *C. albicans*

The prepared SDB was poured into sterile tubes and a small portion of the isolated yeast were suspended in the media, and the concentration of the suspension was established equal to (0.5) McFarland standards by using a McFarland densitometer [34].

The sterile soft lining specimens were deposited in the previously prepared media in the sterilized tubes and incubated for 1hr. at room temperature.

After that, the specimens were taken out of the incubator and removed from the suspension and rinsed with phosphate buffered saline solution for one minute with gentle rocking for eliminating the non-adhered yeast cells , and dried by filter paper [20].

The adhered Candida cells on the PMMA specimens was fixed by using methanol and then stained for 60 sec. with crystal violet, and rinsed again for 30 sec. with PBS solution, then dried by filter paper and examined under inverted light microscope [35,36].

Under the inverted light microscope, the adherent Candida cells enumerated for each sample in three standardized fields and the mean of such fields was taken for each specimen.

RESULTS

Evaluating the adherence ability of *C albicans* to PMMA by examining the stained specimens for each group under the inverted light microscope, The highest mean value of control group (41) while the minimum mean value is (18) for experimental group (5% Tellurium oxide)as seen in (Table 2) and demonstrated in (Figure 1).

Table 2: The mean values and standard deviation of C. albicans adherence test

	N	Minimum	Maximum	Mean	Std. Deviation
Control	5	40	42	41	1
1%	5	38	41	40	1.14
3%	5	22	24	23	0.71
5%	5	17	19	18	1
7%	5	19	20	19	0.55

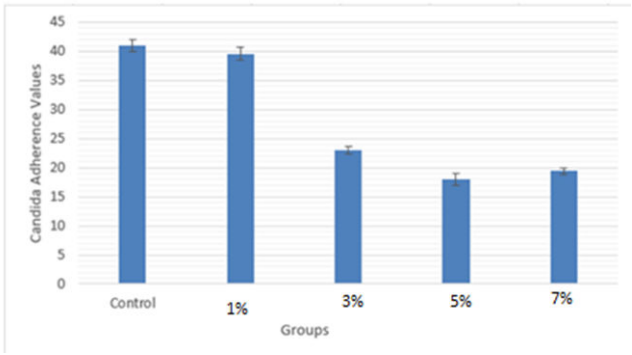

Figure 1: Bar charts of the mean values and standard deviation of *C. albicans* adherence test.

Test of Homogeneity of Variances:

Before starting with ANOVA table multiple comparisons test, the variances of tested groups were analyzed by running the Levene's test of homogeneity. According to primary analysis of data homogeneity, Games-Howell test was selected for multiple comparisons of incorporation part of candida adherence test while Boneferroni test was selected for multiple comparisons of immersion part of candida adherence test.

Evaluating the adherence ability of *C. albicans* (incorporation part)

Evaluation of specimens' groups where made by staining the specimens of each group with crystal violet and then examined under the inverted light microscope. The mean value of control group [41]. The mean value of the first experimental group (incorporation of 1% tellurium oxide) is [39], the mean value of second experimental incorporation group (3% tellurium oxide) is [23], the mean value of third experimental incorporation group (5% tellurium oxide) is [18] and the mean value of fourth experimental incorporation group(7% tellurium oxide) is [19].

Comparison of the means of *C. albicans* adherence test results of the experimental groups using one-way ANOVA was highly significant (Table 3).

Table 3: One-way ANOVA table.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2511.6	4	627.9	765.732	0
Within Groups	16.4	20	0.82		
Total	2528	24			

To compare the mean values among all study groups, Games-Howell multiple comparison test was conducted. There was significant difference between all groupsexcept for the 0.2 group which was non-significant (Table 4).

Table 4: Games-Howell multiple comparisons test.

(I) group		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	1%	1.4	0.57271	0.239	-0.406	3.206
	3%	18.00000*	0.57271	0	16.194	19.806
	5%	23.00000*	0.57271	0	21.194	24.806
	7%	21.60000*	0.57271	0	19.794	23.406
1%	3%	16.60000*	0.57271	0	14.794	18.406
	5%	21.60000*	0.57271	0	19.794	23.406
	7%	20.20000*	0.57271	0	18.394	22.006
0.03	5%	5.00000*	0.57271	0	3.194	6.806
	7%	3.60000*	0.57271	0	1.794	5.406
5%	7%	-1.4	0.57271	0.239	-3.206	0.406

DISCUSSION

PMMA is the most frequent material utilized in the production of mouth prostheses. Denture-related stomatitis (oral candidiasis associated with prosthetic surfaces) is by far the most frequent fungal infection in denture wearers. The most common fungal infections of the oral mucosa are caused by species of the genus Candida, with *C. albicans* being the most common species associated with this infection [37]. *C. Albicans* is classified as normal flora since it is an opportunistic commensal microorganism. This fungus, however, can cause cutaneous, mucosal, or systemic infections in immunocompromised patients [38,39].

Different trace elements, such as selenium and silver ions, have been studied and employed as medicinal agents or dietary supplements in the past few years

[40-42]. Tellurium is a metalloid that belongs to the chalcogen ion family and has been classified as a toxic element [37]. Despite the fact that these metal ions are widely utilized in numerous industries, their medicinal use has not evolved in recent decades due to their toxicity and lack of knowledge about their interactions with living systems [40,43].

The effect of tellurium oxide on Candida adhesion to PMMA was investigated in this study. TeNPs suppress the growth of *Candida albicans* through interfering with the metabolism of membrane sterols.Other studies have found that organo-telluriumcompounds interact with the vicinal sulfhydryl of cysteine in human squalene monooxygenase [44] and inhibit it [44-46]. When *Candida albicans* is exposed to tellurium NPs, it is completely inhibited at a concentration of 2000 g/mL. Furthermore, the results revealed squalene accumulation as well as an increase in the expression levels of the ERG1 gene of the squalene monooxygenase enzyme [47]. Squalene monooxygenase is a rate-limiting enzyme in the biosynthesis of ergosterol and cholesterol.Ergosterol is the primary lipid in fungal membranes, and it plays critical functions in membrane function and integrity [48,49].

From the statistical results of this performed study, there was reduction in the numbers of *C. Albicans* cells that adhered on the PMMA surface containing TEO in comparison with the other control PMMA specimens.

The rise in antibiotic resistance in many bacteria has prompted researchers to concentrate their efforts on the synthesis and creation of several potent compounds to combat microorganism resistance mechanisms. NPs provide an unique and effective “antibiotic” that may be beneficial in combating *C. Albicans* resistance mechanisms.

Biogenic NPs represent a new generation of NPs that function as carriers. These NPs have the capability to direct the toxicity effect to a specific target, potentially reducing collateral damage.

However, the cytotoxic mechanisms of nanoparticles are not fully understood. One of the identified processes is oxidative stress; it is conceivable to study other mechanisms that are similar to oxidative stress. More research is needed to understand the role of nanoparticles in *C. Albicans* resistance mechanisms and whether this exposition can develop new ones.

CONCLUSIONS

From the research provided, it can be concluded that Tellurium oxide powder can be regarded as a strong antifungal material and incorporating the Tellurium oxide powder into heat cure acrylic material can succeed in producing a heat cure acrylic material with antifungal activity against Candida micro-organisms. Also, 3% and 5% experimental groups showed a better activity against *candida albicans* comparing to control, 1% and 7% experimental group.

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