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MICROBIOLOGICAL AND IMMUNOLOGICAL EFFECTS OF MONOMER FREE THERMOSENS AND NANO ZIRCONIA OXIDE REINFORCED DENTURE BASE RESINS ON CONTROLLED DIABETIC DENTURE WEARERS

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ABSTRACT

Background: The overall success of denture base resins depends on both mechanical behaviour and biocompatibility, especially in the rehabilitation of diabetic patients. The growth and nourishment of microorganisms is enhanced in diabetes mellitus specially in presence of dentures resulting in denture stomatitis. This study was prompted to assess the microbiological growth and the development of salivary immunoglobulin A (s-IgA) in relation to newly introduced denture base materials used in the rehabilitation of diabetic patients.

Materials and Methods: Forty eight completely edentulous patients were selected and divided into two groups: group (A), which included controlled diabetic patients and group (B) included non-diabetic patients. Each group was further divided into four sub groups according to the denture base material. Sub group I, patients were received thermoplastic Vertex™ ThermoSens denture bases; sub group II, patients were received heat cure acrylic resin dentures reinforced with nano zirconia oxide particles (nano-ZrO2); sub group III, patients were received conventional heat cure acrylic resin dentures; where sub group VI, patients were received conventional heat cure acrylic resin dentures relined with self-cure acrylic resin. For each participant, non-stimulated saliva samples were collected before denture insertion, and one, two and three months after wearing dentures. Samples were inoculated and incubated both aerobically and anaerobically on selective media for *Streptococcus mutans, Lactobacilli* and *Candida*. Then, isolated microorganisms were identified, the characteristic colony forming units per millilitre (CFUs/ml), were calculated and the salivary immunoglobulin A level was measured by Enzyme-linked immunosorbent assay (ELISA).

Results: The results revealed increase in the CFU values of *Candida, Streptococcus mutans*, *Lactobacilli* and the level of salivary immunoglobulin A in all the assessed patients following denture insertion. However, significant lower values were evident in patients rehabilitated with ThermoSens and nano-ZrO2 reinforced bases compared to heat cure acrylic and self-cure relined bases. Significant increase in CFU values and level of s-IgA were found in diabetic compared to non-diabetic patients after insertion of all denture bases.

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Conclusion: ThermoSens and nano-ZrO2 reinforced denture bases showed less microbial growth and salivary immunoglobulin A level compared to heat cure and self-cure relined bases in both controlled diabetic and non-diabetic patients, hence, more biocompatible.

KEYWORDS: Thermoplastic polyamide, Vertex ThermoSens, Reinforced acrylic, Zirconia oxide nanoparticles, Diabetes mellitus, Candida, Oral bacteria, Salivary Immunoglobulin A.

INTRODUCTION

The human oral cavity provides a unique environment for growth and nourishment of different species of microorganisms including bacteria and fungi [1]. The fast turnover of oral lining epithelia decreases microbial adhesion to the oral mucosa. In contrast, the non-shedding surfaces, as implants and dentures, are more liable to biofilm formation. In denture wearers lacking the proper manual dexterity to eliminate plaque, there is more liability to oral mucosal infections, mainly bacterial and fungal [2] which may contribute to denture stomatitis [3].

Biocompatibility of denture base materials is important especially in diabetic patients having high susceptibility to infection due to reduced immunity[4]. A direct significant correlation between diabetes and oral microbial colonization has been reported[5].

It was pointed out that mechanical cleansing methods and the available disinfection methods are still controversial because they may alter the properties and clinical features of denture bases[6,7]. Recently, surface modification using different coatings or incorporating antimicrobial additives to the denture base materials have been attempted to reduce microbial adhesion and colonization on denture bases[8,9]. The continuous progress in the field of biomaterials has also resulted in the introduction of new materials with enhanced mechanical and biological properties.

Acrylic resin is the most commonly used denture base material,[10] however, low impact strength, fatigue resistance [11], and polymerization shrinkage of resin denture bases result in problems that affect the longevity of removable dentures[12]. Also, the micro porosities encountered within the material encourage microbial adherence and colonization [13] which in turn induce denture stomatitis, thus compromising the efficacy of the material [14]. In addition, the residual monomer remaining after acrylic resin polymerization, that affect the tensile strength, modulus of elasticity and surface hardness of resin denture bases,[15] may diffuse into adjacent oral tissues resulting in irritation and inflammation of the mucosa and may cause allergic response to some denture wearers [16,17].

Many attempts were thus carried out to overcome acrylic resin shortcomings and to enhance its biomechanical properties resulting in recently introduced denture base materials and processing techniques [18].

Currently, thermo-injectable, high impact, flexible or semi-flexible polyamide is introduced as an alternative to the conventional acrylic resin due to its aesthetic physicochemical properties [19]. A newly introduced innovative thermoplastic polyamide material, the Vertex ThermoSens is proved to exhibit superior mechanical properties, minimum dimensional changes and better adaptation as compared to standard polyamide and conventional acrylic resin [20,21]. The flexibility of the material is controllable, hence, it was proposed as removable partial denture material especially as an alternative to metal clasps [22]. The material is also monomer-free thus encouraging its use for patients allergic to acrylic resin monomer [19].

Recently, nanotechnology has been used in the prosthodontics field for material improvement purposes[23]. One of the most common nanoparticle fillers used to enhance the mechanical and physical properties of acrylic resin is nano zirconia oxide

(nano-ZrO2) [24,23]. It has also been proposed to exhibit antibacterial and antifungal properties and may thus play a preventive role in patients susceptible to microbial infection [25,26].

Denture foundation changes and ridge resorption is a usual manifestation associated with diabetic denture wearers. [27]. Thus, Frequent follow up and the need for denture relining is a necessity to cope for denture foundation changes and ridge resorption.

Auto-polymerizing acrylic resin has been commonly used in relining denture bases. That undergoes ill fit. However the material was reported as an unhygienic material because of uncured residual monomer that may cause allergy and soft tissue reaction. In addition, the micro porosities gained by time, thus, harbouring plaque and encouraging the growth of microorganisms [22].

Thus, this study was prompted to assess the biocompatibility of the recently introduced Vertex ThermoSens denture bases and nano zirconia oxide reinforced resin bases compared to the commonly used heat cure and self-cure relined denture bases in controlled diabetic denture wearers.

The mucosal immune system plays an essential role in protecting the human body as an immune barrier and regulator of potential inflammatory reactions. Its main component; the secretory IgA (s-IgA) is used to assess the immune status of the oral mucosa [28]. Alterations in s-IgA concentrations in diabetic patients was reported to possess an impact on the oral health. The dental literature includes controversy regarding the level of s-IgA and the oral condition specially in diabetic patients [29,30] thus the present study also aimed to compare s-IgA levels between controlled diabetics and non-diabetic denture wearers.

MATERIALS AND METHODS

This clinical randomized controlled study was conducted on 48 completely edentulous patients whose ages ranged from 50-60 years. Patients were selected from the outpatient clinic, Faculty of Dentistry Umm Al Qurans University (UQU Dent).

All selected patients had good oral health. The selected patients were free from systemic diseases except twenty four of these patients who were controlled diabetic patients. Patients taking antifungal or antibiotics six months before this study and smokers were excluded. All patients had no previous history of using oral prostheses.

An informed consent was signed by all participants after receiving detailed information about the study. The protocol was approved by the Ethical Committee of the (UQU Dent).

Patients were classified into two equal groups. **Group (A), Test group:** Comprised controlled diabetic patients. **Group (B) Control group:** Comprised non diabetic. Glycaemia control was confirmed by blood glucose test. Depending on the materials used for construction of complete denture bases, **Groups A** and **B** were further randomly classified into four equal sub groups as follows:

Sub group I: Comprised patients who received complete dentures constructed from thermoplastic polyamide Vertex™ ThermoSens denture base material using injection molded technique. **Sub group II:** Comprised patients who received heat cure acrylic resin complete dentures reinforced with nano zirconia oxide particles (nano-ZrO2)**. Sub group III:** Comprised patients who received conventional complete dentures constructed from heat cure acrylic resin. **Sub group IV:** Comprised patients with conventional heat cure acrylic resin base relined with self-cure acrylic resin material.

Upper and lower complete dentures were fabricated for all patients following the conventional clinical method for complete denture construction. Special consideration for processing different materials were as follows:

Sub group I: Thermoplastic VertexTM ThermoSens denture bases (Vertex™ ThermoSens

Rigid, Vertex- Dental B.V. 3705 HJ Zeist, Netherlands) were constructed using thermoject 22 injection moulding unit to produce polyamide denture bases. Maxillary and mandibular casts and the waxed up dentures were invested using dental stone in a special flask. The flask consists of two indexed halves and a round hole through which the injectable material was thermo-pressed. A rod shaped wax sprue was attached to waxed up trial dentures. Wax elimination was carried out, and the flask was placed on top of the injection machine (Thermoject 22 machine, Vertex-Dental B.V. Headquarters Netherlands). The aluminium cartridge containing pre dosed granules of thermoplastic material was attached to the hole of the flask. Both cartridge and flask were heated at 270 ºC- 280 ºC for 18 minutes. The molten material in the cartridge was then injected automatically at a constant pressure of 8.5 bar into the mould. The flask was bench cooled for 30 minutes, dentures were deflasked, finished, polished according to the manufacturer's instructions.

Sub group II: Nano-ZrO2 reinforced acrylic resin denture bases for this group were constructed as follows: Nano zirconia powder (zirconium oxide (ZrO2) nano powder, Advanced Materials, Manchester, USA, with APPS 30-60 nm and a purity of 99.9%) was treated with silane coupling agent (Methacryloxy propyl trimethoxy silane) to permits sufficient adhesion between zirconia nanoparticles and acrylic resin matrix[31]. Silane coupling agent (0.2 wt %with respect to the zirconia powder) was added to 100 ml of distilled water, and both were added to nano zirconia powder (7.5 % by the weight of the polymer), mixed at 50°C. The mixture was then heated at 110°C and allowed to cool [32]. The treated powder was weighed, added to the acrylic polymer (7.5 % by weight), thoroughly mixed with polymer until a homogenous color was attained and stirred for 30 min to confirm the homogeneity and uniformity of color. The acrylic monomer was then added to the prepared mixture. On reaching the dough stage, the mixture was packed into the prepared mould following conventional compression moulding technique and cured by long curing cycle technique.

Sub group III: Heat cure acrylic denture bases (Major base 20, Major Prodotti Dentari S.p.A; Italy). were constructed following the conventional compression molding technique and cured by long curing cycle [33] to give the lowest value of residual monomer content.

Sub group IV: For the self-cure relined dentures, heat cure acrylic dentures were constructed following the conventional procedure. However, before trial packing, tin foil spacer 1 mm in thickness was adapted on the casts to allow even space for the relining material. Dentures were processed following the conventional procedure. The spacer was replaced with self-cure acrylic resin relining material. Dentures were cured at 45°c for 20 minutes in pressure curing unit to prevent porosity of the self-cure relining material.

Laboratory remounting, finishing and polishing was carried out as recommended. During the insertion appointment, dentures were checked to ensure proper fit and extension and to eliminate premature occlusal contacts. Oral and denture hygiene instructions were explained and patients were assured to carry them out on daily scheduled bases. Patients were instructed to use a brush without using any chemical or mechanical cleansing agent. Patients were also instructed to soak dentures in water whenever dentures are not in use.

Saliva sample collection

Four unstimulated salivary samples were collected from each patient; one immediately before denture insertion and the other three samples after one, two and three months from insertion. Before saliva collection, the participants were instructed not to use mouth rinse or ingest any food or drinks for a minimum of 1 hour. A sterile wide mouthed

plastic tubes were used for saliva collection and immediately transported to the laboratory where it is divided into two aliquots; one for immediate microbiological culture and the other kept at -20ºC for IgA testing.

Microbial isolation, identification and counting

Each collected salivary sample was vortexed for 30 second and serially diluted using sterile 0.05 M phosphate buffered saline before plating. Culture media used were Mitis salivarius agar (MSA) (Acumedia, Baltimore, Maryland, USA) supplemented with filtrated 1% potassium tellurite (Difco Laboratories, Detroit, Michigan, USA) for isolation of *Streptococcus mutans,* de Man rogosa sheep (RMS) agar (Oxoid Ltd., Basingstoke, Hampshire, England) for isolation of *Lactobacilli* and Sabouraud dextrose agar (SDA) with chloramphenicol (Oxoid Ltd., Basingstoke, Hampshire, England) for determination of *Candida albicans* and *non albicans*.

One hundred μl from each diluted sample was spread evenly on the three culture media used, MSA and RMS plates were incubated at 37˚C for 48 hours in an anaerobic jar with anaerogas pack system (Oxoid Ltd., Basingstoke, Hampshire, England) while the SDA plates were incubated aerobically at 37˚C for 72 hours. The colonies grown were identified by its characteristic morphology on the media used and *Candida albicans* was differentiated from non albicans biochemically by germ tube test. Identification of all strains was confirmed by automated VITEK 2 Compact instrument (bioMerieux, UK).

Colony forming units (CFUs) of each identified organism were recorded blindly by the same researcher and final count for the sample was calculated by considering the dilution and expressed as the number of colony forming units per milliliter (CFU/ml) of saliva. Final counts were transformed to log CFU and results were presented as the mean of the log CFUs.

Determination of salivary IgA concentrations

The concentration of salivary IgA was assessed by using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Uscn Life Science, Wuhan, China). The previously collected salivary samples were homogenized and centrifuged at 10000 ×g for 15 minutes at 2-8˚C, the supernatant of each sample was stored at –80˚C to avoid loss of bioactivity and contamination. Analysis was performed according to manufacture instructions and finally the colour changes were determined spectrophotometrically at a wavelength 450nm using a microplate reader (State Fax 2100, Awareness, USA). The assay was performed in duplicate and the concentrations of s-IgA in samples were evaluated by comparing the optical density of the samples to the standard curve.

Statistical analysis

All data were tabulated and then statistically analyzed using SPSS software package version 20.0. The distribution of quantitative variables was tested for normality, comparison between two independent data was done using independent t-test. Comparison between multiple data was done using One-way ANOVA test. p-value of less than 0.05 was considered statistically significant.

RESULTS

Candida albicans and non albicans

The results of this study revealed an increase in the mean CFU values of *Candida albicans and non albicans species* in all assessed patients applying the four denture base materials rising by time at different evaluation periods. The difference between mean values from base line to three months post insertion was insignificantly increase in **subgroups I and II** evidence by p value (P more than 0.05) while it was highly significantly increase (P=0.001)

in **subgroup III** and extremely highly significantly increase (P=0.000) in **subgroup IV**. When comparing the mean CFU values in different study periods, the lowest mean CFUs of both *albicans* and *non-albicans* species was found in **subgroup II** and the highest values were in **subgroup IV** with significant difference from other subgroups after one month (P=0.05) and highly significant (P=0.001) or extremely highly significant difference after two and three months (P=0.000) among diabetics and non-diabetics (Table1).

TABLE (1) Means and standard deviations of the four denture base materials regarding the Candida albicans (CFUs x 10^2) and non albicans (CFUs x 10^2) at different evaluation periods among diabetic and non-diabetic denture wearers.

	Diabetic patients (Group A)					***Non-Diabetic patients (Group B)***				
	Vertex ThermoSens resin (Sub group I)	Nano-ZnO2 reinforced resin (Sub group II)	Heat cure resin (Sub group III)	Self-cure resin (Sub group IV)	F (p value)	Vertex ThermoSens resin (Sub group I)	Nano-ZnO2 reinforced resin (Sub group II)	Heat cure resin (Sub group III)	Self-cure resin (Sub group IV)	F (p value)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Candida albicans (CFUs x 10^2)										
Baseline	0.517± 0.244	0.551± 0.011	0.522± 0.213	0.524±0.118	1.669 (0.986)	0.430±0.011	0.421± 0.218	0.412± 0.172	0.401± 0.133	0.764 (0.528)
After one month	0.632± 0.989	0.625± 0.259	1.933 ± 1.01	2.423±0.712	4.271 (0.05)*	0.518±0.188	0.499± 0.227	1.372± 0.365	1.872± 0.581	4.582 (0.05)*
After two months	0.737± 0.991	0.677± 0.314	2.132 ± 1.04	6.771±2.71	9.776 (0.001)**	0.604±0.283	0.533± 0.219	1.974± 0.463	3.820± 0.905	9.385 (0.001)**
After three months	0.742± 0.994	0.691± 0.388	3.427 ± 1.91	11.29±2.823	27.987 (0.000)***	0.649±0.241	0.617± 0.275	2.745± 1.340	6.912± 1.024	11.741 (0.001)**
F (p value)	3.011 (0.06)	1.689 (0.997)	11.89 (0.001)**	44.78 (0.000)***		2.723 (0.07)	2.119 (0.09)	9.996 (0.001)**	12.42 (0.000)***	
Candida non albicans (CFUs x 10^2)										
Baseline	0.478± 0.102	0.491± 0.031	0.487± 0.202	0.511±0.215	0.895 (0.309)	0.359±0.106	0.411± 0.092	0.395± 0.012	0.389± 0.031	0.849 (0.296)
After one month	0.521± 0.133	0.501± 0.116	0.969± 0.287	3.625±0.439	5.847 (0.05)*	0.505±0.182	0.478± 0.135	0.869± 0.211	1.467± 0.502	4.521 (0.05)*
After two months	0.594± 0.209	0.529± 0.217	1.732± 0.480	9.813±1.247	12.479 (0.000)***	0.541±0.217	0.512± 0.227	1.289± 0.319	4.698± 0.653	17.392 (0.000)***
After three months	0.627± 0.246	0.619± 0.228	2.491± 0.992	12.230± 2.318	15.392 (0.000)***	0.609±0.311	0.589± 0.263	1.997± 0.419	7.231± 0.871	17.859 (0.000)***
F (p value)	1.209 (0.997)	0.995 (0.185)	9.995 (0.001)**	19.562 (0.000)***		1.375 (0.998)	0.973 (0.468)	8.071 (0.001)**	16.794 (0.000)***	

SD: standard deviation ***F: one way ANOVA test***

**** Significant difference*** ***** Highly significant difference*** ****** Extremely high significant difference***

Streptococcus mutans and Lactobacilli

Table (2) demonstrates comparisons between the mean CFUs of *Streptococcus mutans* and *Lactobacilli* among groups (**A** and **B**) at different evaluation periods regarding the four denture bases using one way ANOVA test. A statistically extremely high significant difference (P=0.000) in CFU values were reported when comparing the four groups after one, two and three months of denture insertion. Also, statistically extremely highly significant difference (P=0.000) was observed when comparing the evaluation periods in **sub group (III** and **IV**). With respect to **sub group (I and II)**, there were insignificant increase of both *Streptococcus mutans* and *lactobacilli* in diabetics and non-diabetics (P more than 0.05).

TABLE (2) Means and standard deviations of the four denture base materials regarding the *Streptococcus mutans* (CFUs x 10^3) and *Lactobacilli* (CFUs x 10^5) at different evaluation periods among diabetic and non-diabetic denture wearers.

	Diabetic patients (Group A)					***Non-Diabetic patients (Group B)***				
	Vertex ThermoSens resin (Sub group I)	Nano-ZnO2 reinforced resin (Sub group II)	Heat cure resin(Sub group III)	Self-cure resin(Sub group IV)	F (p value)	Vertex ThermoSens resin (Sub group I)	Nano-ZnO2 reinforced resin (Sub group II)	Heat cure resin(Sub group III)	Self-cure resin(Sub group IV)	F (p value)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Streptococcus mutans (CFUs x 10^3)										
Baseline	0.392± 0.19	0.381± 0.10	0.414± 0.21	0.411± 0.011	0.932 (0.215)	0.243± 0.271	0.242± 0.151	0.236± 0.023	0.225± 0.024	0.898 (0.647)
After one month	0.415± 0.214	0.412± 0.181	4.01± 0.52	7.43± 0.938	24.36 (0.000)***	0.301± 0.131	0.288± 0.113	3.092± 0.929	5.814± 0.889	40.98 (0.000)***
After two months	0.433± 0.198	0.421± 0.232	6.42± 0.481	9.611± 1.62	36.93 (0.000)***	0.273± 0.242	0.269± 0.271	4.911± 0.948	6.627± 0.978	49.23 (0.000)***
After three months	0.442± 0.205	0.430± 0.192	6.53± 0.902	9.731 ± 1.93	41.99 (0.000)***	0.389± 0.219	0.372± 0.20	5.718 ± 1.03	6.996± 1.823	46.99 (0.000)***
F (p value)	3.116 (0.06)	2.943 (0.06)	31.31 (0.000)***	45.29 (0.000)***		2.667 (0.09)	2.821 (0.07)	27.74 (0.000)***	39.76 (0.000)***	
Lactobacilli (CFUs x 10^5)										
Baseline	0.319± 0.001	0.321± 0.011	0.319± 0.001	0.312±0.020	0.899 (0.126)	0.191± 0.021	0.189± 0.01	0.199± 0.002	0.191± 0.001	1.021 (0.118)
After one month	0.411 ± 2.71	0.312 ± 0.11	4.12 ± 1.71	7.82 ± 1.60	11.251 (0.000)***	0.225 ± 0.11	0.212 ±0.45	3.91 ± 1.31	6.73 ± 1.62	7.859 (0.001)**
After two months	0.421 ± 0.60	0.361 ± 0.52	9.52 ± 2.21	12.01 ± 2.31	25.325 (0.000)***	0.270 ± 0.15	0.250 ±0.41	6.21 ± 17.5	9.6 ± 66.2	9.759 (0.000)***
After three months	0.438 ± 0.82	0.381 ± 14.3	12.70 ± 2.14	17.33 ± 1.80	24.527 (0.000)***	0.323 ± 0.32	0.279 ±0.26	9.63 ± 1.6	10.2 ± 2.1	27.366 (0.000)***
F (p value)	2.993 (0.118)	3.367 (0.09)	16.252 (0.000)***	22.318 (0.000)***		3.774 (0.07)	3.911 (0.06)	19.265 (0.000)***	23.140 (0.000)***	

SD: standard deviation ***F: one way ANOVA test***

***** Highly significant difference*** ****** Extremely high significant difference***

Using t-test, comparisons between the mean difference of *Candida albicans, non albicans, Streptococcus mutans and Lactobacilli* among diabetic and non-diabetic denture wearers at different evaluation periods regarding the four denture bases were demonstrated**.** There were statistical significances ranged between significance, highly significance and extremely highly significance as shown in **(Table 3).** Regarding the **(Sub group I and II)** at one, two or three months after denture insertion, the mean difference was significant increase evidence by p value (P=0.05). With respect to the **(Sub group III and IV)**, there were significantly increase (P less than 0.05) after one month and extremely significantly increase (P=0.000) after three months.

TABLE (3) Comparison between the mean difference of oral microorganisms at different evaluation periods regarding the four denture base materials among diabetic (group A) and non-diabetic (group B) denture wearers.

	Candida albicans **(CFUs x 10^2)**	***Candida non albicans*** **(CFUs x 10^2)**	***Streptococcus mutans*** **(CFUs x 10^3)**	***Lactobacilli*** **(CFUs x 10^5)**
Vertex ThermoSens resin (Sub group I)				
Baseline	2.409 (0.05)*	3.099 (0.05)*	2.026 (0.05)*	3.521 (0.05)*
After one month	2.957 (0.05)*	2.144 (0.05)*	3.997 (0.05)*	3.575 (0.05)*
After two months	3.115 (0.05)*	2.908 (0.05)*	3.466 (0.05)*	3.452 (0.05)*
After three months	2.393 (0.05)*	2.034 (0.05)*	2.877 (0.05)*	2.119 (0.05*
Nano-ZnO2 reinforced resin (Sub group II)				
Baseline	2.885 (0.05)*	2.913 (0.05)*	3.161 (0.05)*	3.772 (0.05)*
After one month	2.934 (0.05)*	2.175 (0.05)*	3.274 (0.05)*	3.950 (0.05)*
After two months	2.995 (0.05)*	2.877 (0.05)*	2.997 (0.05)*	3.709 (0.05)*
After three months	2.679 (0.05)*	2.691 (0.05)*	2.348 (0.05)*	2.115 (0.05)*
Heat cure resin (Sub group III)				
Baseline	2.797 (0.05)*	2.509 (0.05)*	3.091 (0.05) *	5.338 (0.03)*
After one month	5.638 (0.01)*	4.807 (0.04)*	4.838 (0.04) *	6.219 (0.001) **
After two months	7.014 (0.001)**	6.411 (0.001) **	7.285 (0.001) **	7.093 (0.001) **
After three months	9.312 (0.000)***	8.497 (0.000) ***	7.924 (0.001) **	11.458 (0.000) ***
Self-cure resin (Sub group IV)				
Baseline	2.576 (0.05)*	2.682 (0.05)*	2.408 (0.05)*	2.907 (0.05)*
After one month	4.748 (0.04)*	5.358 (0.01)*	3.846 (0.05)*	6.573 (0.001)**
After two months	9.164 (0.000)***	9.482 (0.000)***	6.171 (0.001)**	7.151 (0.001)**
After three months	11.845 (0.000)***	10.905 (0.000)***	7.364 (0.001)**	9.256 (0.000)***

**** Significant difference ** Highly significant difference *** Extremely high significant difference***

Table (4) demonstrates comparisons between the mean values of s-IgA among groups **(A** and **B)** at different evaluation periods regarding the four denture bases using one way ANOVA test. A statistically extremely high significant difference (P=0.000) in CFU values were reported when comparing the four groups after one, two and three months of denture insertion. The lowest mean value was observed with groups **(II** and **I)** and the highest values was noticed with sub groups **(III** and **IV)**. Also, statistically extremely highly significant difference (P=0.000) was observed when comparing the evaluation periods in sub groups **(III** and **IV)**. With respect to groups **(I** and **II)**, there were insignificant increase in both diabetics and non-diabetics (P more than 0.05).

Table (5) Show comparison between the mean difference of s-IgA among groups **(A and B)** at different evaluation periods regarding the four denture bases. The mean difference between groups (A and B) was significantly increase in **(Sub group I and II)** at one, two or three months after denture insertion evidence by p value (P=0.05). With respect to the **(Sub group III and IV)**, the mean difference was increase (P=0.03) after one month and extremely significant increase (P=0.000) after three months.

TABLE (4) Means and standard deviations of the Salivary Immunoglobulin A (s-IgA) at different evaluation periods among diabetic and non-diabetic wearing the four denture base materials.

Follow up period	Diabetic patient (Group A)					Non- diabetic patients (Group B)				
	Vertex Thermo Sens resin (Sub group I)	Nano-ZnO2 reinforced resin(Sub group II)	Heat cure resin(Sub group III)	Self-cure resin(Sub group IV)	F (p) value	Vertex Thermo Sens resin (Sub group I)	Nano-ZnO2 reinforced resin(Sub group II)	Heat cure resin(Sub group III)	Self-cure resin(Sub group IV)	F (p) value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Baseline	40.35± 3.27	40.14± 2.15	40.98± 2.42	40.77± 2.54	0.779 (0.329)	30.13± 2.19	30.12± 3.28	30.45± 2.37	31.23± 2.82	2.091 (0.245)
After one month	41.96± 3.11	41.81± 2.31	74.28± 4.22	97.59± 5.48	23.653 (0.000)***	31.97± 2.50	30.99± 3.60	49.55± 4.43	85.07± 5.89	36.572 (0.000)***
After two months	42.39± 3.95	42.32± 2.98	118.3± 7.78	104.8± 7.71	47.338 (0.000)***	32.42± 2.98	31.45± 4.88	92.45± 5.61	102.4± 5.58	57.324 (0.000)***
After three months	42.87± 3.14	42.84± 3.44	110.1± 5.07	122.8± 5.11	54.667 (0.000)***	33.41± 3.73	32.11± 4.63	105.4± 3.48	111.3± 7.87	59.931 (0.000)***
F (p) value	3.993 (0.06)	3.912 (0.06)	57.982 (0.000)***	72.378 (0.000)***		3.932 (0.06)	3.871 (0.06)	58.421 (0.000)***	61.140 (0.000)***	

SD: standard deviation

F: one way ANOVA test

****** Extremely high significant difference***

TABLE (5) Comparison between the mean difference of immunoglobulin A among diabetic (group A) and non-diabetic (group B) denture wearers at different evaluation periods regarding the four denture base materials.

	Vertex ThermoSens resin(Sub group I)	**Nano-ZnO2 reinforced resin(Sub group II)**	**Heat cure resin(Sub group III)**	**Self-cure resin(Sub group IV)**
Baseline	3.026 (0.05) *	3.098 (0.05) *	6.428 (0.01) *	2.218 (0.05) *
After one month	4.001 (0.05) *	3.118 (0.05) *	5.219 (0.03) *	4.021 (0.03) *
After two months	3.562 (0.05) *	4.985 (0.05) *	3.942 (0.05) *	8.011 (0.000) ***
After three months	2.311 (0.05) *	2.175 (0.05) *	7.832 (0.000) ***	1.997 (0.000) ***

**** Significant difference*** ****** Extremely high significant difference***

DISCUSSION

Heat cure denture bases and dentures relined with self-cure resin are commonly used in dental practice. However, high rate of denture stomatitis was reported among their wearers due to microbial colonization on its surfaces, especially in diabetic patients[5]. To reduce the risk of such stomatitis as well as to improve its mechanical strength, innovative denture base materials were recently introduced. Hence this study was prompted to assess the microbiological effect of nano zirconia oxide (nano-ZnO2) reinforced acrylic resin and Vertex TermoSens as two recently introduced denture base materials in diabetic patients.

Diabetes mellitus is associated with reduced immunity[4,34] resulting in mucosal changes, with repeated fungal and bacterial denture stomatitis [5]. Thus, the present study selected diabetic patients for assessment of antimicrobial effect of the new denture base materials.

Both bacteria selected were reported to have a main role in the formation and development of denture plaque biofilms [35]. While *Candida* is claimed to be one of the most common opportunistic fungal pathogen in the oral cavity causing denture stomatitis [36,37].

The increase in CFUs of *Candida, Streptococcus mutans* and *Lactobacilli* in the saliva of all denture wearers in the present study could be attributed to the presence of dentures depriving the underlying tissues from the continuous flow of saliva which is effective in removing the microbial pellicle. Also, due to the reduction of oxygen level in the underlying tissues. Which probably contributed to the presence of anaerobic environment thus enhancing the affinity of bacterial and fungal growth[38]. The formation of salivary pellicle including protein with different polarities on denture bases was also reported to alter the hydrophobicity of the denture base material thus encouraging the microbial colonization on the denture surface [39]. This increase in microbial growth noticed after denture insertion is in agreement with the results of other studies that claimed that the complicated shape of acrylic dentures assists microbial plaque accumulation causing stomatitis in up to 88% of denture wearers[40,35,37].

The lowest microbial prevalence evident in patients rehabilitated with nano-ZrO2 reinforced denture bases compared to other base materials could be attributed to its antibacterial and antifungal properties as reported in previous reports[25,26]. The enhanced antibacterial activity of nano zirconia oxide might be attributed to active oxygen created from the nano zirconia oxide, which in turn causes disruption of cell membrane of microorganisms[26].

Nano zirconia oxide can also inhibit the fungal growth through interfering with cell function and causing distortion in fungal hyphae[26]. This decrease was also proved by other investigators who recommended the addition of nano-ZrO2 to self-cure acrylic resin as a possible approach for prevention of denture stomatitis [41]. In addition, the silanization of nano-ZrO2 decreases the apparent porosity and water sorption of the reinforced denture bases which in turn reduce microbial colonization [42,25]. It thus seems advantageous to use nano-ZrO2 in reinforcing acrylic denture.

The reduced prevalence of microorganisms on the ThermoSens denture bases evident in this study could be attributed to the heat-moulding of thermoplastic polyamide material and to its monomer free nature [43]. This probably reduced the formation of micro porosities and hence, the microbial adherence. Also, the smooth surface of this material may have resulted in the reduction in the adherence of microorganisms [44]. This result is in agreement with previous study that also proved reduced microbial growth [45]. However, ***Olms et al.*** [46] proved no difference in microbial colonization on polyamides denture bases compared to heat cure denture bases.

The increased microbial count evident in this study when using self-cure relined bases may be explained by the reduced conversion degree by the chemical initiator during polymerization. This in turn disrupts the surface structure and increases surface roughness of the relining material [47]. Also, the early presence of residual monomer and its evaporation probably results in the formation of microporosities within the cold cure material, which in turn causes plaque accumulation and provides a suitable environment for growth and nourishment of microorganisms [47].

The increased microbial colonization of heat cure denture bases revealed in this study is attributed to the previously reported microporisities resulting from the compression packing technique required for processing. Also, due to the surface roughness and water sorption that enhances biofilm formation and microbial colonization [13].

The significant increase over time in the prevalence of *Candida, Streptococcus mutans,* and *Lactobacilli* in saliva of heat cure and self-cure relined denture wearers sharing in this study is in acceptance with the results of earlier studies providing a direct correlation between time and microbial prevalence in the presence of these denture base materials [47]. However, this increase was insignificant in patients rehabilitated with ThermoSens and nano ZrO2 denoting their biocompatibility [41,48,43].

The result of this study revealed significant increase of microbial count in diabetic versus non diabetic denture wearers which may be attributed to their lowered immunity and due to affinity of microorganisms to nourish in the unstable blood glucose level of diabetic compared to non-diabetic patients [4,34].

The immune system is the physiologic mechanism that allows human body to recognize the response to foreign objects and reactions. In the oral cavity, the mucosal immune system constitutes the first line of immune protection [49]. This action is usually carried out by the salivary immunoglobulin A (s-IgA) which is the predominant secretory immunoglobulin in the oral cavity[50]. Hence, it is used in this study to assess the immune status against bacterial and fungal growth in relation to different denture base materials.

The high level of s-IgA detected in the saliva of all denture wearers after denture insertion is probably due to the protective mechanism against the change in oral ecology and bacterial colonisation on both oral mucosa and dentures in an attempt to combat the microbial metabolism and adhesion to the oral tissue [51,52]. This is in agreement with previous reports that s-IgA acts as the first line of protection against pathogens that attack mucous membrane

in an attempt to prevent the adherence of bacteria and to neutralise their enzymes and toxins [52]. The significant positive correlation between the increase in s-IgA and microbial count, could be explained by the inherent attempt of the immune system to reduce infection.

The increase in the levels of s-IgA could also be correlated to the increase in candida counts which occurred after denture insertion [29]. However, this result disagrees with other studies that concluded that, despite the major role of s-IgA in the immune system, the level of s-IgA was not correlated to either the type or amount of candida prevalent after denture insertion [53].

The increase in the s-IgA level in patients rehabilitated with acrylic resin denture bases was previously proved by many studies and were correlated to clinical manifestations as mucosal irritation and allergic response caused by the monomer content of acrylic resin [54].

The insignificant increase in the level of s-IgA detected in patients rehabilitated with nano-ZnO2 reinforced and ThermoSens denture bases compared to heat cure and self-cure bases is probably due to the lowest microbial CFUs detected on their surfaces in this study that previously explained in nano-ZnO2 reinforced dentures by the antibacterial and antifungal properties of the material and monomer free content of ThermoSens denture bases. The monomer free content of ThermoSen denture bases produces less mucosal irritation and less need for imitating an immune response[55].

The increase in s-IgA detected after insertion of heat cured dentures is probably due to the microbial growth rather than monomer irritation since leaching of residual monomer was proved to regress after acrylic resin processing. However, the results revealed insignificant increase in s-IgA which could be explained by the meticulous oral and denture hygiene care measures carried out during this short term study. For this reason, it is recommended to carry out long term studies to assess the effect of long term use of dentures and the probability of formation of plaque and or microbial adhesion on different denture base materials.

The significant increase in s-IgA levels detected in this study in controlled diabetic compared to non-diabetic patients is in agreement with previous studies [30,56]. Other studies proved the significant increase in s-IgA only in the presence of denture stomatitis in diabetic compared to non-diabetic patients[57]. This finding was previously correlated to local factors as calculus and bacterial plaque accumulation usually clinically evident in diabetic patients [58].

CONCLUSION

Based on the outcomes of this study, the following could be concluded:

1. The insertion of complete denture induces an increase in the level of salivary *Candida, Streptococcus mutans* and *Lactobacilli* as well as salivary immunoglobulin A secretion.
2. Nano zirconia oxide reinforced acrylic bases and ThermoSens bases cause a significant lower level of salivary *Candida, bacteria* and s-IgA compared to heat cure resin and self-cure relined denture bases.
3. Complete denture insertion causes more increase in the level of salivary *Candida, bacteria* and s-IgA in diabetic patients compared to non-diabetic patients.
4. The recently introduced nano zirconia oxide reinforcing and ThermoSens denture bases were associated with insignificant increase of salivary level of *Candida, bacteria* and s-IgA even after three months of insertion. These results reflect their better microbiological and immunological effects in diabetic patients.

RECOMMENDATION

Further long term studies and more clinical assessments are recommended to encourage the use of Vertex ThermoSens and nano zirconia reinforced resin as denture bases.

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