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RESEARCH ARTICLE

MICROBIOLOGICAL ASSESSMENT OF CAD/CAM FABRICATED DENTURES & ITS CORRELATION WITH SURFACE PROPERTIES: A RANDOMIZED CLINICAL TRIAL

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Abstract

Background: Computer-aided design and computer-aided manufacturing (CAD-CAM) techniques have lately become a popular treatment option for complete dentures fabrication. The two principal CAD-CAM techniques; milling and 3D printing used in complete dentures construction have been approved and documented in showing clinically good results. Surface characteristics of dentures fabricated by these new techniques have a great effect on microbiological adherence to denture fitting surfaces.

Aim: As other clinical trials and/or in-vitro studies evaluating the microbiological effect and its correlation with the surface roughness of the two advanced manufacturing techniques and comparing it with the conventional technique are lacking. Thus, this study aimed to further assess the microbiological and surface properties of different widely used denture base materials.

Methodology: Thirty-six completely edentulous patients were selected and divided randomly into three groups; Group I patients received conventional complete denture, Group II patients received CAD/CAM milled complete dentures and Group III patients received 3D printed complete dentures. All denture`s surface roughness were evaluated, also all patients were recalled after 3, 9 & 12 months respectively to evaluate the microbiological adherence.

Results: Microbiological count significantly increased ($P < 0.05$) after 12 months in all groups, after 12 months there was a significant difference ($P < 0.05$) between three groups as group II (Milled) was significantly the lowest, then the group I (conventional), while group III (3D printed) was significantly the highest. Regarding surface roughness of group II (milled) was significantly the lowest, while group III (3D printed) was significantly the highest. Finally, there was a strong positive significant correlation between microbiological adherence and surface roughness in all groups as ($r > 0.5$).

Conclusion: Group II (Milled) appeared to be the best regarding microbiological adherence and surface roughness followed by the group I (conventional) and finally group III (3D printed). Furthermore, it was evident that surface roughness has a great effect on

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microbiological adherence regardless of the fabrication technique utilized.

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Introduction:-

Although several drawbacks are associated with the conventional denture fabrication method, complete denture is still the most widely used treatment option among edentulous patients. Surface roughness is one of the most common problems associated with complete dentures due to its noticeable effect on microbial biofilm accumulation, as any increase in surface roughness allows for microbial adhesion easily, which is in turn difficult to be eliminated from narrow inaccessible areas as pits and pores. [1-3]

The microbial adhesion to denture bases is directly correlated to its surface roughness and its surface-free energy after fabrication. Meanwhile, surface properties as surface charge, substratum chemistry, and substratum stiffness have a little effect on microbial adhesion that may harbor opportunistic pathogens such as *Candida albicans, Streptococcus mutants & Staphylococcus Mutans,* which may initiate several diseases such as denture Stomatitis as demonstrated by several epidemiological studies which revealed that 20%-70% of patients wearing removable denture prostheses suffer from denture stomatitis [4-6]

To overcome the problems associated with the conventional method of denture fabrication and to improve polymethylmethacrylate (PMMA) material properties, many attempts have been made and recently digital dentures fabrication has been widely utilized to improve the surface roughness & minimize microbial adhesion to denture materials as possible [7,8]

The digital methods of denture fabrication represent the latest advancement that has been introduced using the computer-aided-design/computer-aided-manufacturing (CAD/CAM) technology including 3D printing and milling, which is considered as the new era for removable prosthodontics candidates. Moreover, it involves the digitization of the patient scanned intraoral structures using special software. Finally, the digitally designed denture is saved as a Standard Triangulation Language (STL) file and afterward, it is manufactured using either subtractive manufacturing (SM) or additive manufacturing (AM). [9]

Subtractive manufacturing (SM) or CAD/CAM milling has been used to produce dental prostheses from a solid block such as crown, bridges, implants, and removable dentures. This milling method of a ready-made disk under high pressure presents a high degree of accuracy because there is no polymerization shrinkage, reduces the number of pores, improves adaptation, and offers better surface characteristics than conventional acrylic resin which accordingly enhances its biocompatibility and lessens its microbial adhesion susceptibility. [10,11]

Furthermore, milled prostheses offered other advantages as, fewer visits number, more speedy denture fabrication with fewer work process steps which in turn reduce the rate of error incidence. In addition, duplication of old dentures is easier than conventional methods. On the other hand, the main disadvantage is related to financial aspects as each denture base resin disc allows for only one denture base fabrication and the utilized milling burs become extremely dull because of the lengthy milling time required for each denture base. [12,13]

On the other hand, additive manufacturing (AM) or 3D printing is an advanced method of manufacturing a dental prosthesis by laminating and molding photopolymerizable resin and metal powder. Also, it is widely used for making surgical guides and printing models accurately. Recently, it has been used in the fabrication of artificial teeth and a denture base separately using a three-dimensional (3D) printer and finally bonding both together with a special type of photopolymer resin. Moreover, the 3D printers' cost is much less with shorter printing time than the milling technique, less rotary tools wear, less waste of raw materials and furthermore, it allows for the manufacturing of several dentures simultaneously. Thus, it is more economical with greater opportunities of usage in dental laboratories and clinics [11,14]

The present study aims to assess and compare the microbiological properties and surface roughness of 3D printed and CAD/CAM milled complete dentures as compared to the conventional heat-cured dentures treatment modality.

Materials & Methods:-

Study design:

This randomized clinical trial was carried out in the Department of Removable prosthodontics Faculty of Oral and Dental Medicine, MSA University and in the Department of Fixed & Removable Prosthodontics at National Research Center. The participants of this study were thirty-six patients who randomly and equally distributed among three groups according to complete denture fabrication technique: (1) conventionally constructed complete denture, (2) CAD/CAM milled complete denture, (3) 3D printed complete denture.

Ethical Approval:

This study was designed and approved by Research Ethics Committee – Cairo university (registration number: 1798), Cairo-Egypt, which is in accordance with The Helsinki Declaration of 1975. All participants were informed about the nature of this research work, clinical steps and their approvals were obtained through written consent (Ethics committee at National Research Center).

Sample size calculation

The sample size was calculated using PS program according to the previous study ***(Dina Kholiefand Shereen 2019),*** whenthe standard deviation is 1.6, the true mean difference between experimental and control groups is 2.1 with 80% power & 0.05 type I error probability, accordingly the minimally accepted participants were 12 per group after adding 20% for dropout. The total needed participants in this trial were 36 patients in three groups. [15]

Inclusion & exclusion criteria:

All selected participants were completely edentulous patients fulfilling inclusion criteria by recording full medical and dental histories in addition to intra-oral examination. All included patients should be cooperative, males, 45-60 years old patients, free from systemic diseases or oral conditions, good oral hygiene, with Angle`s class I maxilla-mandibular relationship, with adequate inter-arch space and firm sound mucoperiosteal residual alveolar ridges of adequate height and width. Smokers or patients receiving any medication such as antifungal, antibiotic, and antidepressant drugs in the 6 months preceding the study were excluded. Only those who showed cooperation and acceptance for compliance to treatment and recall were accepted in the study.

Randomization, allocation, and blinding

Patients included in the current trial were randomly allocated in one of the 3 groups (simple randomization 1:1 ratio) through sequence generation and allocation concealment. Sequence generation was performed using Rondom.org.online software, while allocation concealment was performed using folded paper & envelope technique. The statistician was blinded about the manufacturing technique of the received denture.

Patients grouping:

Denture construction:

Group I (conventional heat-cured acrylic resin denture):

Preliminary impressions were made using irreversible hydrocolloid impression material. Silicone impression material (poly-C-silicone impression material, thixoflex M, medium, Zhermack, Italy) was used final impressions making after border molding with (Putty-C-Silicone). Impressions were disinfected, boxed, and poured in dental stone.

Then maxillary and mandibular casts were mounted on the articulator using a maxillary face bow, and the centric jaw relation record was made for the patient at the predetermined vertical dimension of occlusion by using the wax wafer technique. Crosslinked acrylic resin teeth were selected according to the patient`s demands then, setting-up of the artificial teeth was carried out. Waxing up was done, and the waxed-up denture was checked in the patient’s mouth.

The denture was fabricated from heat cure acrylic resin (Acron Duo, Associated Dental Products Ltd., Kemdent, Purton, Swindon, Wiltshire, UK). Denture processing was done using a long polymerization cycle, 9 hours in a water bath at 73°C ±1°C, followed by 30 minutes in boiling water as recommended by the manufacturer then, deflasking, finishing, and polishing of the dentures were done following the conventional routine method.

Finally, denture delivery was carried out and the finished denture was checked for proper extension, retention, and stability. The patient was informed about the proper protocol for denture insertion and oral hygiene measures. Routine follow-up appointments were scheduled for two days and one week after placement.

Group II (Milled complete denture using CAD/CAM technique):
After the initial examination, primary impression, final impressions & maxillo-mandibular relationship were recorded following the conventional steps of complete dentures. The master models and the occlusal rims were prepared for scanning with scan spray. Scanning was performed with an optical 3D scanner (3shape E2, Holmenskanal 7 Copenhagen).

The files from the laser-scanned master models and connected occlusal rims were translated into stereolithography (STL) files as presented in figure (1). Complete dentures were virtually designed in CAD software (EXOCAD GmbH, Germany) in upper and lower permanent denture bases with suitable teeth dimensions chosen from the software library as presented in figure (2). The virtual denture (with the digital smile design integrated) was then sent as an electronic preview to the clinician and patient via social media (email, WhatsApp, or Facebook messenger) for approval.

The digital denture was presented as four STL files, upper denture base, and lower denture base, upper and lower set of teeth. In both CAD CAM groups, the teeth STLs were milled fabricated by the command software of the milling machine (SHERA eco-mill 5x milling machine) from pre-polymerized resin acrylic blanks (TSM Acetal Dental) as shown in figure (3). After milling procedure, the teeth were removed from the blank as well as their support rods, finished and polished to be ready for bonding them to their perspective sockets of the denture bases.

The upper and lower denture base STLs with their perspective sockets were also milled from another PMMA denture blanks, then finished and polished according to the manufacturer instructions to be ready for the teeth sets to be bonded and inserted in their perspective sockets as shown in figure (4). Assessment of the occlusion was made with articulating paper, chair-side adjustments were made and minor premature contacts were corrected.

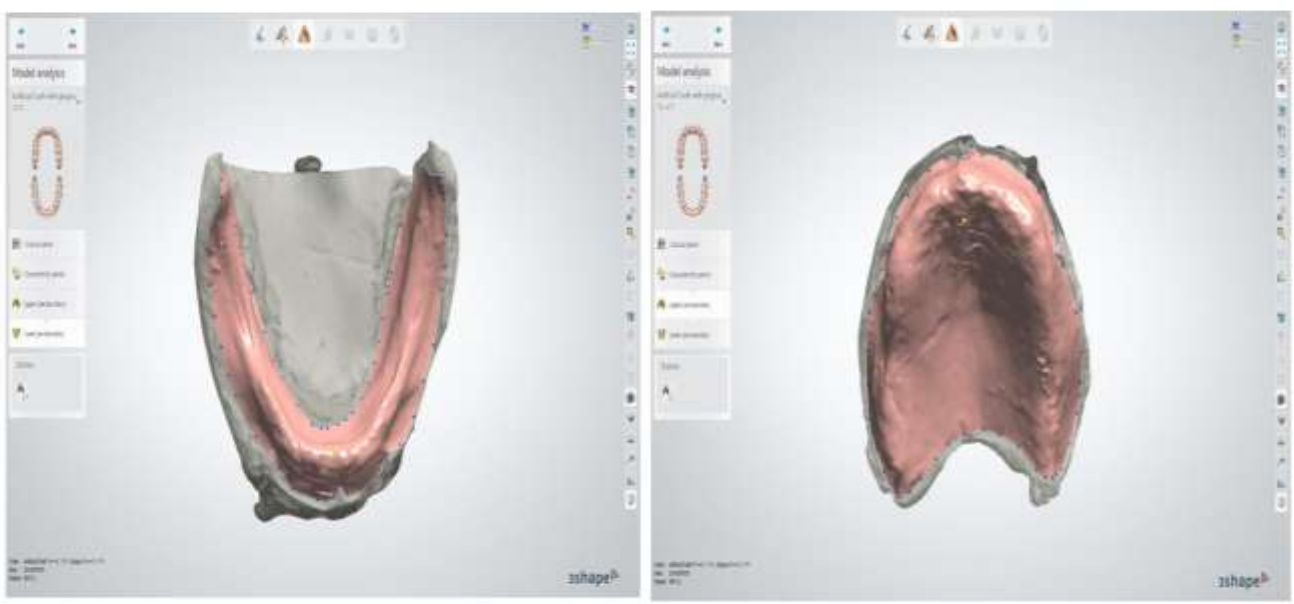

Figure (1):- The stereolithography (STL) file translated from the laser-scanned master models and connected occlusal rims.

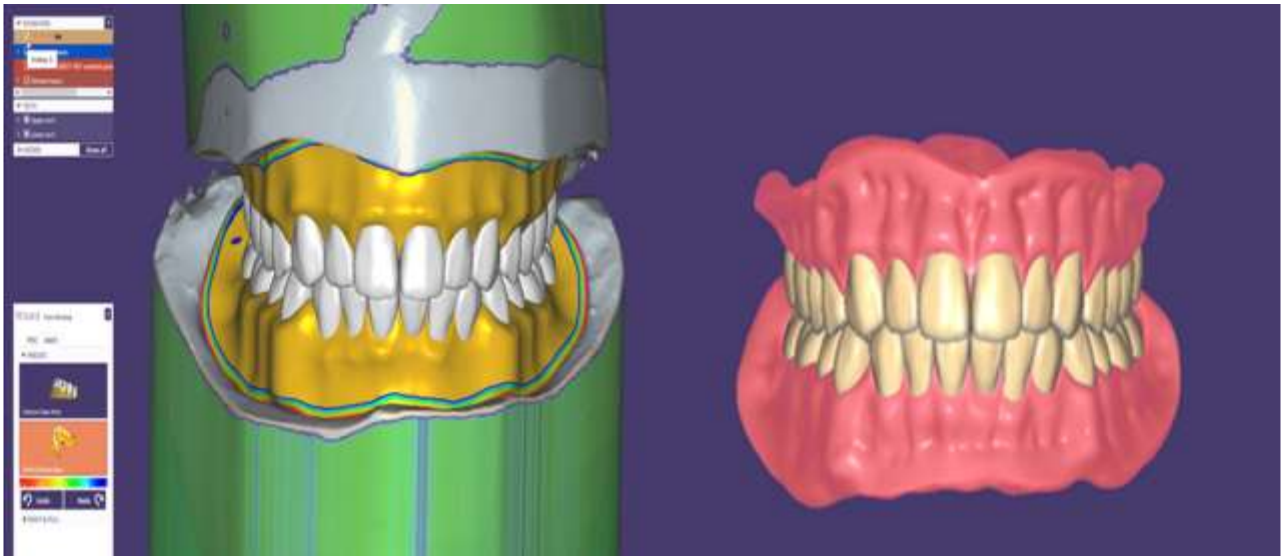

Figure (2):- Virtual designs of the complete denture by using CAD software.

Figure (3):- Milled upper and lower set of teeth.

Figure (4):- Milled denture bases.

Group III (3D printed complete denture using CAD/CAM technique):
For the 3D-printed workflow, the scanned denture STL files were 3D then designed using (EXOCAD GmbH, Germany). A subsequent preview was generated after the incorporation of any changes suggested by the clinician and approved. Once the design was approved, the file sent to the 3D printer.The denture bases were fabricated from a printable resin (NextDent Denture 3D+; NextDent B.V.) by using a 3D-printer (NextDent 5100; NextDent B.V.) that utilizes Digital Light Projection Manufacturing (DLPM) equipment. The denture bases were printed in a vertical orientation with a layer thickness of 100 microns, with the location and number of support struts being automatically generated by the software program as shown in figure (5). After each layer was cured, the 3D printer's platform with the cured structure attached moved upper and another layer of uncured liquid resin was spread over the bottom until the final construction was built. After printing, the denture bases were separated from the build platform with a putty knife and the support struts were removed. The bases were ultrasonically cleaned in isopropyl alcohol for 5 minutes, dried, and then placed in an ultraviolet light unit (LC-3DPrint Box; NextDent B.V.) for 30 minutes for post-polymerization. Teeth set STL files were milled and bonded to the printed denture bases according to the manufacturing instructions as mentioned in the milled group.

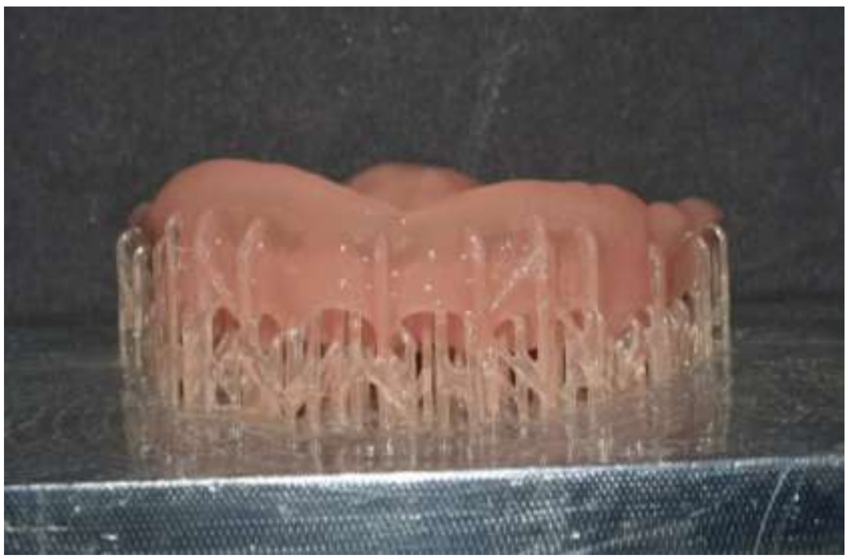

Figure (5):- 3D printed denture bases denture bases.

Patients' instructions & follow up:
The patients were instructed not to change their routine oral hygiene measures and not to take out the dentures at least four hours before the sampling.

Microbiological assessment:
Swabs were obtained by vigorous rubbing of all the fitting surfaces of the maxillary complete dentures by using sterile cotton-tipped wooden swabs for each patient, for 30 sec. to facilitate microorganism's isolation. The swab was inoculated immediately in a sterile glass tube containing 1 ml of sterile saline and transferred into a dry container to the laboratory within one hour.

The sample was then plated on the Sabouraud's dextrose agar for isolation of *Candida Albicans*, while MacConkys agar is used as a differential media for isolation of *Staphylococcus Aureus (S. Aureus), and Streptococcus Mutans (S. Mutans).* The plates were placed in a special incubator * (VWR Shel-Labs 1530 Lab Incubator-China) at 37℃ after 48 hours incubation period, the dishes were taken out from the incubator and were observed for the tested microorganisms.

The microbial evaluation was made through counting the number of colonies on the surface of the agar gels and Colonies forming unit per ml (CFU/ml) was calculated according to the following formula: CFU/ml= total number of colonies counted in the plate X inversion of the saline dilution X inversion of the cultured volume X 1000.

Surface roughness evaluation:
Samples were photographed by using the USB Digital microscope with a built-in camera as presented in figure (6) and connected to an IBM compatible computer using a fixed magnification of 50x. Microsoft office picture manager was used to specify and standardize roughness measurement area. Cropped images have been analyzed using WSxM software**18.

Figure (6):- Digital microscope with a built-in camera.

Statistical analysis
It was performed using Statistical Package for Social Science (IBM SPSS) version 23 and Graph Pad Prism version 8.0.2 (263). All data were presented as mean, standard deviations. Independent t-test was used to compare between three groups. While repeated measure ANOVA test was used to compare between different follow up periods among each groups separately. The p-value ≤0.05 was considered significant.

Results:-

Comparison between microbial changes during follow-up period among 3 tested groups was made using Repetitive One-Way ANOVA followed by Tukey1s Post Hoc test which revealed a significant increase in all microorganisms among three groups after 12 months as $P < 0.05$, as presented in table (1).

Also, different groups comparison was performed by using One Way ANOVA test which revealed a significant difference as $P < 0.05$, followed by Tukey`s Post Hoc test which revealed that, changes from baseline – 3 months group II (Milled CD) was significantly the lowest while there was insignificant difference between group I (conventional) and group III (3D printed) in Strept.Mutans&Staph. Auras, while in Candida Albicans there was a significant difference between all groups as group II (milled) was the lowest and group III (3D printed) was the highest.

Regarding microbiological changes from 3 months – 9 months, significant difference was only found between groups II & III, as group II (milled) was significantly the lowest and group III (3D printed was significantly the highest in Strept.Mutans & Staph. Auras, while in Candida Albicans group II (milled) was significantly the lowest while there was an insignificant difference between group I & III.

While in microbiological changes from 9 months – 12 months, there was a significant difference between all groups as group II was significantly the lowest while group III was significantly the highest as presented in table (2) and figure (7).

The surface roughness of the tested groups was compared by using One Way ANOVA test which revealed a significant difference between them followed by Tukey`s Post Hoc test which revealed that group II was significantly the lowest while there was an insignificant difference between group I & III. Moreover, the correlation between surface roughness and microbial adherence was calculated and revealed strong ($r>0.5$) positive (+) significant ($P<0.05$) correlation in all groups, as presented in table (3) and figure (8).

Table (1):- Microbiological & candidal changes during different follow up visits in all groups:

Group	Microorganism	Baseline – 3 months		3 months – 9 months		9 months – 12 months		Repetitive One- Way ANOVA P value
		MD	SD	MD	SD	MD	SD	
-Group I Conventional complete denture	Strept.mutans	63.7 [a]	10.3	210.3 [b]	55.7	569.4 [c]	151.2	0.0001*
	Staph. Auras	47.6 [a]	8.7	183.7 [b]	47.5	501.9 [c]	137.4	0.0001*
	Candida albicans	10.5 [a]	1.1	150.6 [b]	33.4	425.8 [c]	111.3	0.0001*
Group II Milled complete denture	Strept.mutans	51.2 [a]	8.1	170.2 [b]	41.1	510 [c]	120.2	0.0001*
	Staph. Auras	34.2 [a]	6.5	143.1 [b]	37.4	410.1 [c]	117.1	0.0001*
	Candida albicans	6.2 [a]	1.3	111.2 [b]	23.2	370.5 [c]	90.8	0.0001*
Group III 3D printed complete denture	Strept.mutans	66.1 [a]	11.2	222.5 [b]	58.1	578.1 [c]	149.2	0.0001*
	Staph. Auras	54.6 [a]	8.5	201.4 [b]	41.5	509.1 [c]	139.1	0.0001*
	Candida albicans	12.5 [a]	2.2	160.1 [b]	41.1	430.1 [c]	115.1	0.0001*

MD; mean difference between two successive intervals SD: standard deviation

*P value was significant as it was ≤ 0.05 by using Repetitive One- Way ANOVA test.

Means with the same superscript letters were insignificantly different as P > 0.05 by using Tukey`s Post Hoc test.

Means with different superscript letters were significantly different as P < 0.05 by using Tukey`s Post Hoc test.

Table (2):- Comparison between three groups regarding Microbiological & candidal changes during different follow up visits.

Group	Microorganism	Group I Conventional complete denture		Group II Milled complete denture		Group III 3D printed complete denture		One- Way ANOVA P value
		MD	SD	MD	SD	MD	SD	
Baseline – 3 months	Strept.mutans	63.7 a	10.3	51.2 b	8.1	66.1 a	11.2	0.001*
	Staph. Auras	47.6 a	8.7	34.2 b	6.5	54.6 a	8.5	0.0001*
	Candida albicans	10.5 a	1.1	6.2 b	1.3	12.5 c	2.2	0.0001*
3 months – 9 months	Strept.mutans	210.3 ab	55.7	170.2 a	41.1	222.5 b	58.1	0.04*
	Staph. Auras	183.7 ab	47.5	143.1 a	37.4	201.4 b	41.5	0.006*
	Candida albicans	150.6 a	33.4	111.2 b	23.2	160.1 a	41.1	0.002*
9 months – 12 months	Strept.mutans	569.4 a	130.1	450 b	115.4	578.1 c	132.2	0.03*
	Staph. Auras	501.9 a	137.4	362.7 b	117.1	509.1 c	139.1	0.01*
	Candida albicans	425.8 a	111.3	314.8 b	90.8	430.1 c	115.1	0.01*

MD; mean difference between two successive intervals SD: standard deviation

*P value was significant as it was ≤ 0.05 by using Repetitive One- Way ANOVA test.

Means with the same superscript letters were insignificantly different as P > 0.05 by using Tukey`s Post Hoc test.

Means with different superscript letters were significantly different as P < 0.05 by using Tukey`s Post Hoc test.

Table (3):- Comparison between three groups regarding surface roughness and correlation with microbiological adherence:

	Surface roughness (µm)			
	M	SD	r	One Way ANOVA test P value
Group I Conventional complete denture	0.32 a	0.09	0.87*	0.001*
Group II Milled complete denture	0.21 b	0.07	0.91*	
Group III 3D printed complete denture	0.35 a	0.11	0.93*	

M: mean SD: standard deviation

*P value was significant as it was ≤ 0.05 by using Repetitive One- Way ANOVA test.

r: Pearson's correlation

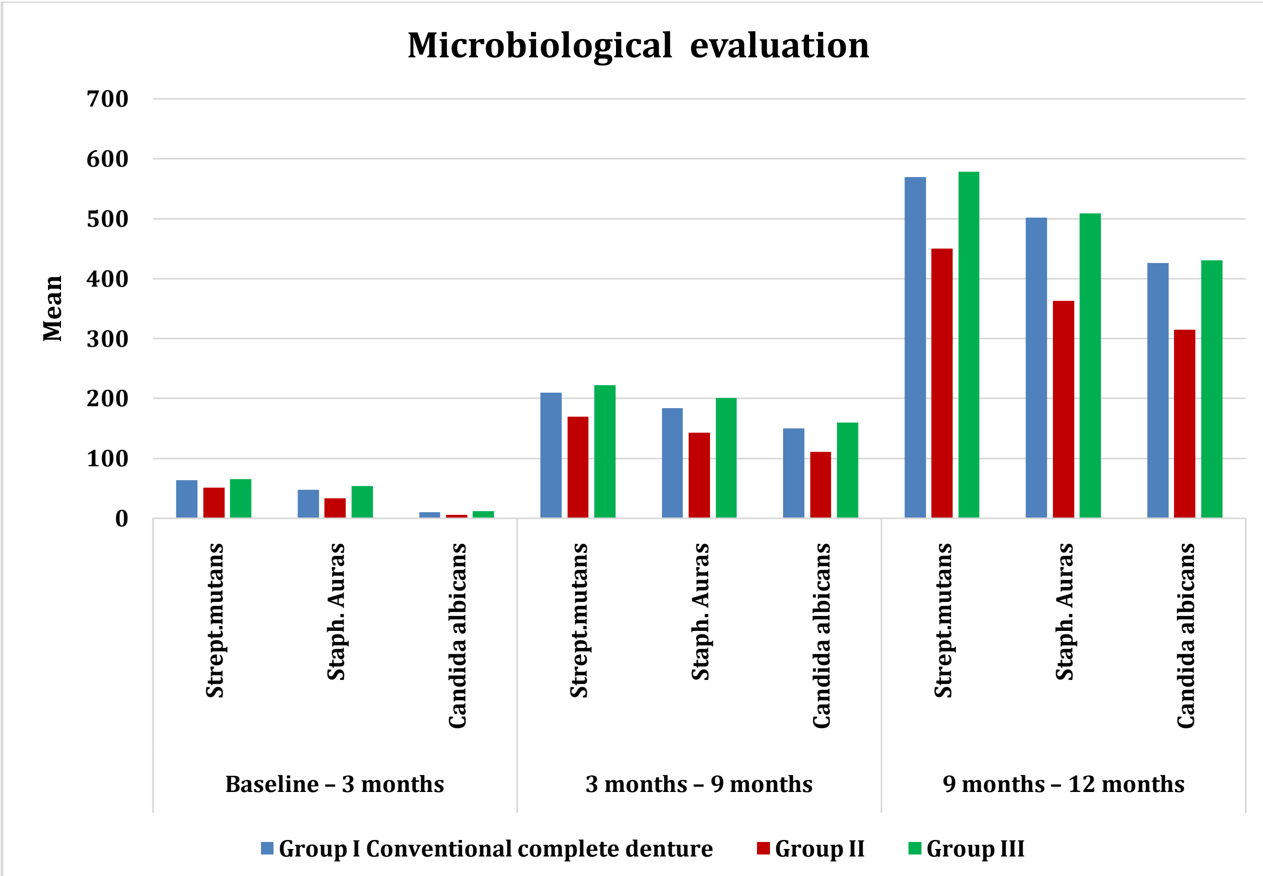

Figure (7):- Bar chart representing microbiological changes of count Strept.Mutans, Staph. Auras & Candida Albicans among different intervals in three groups.

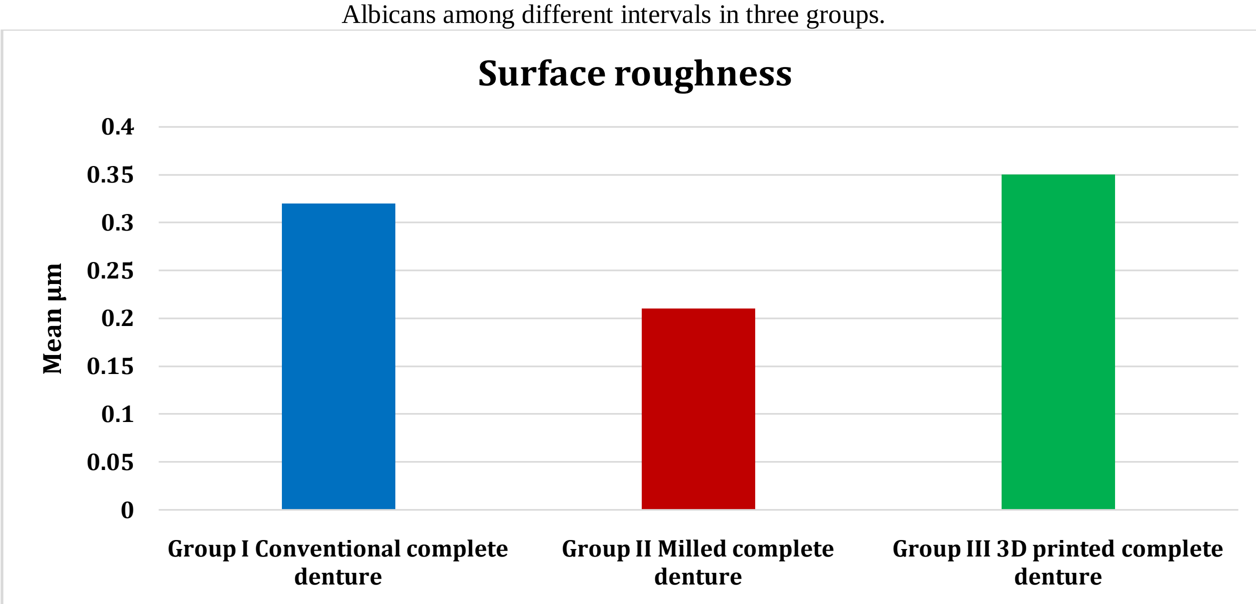

Figure (8):- Bar chart representing surface roughness (µm) in three groups.

Discussion:-

Despite the rapid advancements in dental implants, many completely edentulous patients cannot afford or are not good candidates for implant therapy thus, they have no other options except conventionally fabricated complete dentures treatment modality which is the cheapest and easiest alternative. In this study comparison between 3D printed & milled dentures regarding microbiological adherence & its relation to surface roughness was performed as it has rarely been reported and limited by in vitro designs and heterogeneous methodologies.[16]

The rapid progress of digital dentistry allowed for CAD/CAM technology usage for dentures construction where it converts all clinical steps into digital workflow, the current study combines the advantages of CAD/CAM technology and conventional techniques. All clinical steps converted to digital steps except for dental impressions; as the workflow is completely CAD/CAM driven which helps in elimination of most of the disadvantages of each laboratory step required for conventional denture fabrication and helps in work simplification in addition to, reduction of the number patient visits. Meanwhile, the dental impression was obtained following conventional technique to avoid intraoral scanning of edentulous arches which represents a great challenge owing to soft tissues dynamic movements, that influence the quality of the direct digital impressions making.[17-19]

Heat-polymerized acrylic resin was used as a control group, because it is the most popular denture base material, and it has been found that heat polymerized acrylic resin has more compatible chemical properties than auto-polymerized acrylic resin. [20]

Furthermore, using pre-polymerized acrylic resin block which is a high-density polymer based on highly cross-linked PMMA (polymethyl methacrylate) acrylic resins in CAD/CAM technique is considered as the main advantage over the conventional technique, as it is eliminating shrinkage possibility and further presents a decrease in the porosity and surface roughness than conventional heat-cured acrylic resin. [21,22]

However, it is well known that all removable dental prosthesis act as a reservoir that harbors a mixed species of bacterial biofilm, as the denture base is easily colonized by oral endogenous bacterial and candida species as well as extra-oral species. Thus, this microbial reservoir can be the main cause for denture-related stomatitis, aspiration pneumonia, and cardiovascular diseases particularly bacterial endocarditis and many other life-threatening infections, mainly in elder patients. [23-25]

Therefore, the prevention of microbiological and candida biofilm formation is an issue of extreme importance. Incorporation of antimicrobial nanoparticles into the denture base acrylic resin has been addressed by several researchers. However, these nanoparticles may affect the material physical properties. Other researchers have used various coating materials to prevent microbial adhesions but without doubt, these coatings will wear off with time. Therefore, the need for improved materials and methods of fabrication is still of high priority. Accordingly, investigating oral microflora for the removable dental prostheses wearers and factors affecting it as surface characteristics becomes a very important issue. [6-8]

As the intra-individual variations in the bacterial counting were very critical. Therefore, the mean difference between the microorganisms counts before insertion of the denture and, after 3, 9 and 12 months was calculated. The results of the current study revealed that microbial count significantly increased in all groups after 12 months, which may be attributed to the surface roughness of the materials, as the aging process increases the roughness of the denture base surface. [26]

In this study, milled conventional denture revealed lower significant microbial flora after 1 year than conventional denture which may be due to less residual monomer release and less porous pre-polymerized PMMA acrylic resin blocks used in manufacturing due to the use of polymerized acrylic resin under special conditions, which is high pressure and temperature, accordingly, the CAD/CAM dentures may reduce the microbial colonization, compared to conventional denture processing techniques. This is in accordance with other studies, which reported that the initial adhesion of microorganisms usually begins in the rough surface pores , which are known to provide protection against shear forces and provide time for irreversible adhesion of microbial cells to the surface. Murat et al. showed that CAD/CAM PMMA-based polymers have less surface roughness when compared to conventional polymerized PMMA and thus, such materials reveal much less Candida adhesions. [7-10]

Moreover, the milled complete dentures revealed lower significant microbial flora than 3D-printed dentures after one year, which may be attributed to the fact that 3D printing is less accurate than milling as demonstrated by several studies which concluded that milled dentures were superior to the 3D-printed complete dentures in terms of trueness, as milled dentures showed higher trueness & precision; (3D printing was less true than milling by 17–89 μm and less precise by 8–66 μm). [11-13]

The results of this clinical study suggest that, 3D printed dentures may enhance microbial adhesion compared with conventional heat-cured dentures, thus increasing the risk of developing denture stomatitis and other Candida albicans related problems whereas CAD/CAM milled dentures decrease those risks. [11, 27]

It is to be mentioned that printing orientation has a significant influence on the printing accuracy, flexural strength, roughness and response to C. Albicans. Thus, to fabricate products with appropriate properties, the printing orientation should be carefully selected. [27]

Therefore, both milling and 3D printing may be considered clinically acceptable. However, for clinical recommendations, a comprehensive evaluation of all advantages and disadvantages, including the equipments used and infrastructure costs is required, with special attention given to accessibility and usability especially in a worldwide scenario where millions of people, particularly in developed countries are in an utmost need for feasible removable prostheses. [28]

Conclusions:-

Within the limitations of this clinical study, the following conclusions were drawn:

1. Milled dentures revealed to be the best regarding microbiological adherence and surface roughness.
2. 3D printed dentures are better than milled dentures regarding cost-effectiveness.
3. Surface roughness has a great effect on microbiological adherence regardless of the fabrication technique utilized.

Conflicts of interest

There are no conflicts of interest to be declared.

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Nil.

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