

Comparison of predominant cultivable microflora on acrylic denture base resin cured by two different techniques



Sulaimani Dental Journal

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Abstract

Objectives: To characterize the prevalence of normal microorganisms on complete upper dentures on successive weeks after insertion reaching a stable condition. Also, to find out the effect of the microwave curing system on the periodic microbiological growth on complete upper dentures and compare them with the results obtained from the water-bath curing method.

Materials and Methods: Ten healthy fully edentulous patients were selected. A comparative micro flora on acrylic resin samples cured by two ways: the water-bath curing method and microwave curing energy. The samples fixed in the fitting surface of complete upper denture. The micro flora were studied during four experimental periods starting from zero time, the time just before the insertion of new complete dentures till the 6th week of denture insertion. The data were analyzed using SPSS software with T-test, and the differences were considered statistically significant at $P < 0.05$.

Results: Various microorganism species isolated from both samples, include streptococci, staphylococci, Gram-positive rods, Gram-negative rods and cocci.

The microwave samples appeared to support significantly more aerobic Gram-positive rods and anaerobic Gram-negative rods, but less aerobic and anaerobic streptococci, Gram-negative cocci, and aerobic staphylococci. No candida growth could be identified before and after denture insertion. In spite of oscillate population of the bacteria, it has been noted it tends to stabilize on two subjects at the 10th week.

Conclusions: It was difficult to decide which one of the either methods of curing acrylic resin is better in harboring fewer microorganisms. The number of both aerobic and anaerobic microorganisms significantly increased as the period of wearing denture is prolonged. Early plaque contains different kinds of bacteria; the majority was Gram-positive rods. No candida growth was detected.

Keywords: Microorganisms, microwave, water-bath, acrylic resin, complete denture.

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Received: September, 2015

Accepted: November, 2015

Published: December, 2015

Cite this article as: AL-Kaisy N, Salem SA. AL-Zubaidy TS. Comparison of predominant cultivable microflora on acrylic denture base resin cured by two different techniques. Sulaimani Dent J. 2015;2(2):113-120.

Introduction:

A water-bath curing unit is most commonly used to process acrylic resin denture base materials⁽¹⁾. Processing the denture base materials by other methods was also used. Among these the water vapor method, dry heat, chemical curing by pouring technique and visible light curing method⁽²⁾.

The polymerization of poly methyl methacrylate resin by using microwave energy was also recommended, as a good source of curing acrylic resin, since it requires easy processing procedures, cleaner, and time-saving^(3,4). Several investigators studied the mechanical and physical properties of resins cured by the microwave oven and compare them with those cured by ordinary water-bath. These properties include hardness, porosity⁽⁵⁾, transverse strength and flexural strength⁽⁶⁾, and adaptability⁽⁷⁾. They could demonstrate

that the important physical and mechanical characteristics of the microwave-cured resin are approximately the same as the resin cured in a conventional water-bath. However, to the author knowledge, a comparison of predominant micro flora on dentures cured by both curing cycles did not get attention previously.

The normal mouth in dentulous and edentulous denture users contains different kinds of micro flora in large numbers with different species, which, plays an important role in maintaining a healthy oral mucosa⁽⁸⁾. They may reach to about 700 bacterial and fungal species that the majority have not yet been isolated and differentiated⁽⁹⁾.

In spite of the clinical importance, knowledge about denture plaque mainly restricted to the presence and possible role of yeast. However, the microflora of denture plaque has been shown to be predominantly bacterial caused by Gram-positive and Gram-negative cocci forms, and *Candida* was only rarely observed⁽¹⁰⁾.

Once the early colonization of the fitting surface of the denture, particularly the maxillary denture occurred, microbial succession would take place in the normal mouth and cause bacterial plaque. Denture plaque considered to be the most significant of all the oral deposits and has identified as the primary etiologic factor of denture stomatitis⁽¹⁰⁾. And in the establishment of several systemic infections such as urinary tract infections, pneumonia, abscess and even endocarditis^(11,12).

Dental prostheses may act as a potential source of infection in elderly denture users. Many adaptable microorganism species are capable of adhering and surviving on the denture acrylic resin surfaces. Among these are *Streptococcus*, *Staphylococcus*, *Candida*, *Lactobacillus*, *Pseudomonas*, *Enterococcus*, and *Actinomyces* associated with denture biofilm⁽¹³⁾.

The purpose of the study is to study the prevalence of normal predominant microorganisms on complete dentures on successive weeks reaching a stable condition. And to find out the effect of the microwave energy as a method of curing acrylic resin on the periodic microbiological growth on complete upper dentures and compare them with the results obtained from the ordinary water-bath curing method.

Materials and Methods:

Ten patients (eight males and two females) with age range from (52-75) years with mean age of (60.51) years included in this study. The participants attended the Department of prosthetic dentistry, College of Dentistry, University of Baghdad (Table 1).

All participants had healthy oral mucosa and prepared to receive newly constructed dentures. Individuals with poor medical condition or taking drugs (antibiotics or antimicrobial), which alter the normal micro flora were excluded.

Acrylic resin samples (Permacyl, type 1 Coe laboratories Inc. Chicago, IL 6058) measured 5mm in diameter and 2mm thickness were prepared from a wax sheet. The flasking and the packing for the wax samples were carried out using two different techniques: the water-bath (W.b) and the microwave (M.w) curing methods. W.b samples cured for 7 hrs. at 70°C followed by 3 hrs. at 100°C⁽¹⁴⁾. While the M.w samples cured at 80 watts for 10 min, then the flask was turned for another 10 min at 180 watts⁽¹⁵⁾ using National N.N 7407 microwave oven.

A preparation has done inside the fitting surface of the denture to accommodate the acrylic samples by using disk like stone bur. The size of the disk was within the same size of wax samples. The acrylic samples were fixed by using sticky wax a day before the insertion of the denture. Finally, the denture together with the samples kept in distilled water until the time of insertion (Fig.1).

For the acrylic samples to undergo the same environmental condition in the same oral cavity, the same patient should harbor both samples together at the same time. Five water-bath curing samples fixed in the right side of the denture, while, on the other side, the corresponding numbers of microwave curing samples were fixed (Fig.1).

To observe the changes in the number and prevalence of denture flora, the samples were studied during four experimental periods. Starting from zero time (the time just before the insertion of new dentures, by taking a cotton-tip swab from the palate of the patient). The first two visits have a one-week interval between them while the last two visits have two weeks interval (Fig.1).

Table 1: Descriptive information of the studying sample. M, male; F, female; --, no previous denture.

Patient's number	Sex	Age	Age of previous denture	Years of edentulism
1	F	52	--	1
2	M	52	1	3
3	M	54	2	5
4	M	55	5	10
5	F	60	--	0.5
6	M	60	--	2
7	M	63	4	1.5
8	M	64	6	2
9	M	70	15	12
10	M	75	10	11

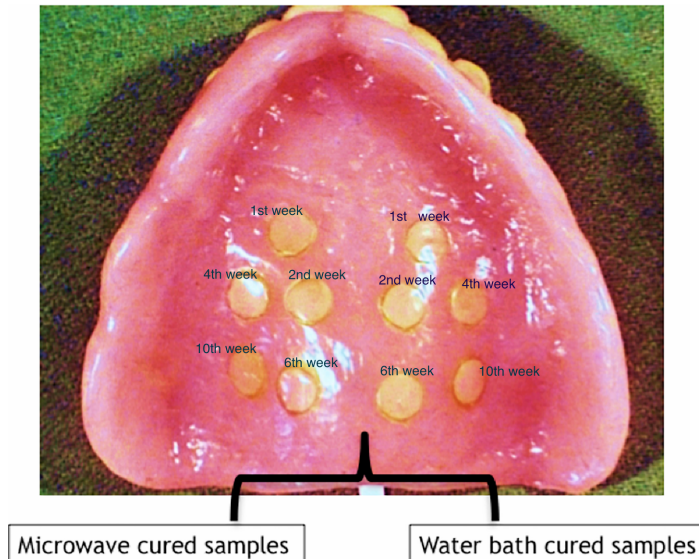


Figure 1: Water bath and microwave cured acrylic samples in the fitting surface of upper complete denture with samples removal period.

The participants were instructed not to brush the inner surface of the dentures. Moreover the use of antiseptic materials for the dentures and wearing the denture at night were avoided. These instructions were given to allow normal biofilm accumulation and colonization of the intaglio surface of prostheses by microorganisms. Those who had old dentures were instructed to leave them three days before the insertion of the new ones.

At each visit after denture insertion, one sample of each curing cycle was removed from the denture in a septic condition and inserted into a sterile transport broth of 5 ml of brain heart infusion (BHI) broth (Difco). Then sent to a microbiological laboratory for microbiological analysis within 2 hours.

Viable microorganisms counts were calculated as number/sample by plating out 1 ml of diluted suspension to 10^5 (which was determined from a pilot study that it is the most practical dilution) for microbial categories and cultured on Blood agar (oxoid,UK), MacConkey's agar (biolife,UK). These plates were incubated for 48 hrs at 37°C under aerobic conditions; another set incubated in anaerobic jar with gas pak. While for fungi growth, the Sabouraud's Dextrose agar (oxoid,UK) was used by spreading 1 ml of 10^5 suspensions and incubated in the air for 4-6 days at 37°C.

Different distinct colonies of predominant cultivable flora were picked up for isolation. Each colony was studied morphologically by staining the colonies by Gram stain. The biochemical tests were

used mainly for streptococci, while other tests such as coagulase and catalase were used for confirmation of other microorganisms such as staphylococci, Gram-positive rods, Gram-negative rods, and Gram-negative cocci. The classification of yeast (candida) depended only on their colonial morphology on Sabouraud's agar and microscopical appearance.

At last visit and after removal of last sample, relining of the upper denture was accomplished for participants.

The data analyzed by SPSS software using T-test, and the significant level set at $P < 0.05$.

Results:

Several types of microorganisms were isolated from the inner surface of the denture at zero time, the time just before denture insertion in the patient's mouth after taking swab from the middle palatal area (Table 2).

The total number of microorganisms (both aerobic and anaerobic) was increased significantly as a period of wearing denture is prolonged (0-6 weeks), regardless the method of denture curing. Besides, there was a high statistical difference in aerobic microorganisms count between the two methods (Fig. 2).

The total aerobic and anaerobic streptococci were decreased after one week of denture insertion on both samples (Fig. 3). After this period the samples cured by water-bath showed a marked increase in the number of

Table 2: Prevalence of isolated microorganisms from patients' palate in percentage before denture insertion.

	Strep.	Staph.	G-ve rod	G+ve cocci	G-ve rod	Candida
Aerobic	57.1%	71.4%	85.7%	42.8%	0%	0%
Anaerobic	57.1%	57.1%	57.1%	28.5%	0%	0%

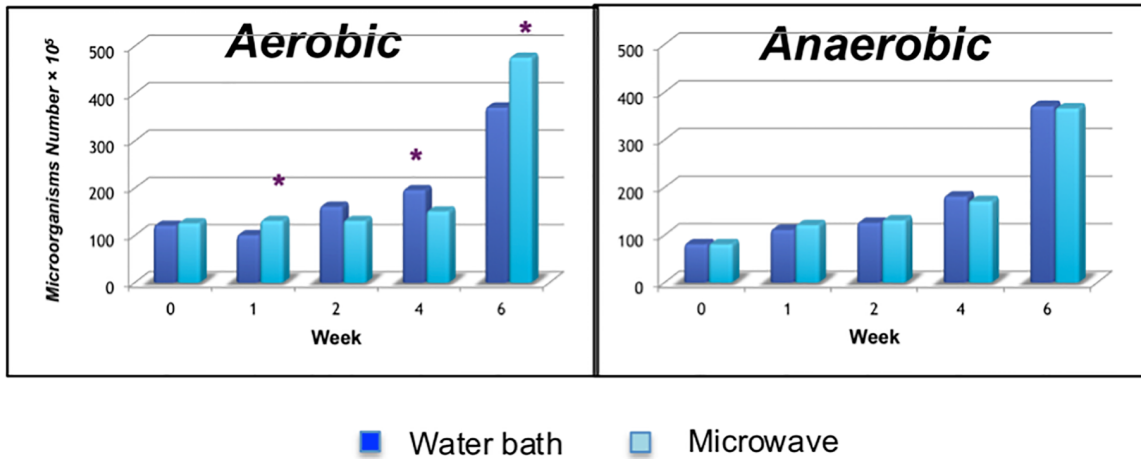


Figure 2: Comparison of total microorganisms count between microwave and water bath curing methods. *Significant difference at $p < 0.05$.

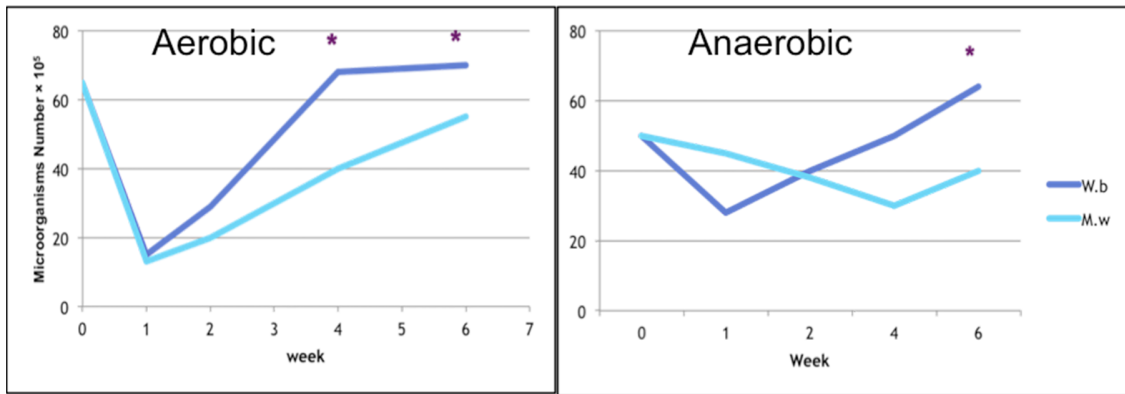


Figure 3: Comparison of total Streptococcus distribution between the two curing methods. *Significant difference at $p < 0.05$.

streptococci until it exceeds the preinsertion level at 6th week. At the 6th week of denture insertion, the M.w curing samples had significantly less number of streptococci than W.b curing samples.

The total anaerobic staphylococci were decreased at the 1st week and increased in the next weeks. The aerobic staphylococci showed different behavior, they tended to decrease after the 2nd week. M.w samples showed a significant decrease in aerobic staphylococci at the 2nd and 6th week period (Fig. 4).

The aerobic and anaerobic Gram-positive rods, begun to increase gradually from no growth at all before denture insertion, till it jumped to its highest value at the end of the 6th week. The M.w samples significantly harbored many anaerobic Gram-positive rods than W.b samples till the 6th week. At that time, they showed no significant differences although the aerobic Gram-positive rods showed a significant difference at that time (Fig. 5).

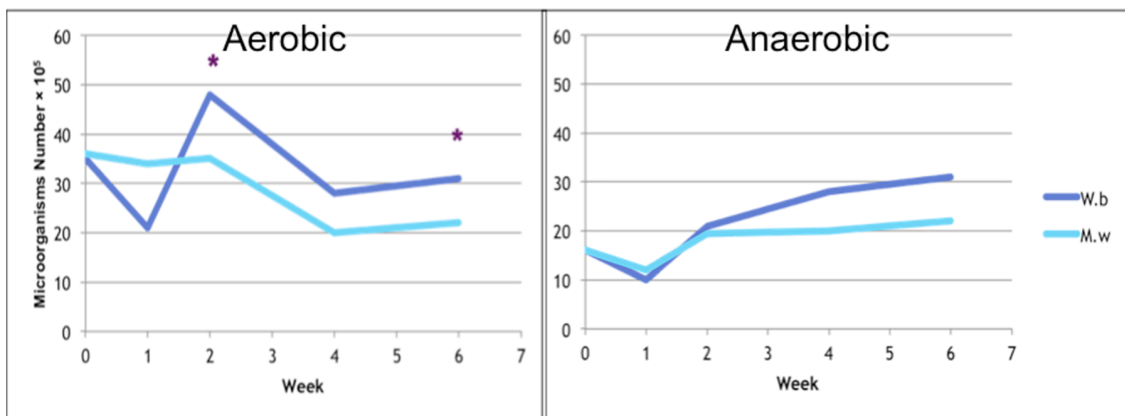


Figure 4: Comparison of Staphylococci distribution between the two curing methods. *Significant difference at $p < 0.05$.

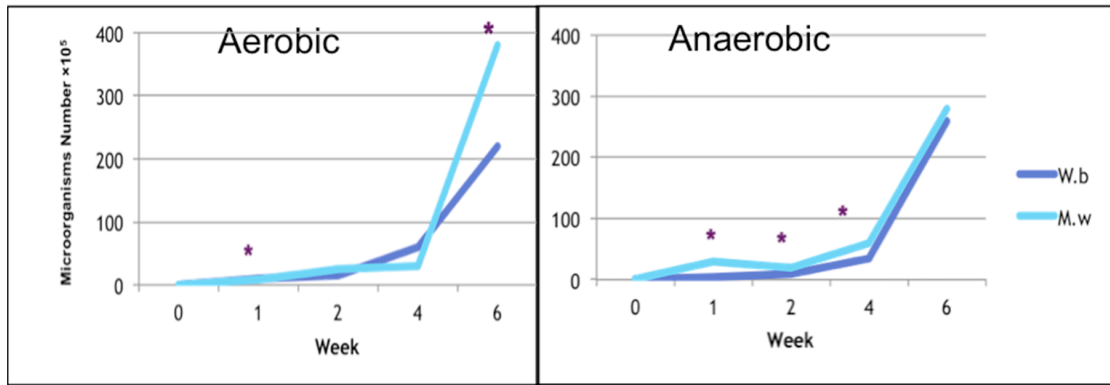


Figure 5: Comparison of Gram-positive rods distribution between the two curing methods. *Significant difference at $p < 0.05$.

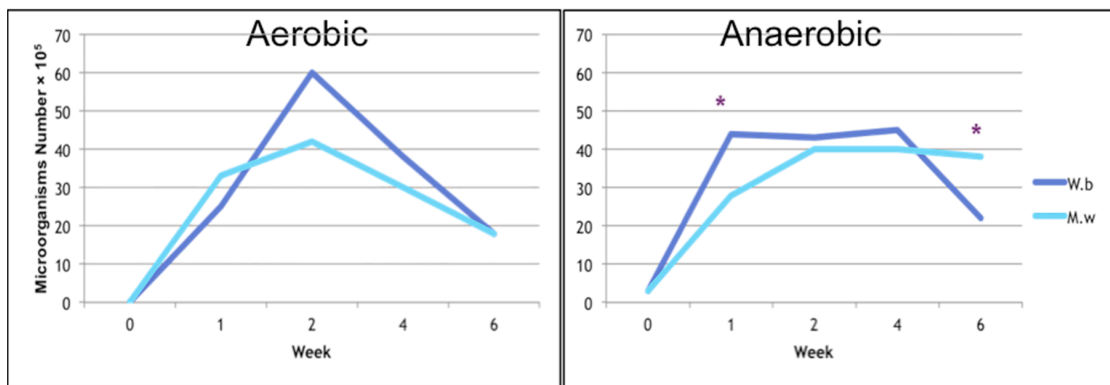


Figure 6: Comparison of Gram-negative rods distribution between the two curing methods. *Significant difference at $p < 0.05$.

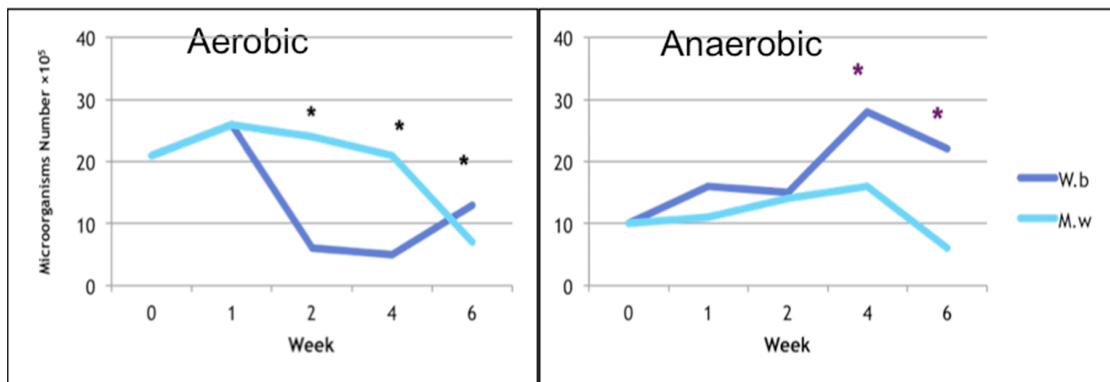


Figure 7: Comparison of Gram-negative cocci distribution between two curing methods. *Significant difference at $p < 0.05$.

While Gram-negative rods increased markedly after denture insertion, until the 2nd week, then they showed a tendency for high reduction at the end of the 6th week. There were no significant differences in their aerobic count on both samples, whereas the anaerobic Gram-negative rods revealed a significant increase only on the M.w samples at the 1st and 6th week (Fig. 6).

Water-bath samples had a significant sharper reduction in the number of aerobic Gram-negative cocci at the 2-4 weeks. On the contrary, the Gram-negative cocci started to increase throughout the four weeks after the insertion of the denture. However, the M.w samples show a significant reduction in Gram-negative cocci at the 6th week (Fig. 7).

There was no yeast detected in the patients' mouth before the insertion of the denture. Five patients reflected negative growth before and after denture insertion. The positive appearance between the visits occurred with out any regularity, as shown in table 3.

In summary, at the end of the 6th week of denture insertion, The Gram-positive rods get almost the highest number among other types of bacteria. M.w samples harbored significantly more aerobic Gram-positive and negative rods but significantly fewer streptococci, staphylococci (aerobic), and Gram-negative cocci (Fig. 8).

Table 3: Comparison of candida growth on Sabouraud's agar between water bath and microwave methods. --- no growth; ☑ very few colonies; ☑☑ few colonies.

	0 time	Method	1 st week	2 nd week	4 th week	6 th week
P1	---	W.b	---	---	---	---
		M.w	---	---	---	---
P2	---	W.b	---	---	---	---
		M.w	---	---	---	☑
P3	---	W.b	☑☑	---	☑☑	---
		M.w	☑☑	---	---	---
P4	---	W.b	---	---	---	---
		M.w	---	---	---	---
P5	---	W.b	---	---	---	---
		M.w	---	☑	---	---
P6	---	W.b	---	☑	---	☑
		M.w	---	☑	---	☑☑
P7	---	W.b	---	---	---	---
		M.w	---	---	---	---
P8	---	W.b	---	---	---	---
		M.w	---	---	---	---
P9	---	W.b	---	---	---	---
		M.w	---	---	---	---
P10	---	W.b	---	☑	---	☑
		M.w	---	---	---	---

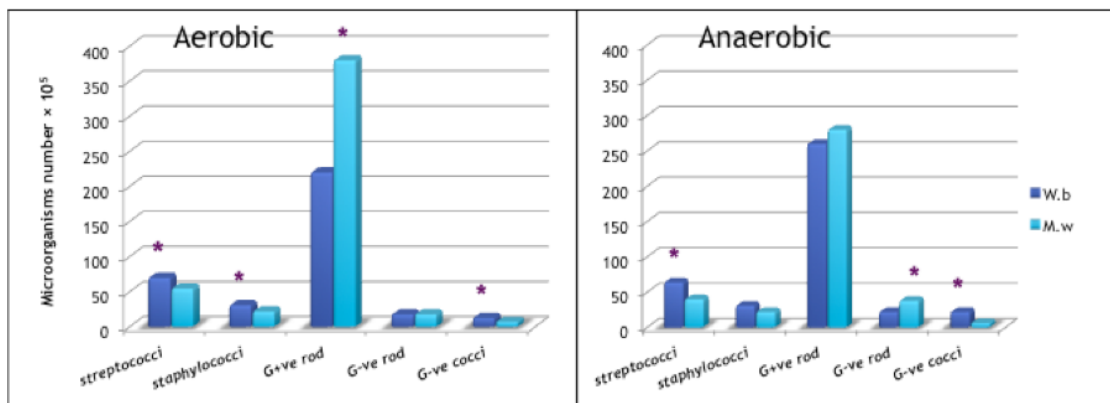


Figure 7: Comparison of Gram-negative cocci distribution between two curing methods. *Significant difference at p<0.05.

Discussion:

To the author knowledge, there is meager information about the comparison of two different methods for curing namely, M.w and conventional curing methods on the periodic qualitative and quantitative microbiological growth on the acrylic denture resin samples.

There is a correlation between the period of wearing the dentures and the number of bacteria. The

number of bacteria increases when the denture worn for a long period, a similar conclusion was also obtained by previous study⁽¹⁶⁾.

The recovery of anaerobic bacteria in addition to aerobic in all testing periods, is in agreement with a previous study conducted by Hardie⁽¹⁷⁾.

The microwave samples harbored significantly more aerobic microorganisms after the 6th week of denture insertion. This significant difference may be due to the higher porosity of samples cured by microwave energy than the samples cured by conventional W.b method⁽⁵⁾.

There was a gradual increase of the aerobic streptococci after one week of denture insertion, which may be due to the appearance of new species of streptococci in addition to the formal ones. Its count remains slightly higher than other types of bacteria. Nevertheless, Gram-positive rods reflect the obvious highest counts among other bacteria throughout the experiment periods. This finding disagrees with previous studies that found that Streptococcus is a predominant microorganism in edentulous subjects wearing complete dentures^(18,19). The W.b samples appeared to harbor significantly more streptococci at the end of 6th week, the habitat condition of W.b curing samples promotes their aggregation and growth.

Staphylococci found in considerable number in denture wearer patients⁽²⁰⁾ as well as in edentulous patients⁽²¹⁾. At the end of the 6th week period, W.b samples harbor significantly more aerobic staphylococcus. Their numbers remain around the pre-insertion level.

The sharp increase in the number of Gram-positive rods after the 4th week of denture insertion is in agreement with other studies, which stated that the count of these bacteria has been shown to be very few or absent in edentulous people, but after denture insertion, the number of these organisms significantly increased^(22,23).

The results of the current study showed that there were a detectable numbers of aerobic and anaerobic Gram-negative rods after denture insertion. This disagree with other studies stated that these microorganisms only transiently present and usually observed in low number in denture plaque^(22,24).

The increasing of aerobic Gram-negative cocci after one week of denture insertion and its subsequent reduction is in agreement with Ritz⁽²⁵⁾, who stated that aerobic Gram-negative cocci had been found to be part of dental plaque and more prominent in younger plaque.

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Candida in the present study were not detected in edentulous persons before denture insertion⁽²⁶⁾ nor denture plaque⁽²²⁾. It seemed that the environmental condition of both samples at the early stage of denture insertion did not favor the establishment of fungi and yeast. After one month of denture insertion candida could only be detected in small proportions of three patients and this disagrees with previous study⁽²⁷⁾.

It is better to conduct a comparison study of the microbial growth of denture plaque after one month of denture insertion and more. At this time, the bacterial growth becomes more stable. And hence, a precise decision about the superiority of one of the curing methods concerning the microbial growth could be concluded.

Conclusions

Microwave samples harbored more aerobic microorganisms at the 6th week after insertion, which may be due to higher porosity. However, there were no statistical differences in total anaerobic. Concerning the predominant microorganisms species, the statistical differences between the two methods occur as follow:

Microwave samples appear to harbor less aerobic and anaerobic streptococci, Gram-negative cocci, and aerobic staphylococci. In contrast, these samples harbor more aerobic Gram-positive rods and anaerobic Gram-negative rods. Other species (aerobic Gram-negative rods, anaerobic staphylococci and Gram-positive rod) show no statistical differences with water-bath samples.

Acknowledgment

My appreciations go to Mr. Nasir Medidan, Community health Department, Institute of Medical Technology/Baghdad, for his excellent microbiological laboratory assistance and patience throughout the whole steps of laboratory work. Special thanks to all members of the Community health Department, Institute of Medical Technology for their continuous help and support to achieve this work.

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