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Microbial evaluation of heat cured silicone versus heat cured acrylic resin in maxillary obturator

"A crossover clinical trial"

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Abstract

Purpose: The aim of this trial was to compare the microbial colonization of heat-cured silicone and heat-cured acrylic resin in obturators restoring acquired maxillary defects.

Material and methods: The experiment was carried out on six partially edentulous patients having unilateral total maxillectomy defects approaching midline (class I Aramany classification) who are in need of definitive obturator. Selected patients received metal framework prosthesis with heat-cured acrylic resin bulb extending into the surgical site. At the time of delivery, a swab was obtained from the patients who represent the baseline for the group I. Patients were recalled after two and four weeks from wearing the obturator for microbiological evaluation and a swab was taken each time from the same place. In group II, the heat-cured acrylic resin was replaced with heat-cured silicone and a swab was obtained on the day of insertion as a baseline for group II. Patients were recalled after two and four weeks from the insertion of the relined obturator for microbiological evaluation and a swab was taken each time from the same place. Swabs were obtained from the nasal surface of the surgical defect and immediately cultivated into three different media Blood Agar, Sabouraud Dextrose Agar, and Macconkey media and incubated for microbiological evaluation. The identification and quantification of the isolated microorganisms were performed using the conventional microbiological cultivation method. Finally, the collected data were tabulated and statistically analyzed.

Results: Statistical analysis of the collected data showed, that the difference between the two groups was insignificant. However, patients were more satisfied with obturators lined with heat-cured silicone.

Conclusion: It was concluded that within the limitations of this study, both acrylic resin and resilient lining materials could be used as a material for obturator construction in maxillofacial cases. However, longer follow-up period might show different results.

Keywords: Acquired maxillary defect, Obturator, Heat cure acrylic resin, Heat cure-silicon

Background

Restoring patients with maxillofacial imperfections is one of the most challenging treatments of the stomatognathic system. Usually, maxillary defects result from surgical elimination of oral tumors. The main objective of prosthetic obturation is the closure of the maxillary defects by an obturator in order to avoid hyper-nasal speech and fluid escape into the nasal cavity. Since the construction of an obturator for a maxillary defect requires optimum retention, stability, and obturation of the defect thus the weight of the obturator must be kept as minimum as possible to counteract the dislodging pull of gravity. This could be achieved by constructing a hollow bulb

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obturator. Furthermore, relining of the palatal part of the obturator with a soft liner greatly enhances the comfort of the patient as it is flexible and protects the integrity of the adjoining moving tissues (Shah et al. 2012).

Patients with acquired maxillary defects suffer from traumatized mucosa with less tolerance to masticatory forces, therefore, resilient liners are essential for their Cushing effect and ability to distribute the stresses evenly and uniformly at the mucosa lining interface (Pavan et al. 2010).

Fabricated a perfectly adapted silicone obturator engaging favorable undercuts within the defect. Regarding the retention, stability, and fluid leakage of the prosthesis, the patient was satisfied and during the follow-up appointment expressed remarkable improvement in the speech and prosthesis comfort (Guttal et al. 2010).

The adherence of microbes to host cells or polymers such as acrylic resin and soft liners is necessary for colonization and the development of pathogenesis and infection (Bulad et al. 2004). Oral mucositis induced in maxillofacial patients receiving radiotherapy may be explained by the alteration of oral flora and microbial colonization due to xerostomia induced by radiation (Stokman et al. 2003).

Investigations have reported that continuous swallowing or aspiration of microorganisms from denture plaque exposes patients to the risks of unexpected infections (Tari et al. 2007). Different Gram-positive and Gram-negative micro-organisms are present in the microflora of denture plaque. Numerous pathogenic and opportunistic bacteria and fungi from the patient's prosthesis were identified. Staphylococcus species were the dominant, Gram-positive cocci and wide arrays of Gram-negative rods were identified, including Pseudomonas aeruginosa, Enterobacter cloacae, and Klebsiella pneumonia (Pavan et al. 2010).

There's a huge body of evidence demonstrating that Candida is able to adhere to acrylic resin. Typically the first step that will lead to the growth of denture stomatitis of the adjacent mucosa. Candida adheres specifically or through a layer of denture plaque to denture base (polymethylmethacrylate, PMMA). Without this adherence, micro-organisms would be eliminated from the oral cavity when saliva or food is being swallowed (Pereira-Cenci et al. 2008). Adherence of Candida to acrylic resin denture materials is influenced by the degree of surface roughness in addition to other factors such as the presence of other microorganisms and diet rich in sucrose (Karaagaclioglu et al. 2008). They added that C. Albicans, being a relatively hydrophilic species, adheres to the surfaces in larger amounts as the surface wettability increases. Several studies have revealed that rough acrylic resin surfaces are more responsible for bacterial accumulation and plaque formation than smooth surfaces (Ivković et al. 2013). Adhesions of microorganisms on the surface of soft liners depend on the surface topography and composition of these materials. It was found that soft liners are more favorable for microbial colonization than acrylic resin leading to surface deterioration (Valentini et al. 2013).

Microbiological investigations have been used widely in prosthodontics research. Samples taken from appliances, teeth, ridge, or implants were investigated for several reasons and by several methods (Steinebrunner et al. 2005). Hence, this trial was performed to an ecological evaluation of the oral environment and bacterial or candidal growth when hard acrylic resin of the obturator bulb is changed with heat-cured silicone. The research question stated here was "In maxillectomy patients will obturator with heat-cured silicone will result in less microbial colonization than conventional obturator? This trial was performed following verifications made in the Consolidated Standards of Reporting Trials (CONSORT), statement for reporting RCTs.

Methods

Trial design and setting

The study was designed to be crossover clinical trial.

Six partially edentulous patients having unilateral total maxillectomy defects approaching midline (class I Aramany classification) were selected from the outpatient clinic, Prosthodontics Department, Faculty of Oral and Dental medicine Cairo University or referred from the National Cancer Institute.

- Control group (Group I): all the patients first received definitive obturator which fabricated using conventional heat cured acrylic resin.
- Study group (Group II): after that the obturator was removed from all the patients and then relined with heat cured soft silicone material.

Trial registration

The study protocol was approved by Evidence-based Dentistry Committee, Prosthodontics Department Board and Ethics Committee of Faculty of Oral and Dental Medicine, Cairo University.

Eligibility criteria

Inclusion criteria

1. Partially edentulous patients having unilateral total maxillectomy defects approaching midline.
2. At least four months were elapsed from the date of surgery.

3. Adult patients with age ranged between 20 and 60 years old with an average age of 45 years.
4. All patients had a full set of natural teeth on the intact side of the arch and intact opposing arch.
5. Cooperative patients and follow the instructions.
6. Remaining palatal mucosa was free from inflammatory conditions.

Exclusion criteria

1. Patients with systemic disorders that might disturb oral ecology were excluded, such as diabetes mellitus, blood diseases, T.B.
2. Patients were not receiving chemotherapy or radiotherapy or any drugs that could affect bacterial balance during the study period.
3. Smoking patients. Because it will affect the healing process.
4. Uncooperative patients. Because it will not return for follow-up

Patient examination

Patient's assessment was done to determine whether the patient met the study inclusion criteria. These assessments include a medical history questionnaire, a clinical examination, and radio-graphic assessment.

Patient consent form

Diagnostic data, suggested treatment and alternatives were reviewed with participants for this study. Illustrative consultation, treatment period, prosthodontics device and ultimate difficulties as well as hazards were all written in a consent form. The patients were fully educated about the possible consequences of the proposed research and signed a special written consent form designed for this purpose. All patients were requested to sign an informed consent form; this was translated into the Arabic language to be understood by the patients. The trial was conducted in accordance with the Declaration of Helsinki (2008).

Interventions and study procedures

A conventional obturator was fabricated for all patients following the traditional steps.

Construction of the definitive obturator

A suitable maxillary perforated stock tray was selected according to patient arch form and size. The tray was modified either by reduction or addition of modeling wax[1] in order to cover the area of defect and allow the impression material to extend to the required borders.

Training appliances and muscle relaxants were prescribed for patients suffering from trismus. Topical anesthesia was applied to the defect to reduce pain during procedure and undesirable undercuts were blocked out with vaselinated gauze.

Upper and lower primary impressions were made using irreversible hydrocolloid impression material and poured into dental stone[2] to obtain study casts.

Surveying[3] of the maxillary diagnostic cast was carried out.

A. Mouth preparation

Mouth preparation was done according to the planned design.

Support

Support was achieved through the palatal plate major connector, in addition to multiple occlusal rest seats were prepared distal to the first premolar, mesial to the second premolar, distal to the first molar and mesial to the second molar. A cingulum rest was prepared just above the cingulum of the canine tooth.

Retention

Retention was achieved through double Aker's clasps on the premolars and molars with alternating buccal and lingual retention.

Bracing and reciprocation

Bracing and reciprocation were obtained through the double Aker's clasps and the minor connectors.

B. Final impression

A custom made acrylic tray with wax spacer[4] was fabricated with a wax spacer. Any undesirable undercuts in the defect side was blocked out using vaslinized gauze.

A rubber base adhesive was applied to the fitting surface of the special tray and the final impression was made using medium body rubber base.[5]

The Impression was disinfected and assessed for extension, anatomical landmarks, rolled borders and surface details.

[1] ANUTEX modeling wax, Associated Dental Products LTD, England.

[2] Bayer Dental Co, D-5090, leverkusin. West Germany.

[3] Bio-Art´s Surveyor model B2, Brazil.

[4] ANUTEX modeling wax, Associated Dental Products LTD, England.

[5] Impregum F, Polyether impression material medium consistency, ESPE, Germany.

The final impression was then boxed and poured into dental stone[6] to obtain the master cast.

C. Framework construction

On the obtained master cast, relief and block out were made. The planned design was then transferred to the refractory cast and wax pattern was fabricated.

The Refractory cast was then invested, burnt out and cast. Framework was trimmed, finished, polished and tried in the patient's mouth.

The fitting surface of the metal framework was coated with pressure indicating paste (PIP)[7] before insertion and any interference was eliminated. It was checked for fitness, retention, extension, stability and finally it was checked for occlusion.

After metal framework try in, framework with trial denture base and occlusal rim were fabricated.

D. Centric relation record and setting up of teeth

The Framework with trial denture base and occlusal rims were inserted in the patient's mouth and asked to close with gentle force on softened wax[8] so that the occlusal imprints of the opposing teeth are recorded. Then the upper and lower casts were mounted on a semi adjustable articulator.[9]

The teeth[10] shade, size and form were determined; setting up of artificial teeth was carried out and arranged following the guide lines of the lingualized concept of occlusion.

E. Final try in stage

The waxed up definitive obturator teeth was tried in patients mouth and checked for retention and comfort. Extension of the posterior and lateral borders of the obturator and restoration of the normal facial contour were also evaluated.

F. Processing of the obturator

Definitive obturators were fabricated using conventional heat cured acrylic resin[11] (group I). During packing a hollowed obturator bulb was constructed using the lost salt technique. A long curing cycle was performed (74 °C for 9 h).

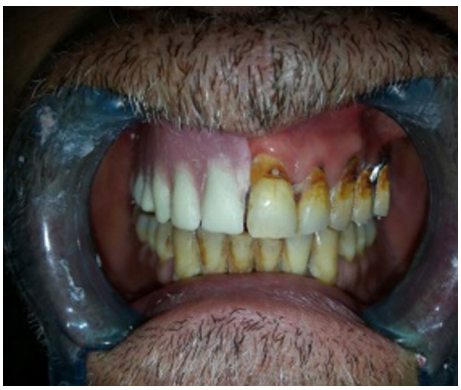

Fig. 1 Obturator insertion (frontal view)

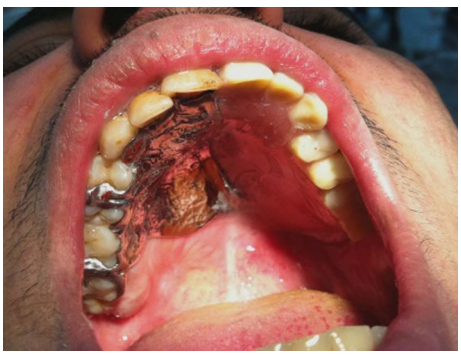

Fig. 2 Obturator insertion (occlusal view)

Adequate time was allowed for proper cooling of the flask after curing prior to the deflasking procedure. The obturator was highly finished and polished.

G. Obturator insertion

Finished obturator was checked carefully for blebs, bubbles, artifacts in either metal or acrylic and borders were checked for sharp edges.

At the time of delivery (Figs. 1, 2), the prosthesis was checked intra-orally for proper extension, retention, adaptation, pressure areas, and occlusion. The patient was instructed to come back in the next day and any necessary adjustment was carried out.

Patient instructions

One week before obturator insertion, patients were instructed to remove the interim prosthesis all day except during eating, perform Chlorohexidene mouthwash in addition to Penicillin 500 mg. and Metronidazole 500 mg,

[6] Bayer Dental Co, D-5090, leverkusin. West Germany.

[7] Protechno, Advanced Products For Dental Labs, 17,469 VILAMALLA (GIRONA) SPAIN.

[8] ANUTEX modeling wax, Associated Dental Products LTD, England.

[9] A7Plus Articulator, Bio-Art, Brazil.

[10] Acrostone 4007. Acrylic teeth, England.

[11] Acrostone, Denture base material, Acrostone dental facrtory, England.

during this period any other medications that might alter the oral flora were avoided.

After obturator insertion, patients were instructed to wear the prosthesis during daytime, eating and to be removed from mouth for approximately 8 h daily (sleeping hours) to reduce trauma to the underlying mucosa. Patients were instructed to avoid any medications or mouthwashes.

The prosthesis should be cleaned after each meal under running water over a basin filled with water to avoid accidental drop and breakage. While not in use it should be placed in a container with tap water.

Microbiological samples for obturator with heat cured acrylic resin bulb

- At time of obturator insertion: one swab was taken from each patient from the nasal surface of the surgical cavity. It was considered a base line for each patient.
- After obturator insertion: one swab was taken at the following follow-up periods:
  - Two weeks after insertion
  - Four weeks after insertion

Relining of the obturator

After the fourth week swab was obtained, 2 mm of the heat cured acrylic resin bulb were reduced and the obturator was relined with chair side soft liner.[12] The patients were instructed not to remove the prosthesis for the next 48 h and come back for further relining procedures.

While prosthesis still in place, an overall impression using a hydrocolloid impression material[13] in a perforated stock tray was done. The obtained cast with the obturator was flasked. After deflasking, the chair side soft liner was replaced with heat cured soft silicone material[14] (group II) prior to application of silicone liner an adhesive primer with a solvating effect must be used on the denture base.

At the time of delivery, the prosthesis was checked intra-orally for proper extension, retention, adaptation, pressure areas, and occlusion. The patient was instructed to come back in the next day to adjust any problem related to the prosthesis (Fig. 3).

[12] COE-SOFT, soft denture reline material, GC America INC, USA.

[13] Alginate CA 37: superior pink, Alginate impression material Cavex Holland BV.P.o Box.825, 2003 RW Haarlem (Holland).

[14] Molloplast B, Detax company, Germany.

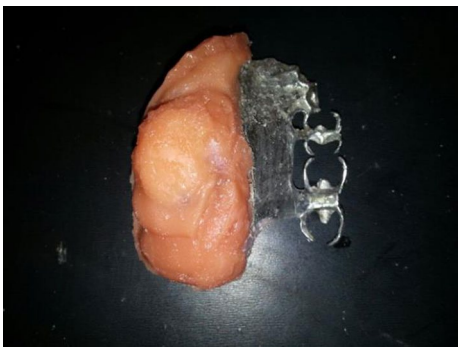

Fig. 3 Obturator prosthesis with heat cured silicone bulb

Fig. 4 Sterile swab

Microbiological samples for obturator with heat cured silicone bulb

- At time of obturator insertion: one swab was taken from each patient from the nasal surface of the surgical cavity. It was considered a base line for each patient.
- After obturator insertion: one swab was taken at the following follow-up periods:
  - Two weeks after insertion
  - Four weeks after insertion

Microbiological procedures

For all patients, microbiological samples were collected and evaluated by semi quantitative culture of microorganisms in the following manner.

Isolation of microorganisms was carried out using gamma sterilized disposable swabs (Fig. 4).

Microbial growth evaluation was made as following:

- The swabs were emulsified in 1 ml sterile nutrient broth then after good shaking; it was added to 9 ml

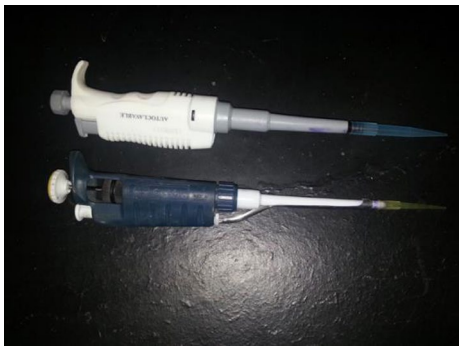

Fig. 5 Micropipette

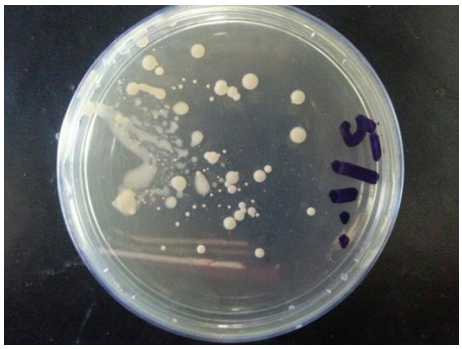

Fig. 6 Sabouraud dextrose agar plates

nutrient broth in a sterile tube making a dilution of 1:10.

- Serial dilutions were done to reach a dilution of 1: 1000. 20 micron from the nutrient broth was taken by micropipette (Fig. 5) and applied to the surface of Sabouraud Dextrose Agar plate, another 20 micron to Blood Agar and Macconkey plates and distributed on them by glass rod. Then these plates were incubated at 37 °C for 24 h in an incubator.
- After incubation, the plates were examined for the growth of Candida on Sabouraud Dextrose Agar plates, Staphylococcus aureus on Blood Agar plates and Gram negative on Macconkey plates. Colony Forming Unit (CFU) were counted (number of Candida, Staphylococcus aureus and Gram negative /sample was calculated)
- Identification was done by morphological examination and gram-staining.

Morphological examination: Candida (Fig. 6)

- Appear on Sabouraud Dextrose Agar plates as cream colored pasty colonies.
- The Colonies have a distinctive yeast smell.
- Appear as large dark violet budding organisms in gram stain.

Staphylococcus aureus (Fig. 7)

- Appear on Blood Agar plates as yellow or occasionally white 1–2 mm in diameter surrounded with a clear zone of complete hemolysis (β hemolysis).
- Pigment is less pronounced in young colonies.
- Colonies are slightly raised and easily emulsified.
- Appear as gram positive cocci arranged in clusters.

Fig. 7 Blood agar plate

Gram negative bacteria

- Appear pink to red or pale on Macconkey Agar plates.
- Appear as Gram negative bacilli in gram stain

Statistical analysis

In this study, data of Candidal and Bacterial colonies were coded, edited, collected, and analyzed as means and standard deviations for both groups (before and after relining) before insertion as base line, 2 weeks and 4 weeks follow-up periods.

Statistical analysis was carried out using Microsoft Excel 2010 program. While testing significance was performed using SPSS® 20 (Statistical package for scientific studies, SPSS, Inc., Chicago, IL, USA) and Minitab ® statistical software Ver. 16.

Collected values were calculated according to the equation, CFU/ul = Total number of colonies counted in the plate X inversion of the saline dilution (1000)/10.

Table 1 Comparative study of candidal growth between both groups at each time interval (mean difference)

	0–2		0–4	
	M (CFU)	SD	M (CFU)	SD
Hard acrylic resin Group I	300	394.65	315	373.57
Heat cured silicone Group II	200	104.42	290	651.23
p-value	0.56*		0.84*	

* *p*-value less than 0.05 (typically ≤ 0.05) is statistically significant. *p*-value more than 0.05 (typically ≥ 0.05) is statistically insignificant

Table 2 Comparative study of bacterial growth between both groups at each time interval (mean difference)

	0–2		0–4	
	M (CFU)	SD	M (CFU)	SD
Hard acrylic resin Group I	1460	2033	510	1481
Heat cured silicone Group II	906.67	663.93	846.67	1166.474
p-value	0.54*		0.67*	

* *p*-value less than 0.05 (typically ≤ 0.05) is statistically significant. *p*-value more than 0.05 (typically ≥ 0.05) is statistically insignificant

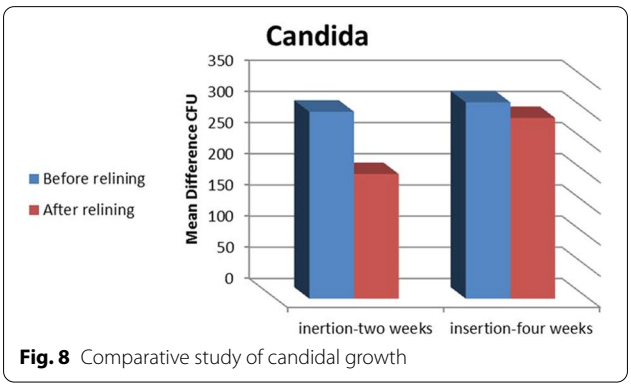

Fig. 8 Comparative study of candidal growth

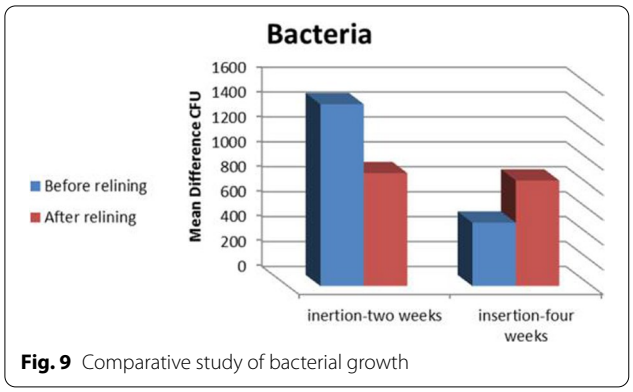

Fig. 9 Comparative study of bacterial growth

Data were explored for normality using Kolmogrov-Smirnov test and Shapiro–Wilk test. Exploration of data revealed that the collected values were not normally distributed.

Kruskal–Wallis test followed by multiple comparisons test were performed to test the significance between the follow-up periods within each group to detect the effect of time on candida and bacterial growth.

In addition, Mann–Whitney *U* test was performed to test the significance between both groups at each follow-up periods to compare the candida and bacterial growth between both groups.

A probability level of $P \leq 0.05$ was considered statistically significant.

Results

Comparison between both groups

Regarding Candidal colonization

For evaluation of candidal growth change for each follow-up period, mean difference was calculated of each time interval of each group.

Mann–Whitney *U* test was performed to detect the significance between both groups at time interval which revealed that there was insignificant difference during the follow-up period as showed in Table 1 and Fig. 8.

Regarding bacterial colonization

For evaluation of bacterial growth change for each follow-up period, mean difference was calculated of each time interval of each group.

Mann–Whitney *U* test was performed to detect the significance between both groups at time interval which revealed that there was insignificant difference during the follow-up period except at 2–4 month time interval which was significant as showed in Table 2 and Fig. 9.

Discussion

This study was performed on six partially edentulous patients having unilateral total maxillectomy defects approaching midline (class I Aramany classification) who undergo surgery at least six months before the study was initiated, which is quite sufficient to prepare the patient physically and emotionally for the prosthetic intervention (Singh et al. 2013). This trial was accepted by the Ethical Committee, Evidence-based Dentistry Committee, and Prosthodontics Department Board of Faculty of Dentistry, Cairo University, Egypt. This study has been planned, performed, and reported, intentionally using the best-presented methodology, according to principles for evidence-based

medicine. The results of this study showed an insignificant difference between both materials used for the construction of the obturator bulb. However, the acrylic resin group showed higher results after two weeks. The increased microbial colonization of the acrylic resin group is in agreement with that postulated by Bettencourt et al. (2010) who concluded that the inferior fit and retention of the obturator bulb of the acrylic resin, in addition to the toxic effect on the oral cells and tissues caused by the residual monomer as a result of the polymerization process, leads to tissue trauma that enhances microbial colonization. This is in controversy with what reported by some authors that resilient liners present greater retention of candida than the acrylic resin. Pereira-Cenci et al. (2008) stated that patients wearing obturators, due to their fear of frequent insertion and removal for good oral hygiene, allow denture plaque to accumulate on the denture base material, according to the study (PMMA). This accumulation allows microorganisms to adhere to the surface, resulting in denture stomatitis. This is in accordance with the results obtained in the present study as the acrylic resin showed greater colonization than the resilient liner. As reported by Verran and Maryan (1997) greater adherence of candida and bacteria was observed in some soft lining materials. However, they found that Molloplast B is the most successful soft lining material in terms of reduced bacterial colonization and this is in agreement with the results obtained in the present study.

Nikawa et al. (2000) reported that resilient liners show changes in their physical properties with the aging of the material which enhances colonization; however, in the present study the short follow-up period did not allow these changes to take place. On the other hand Busscher et al. (1997) reported that in clinical situations colonization of resilient liners is more than hard acrylic resin which is in controversy with the current study. Although it was reported that the less smooth surface of soft liners in comparison to the hard acrylic resin is considered a perfect shelter for microorganisms, the results of this study show no significant correlation. These results are in accordance with the study of Nikawa et al. (2003) who observed no relationship between surface roughness and biofilm formation on different soft lining materials, including Molloplast B. This finding implies that the surface roughness of a material may not be the only factor that governs the adherence of microorganisms. Glass et al. (2001) postulated that the bacteria adhere (in CFU/ml) more to the soft lining materials than C. Albicans. These findings are important as pathogenic bacteria are present in the denture plaque and may play a role in denture stomatitis and in systemic infections. This is in accordance with the results obtained in the present study as the bacterial colonization is greater than the candidal colonization. Clarke (1975) reported that the resilient liners are used to allow uniform distribution of the forces at the mucosal lining interface and to limit tissue trauma that is considered main factor in the development of denture stomatitis. The superior fit and adaptation of the silicone material to the delicate tissues of the surgical site played an important role in minimizing the leakage of ingested food into the defect and the adherence of microorganisms. This could explain the reduced colonization of candida in the resilient liner group. Moreover, the easy removal and insertion of the obturator minimized the friction with soft delicate tissues which reduced tissue trauma and thus reduced colonization. Molloplast B is a permanent soft lining material which can serve for several years before showing surface deteriorations as reported by Schmidt et al. (1986). The short follow-up period was insufficient for Molloplast B to show any surface changes that might affect the oral flora significantly. Therefore, extending the follow-up period may change the results. Patients were asked about their feedback regarding their prosthesis. All patients of the Molloplast B group without exception were greatly satisfied with the comfort, retention, and phonetics during using the prostheses. This may be attributed to the inherent resiliency of the Molloplast B which provided excellent peripheral seal. Also, the flexibility of the material provided easy insertion and removal through the undercuts that allowed greater engagement of soft tissue undercuts and thus improved retention and stability significantly. On the contrary, two patients of the acrylic resin group were not well satisfied with the phonetics, and they experienced discomfort during insertion and removal that necessitated much more adjustment.

Conclusions

Considering the limitation of this study, the following conclusion can be pinched; both acrylic resin and resilient lining materials could be used as a material for obturator construction in maxillofacial cases.

Abbreviations
PMMA: Polymethylmethacrylate; RCT: Randomized Clinical Trial; TB: Tuberculosis; PIP: Pressure indicating paste; CFU: Colony Forming Unit; SPSS: Statistical package for scientific studies.

Acknowledgments
Not applicable.

Author contributions
MAE performed the clinical examination, obturator fabrication and data collection, follow-up visits and multidisciplinary consultation. MHM and AAA were major contributors in clinical steps; they performed the statistical analysis and writing the manuscript. All authors read and approved the final manuscript.

Funding
Self-funding.

Availability of data and materials
The datasets generated during and/or analysed during the current study are available with the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This clinical trial was approved by the research ethical committee faculty of dentistry Cairo University. Number: 10-4-13. The patients accepted to participate in the trial were asked to sign written consent.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Recommendation
Larger well-conducted RCTs with longer follow-up period might show different results.

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