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GROWTH OF CANDIDA ALBICANS ON POLYMETHYL METHACRYLATE AND POLY AMIDE DENTURE BASE RESIN MATERIAL: AN IN-VIVO STUDY

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Abstract

Aim: *Candida albicans* plays an integral role in denture related stomatitis. The aim of this study is to evaluate and compare the growth of *Candida albicans*on polymethyl methacrylate and polyamide denture base resin material.

Materials and Methods: Twenty patients were selected for each group and the removable partial denture was fabricated by using the compression molding technique and the injection molding technique. The swab was collected prior to denture insertion, 3 months and 6 months post insertion. The swab was cultured and Candial CFU count was done.

Results: The mean growth of Candida albicans increased highly significantly ($P \leq 0.001$) with time in both PMMA and polyamide. It was found that polyamide shows more amount of Candida albicans growth in comparison to PMMA prior to denture insertion, after 3 months and after 6 months respectively.

Conclusion: In this study, PMMA clearly proves to be the better option; further research is needed to find the ideal denture base material. Subsequent research endeavors ought to strive to close the noted gaps, utilizing a multidisciplinary methodology.

Keywords: Candida albicans, Polymethyl methacrylate, Polyamide, Denture base resin, Flexible denture.

Introduction

The primary goal of prosthodontists is to replace missing teeth and restore both function and aesthetics for patients. Various treatment options exist for partially edentulous individuals, including acrylic removable dentures, flexible dentures (also known as thermoplastic dentures), cast partial dentures, and fixed dental prostheses. The clinical situation determines the decision to provide a removable denture over other treatment options.Theremovableprosthesisis preferred forlargeormultiple edentulous areas, remaining dentition with severe periodontitis,severely resorbedalveolar ridges, patients with physical or emotionalvulnerability, limited economic status and patient's preference for the prosthesis [1,2,3].

Polymethyl methacrylate(PMMA) partial dentures are the conventional material for restoring missing teeth. However the residual monomer can cause cytotoxicityinallergic individuals, and its likelihood increases with a greater amount of monomer, a shorter water storage time of cured denture, and polymerization of PMMA. Rather than thermal curing on the other hand thermoplastic materials are beneficial in challenging conditions such as denture resins allergy, and angulated remaining teeth with undercuts or reduced mouth openingcases. Furthermore, the injection molding technique used for flexible denture(synonyms: thermoplastic dentures, non-metal clasp dentures) fabricationis morestablethan the compression moldingtechnique [4,5].

The flexible denture material was first developed in the 1950s andconsisted of various grades of polyamides, acetal, fluoropolymers, and thermoplastic materials based on polycarbonate [4,6].Differentflexibledenture materials are commercially available in the market. These areValplast, Deflex, Bre flex, Lucitone FRS, Flexite, Flexite plus, Jet carboresin,Proflex [6,7,8].

But even after so many advantages of flexible dentures, there aresome limitations with their use after some time, including debonding ofacrylic teeth, high surface roughness, discoloration, technique sensitivity,difficulty to adjust,polish,andlowerhardness [9].

The surface attributes of denture base materials are importantsince studies [10,11,12] of denture base materials have demonstrated a straight connection between rougher surface, plaque built up, and speciesofCandida colonization. In cases of denture-induced stomatitis, there has been a risein the presence of Candida species [10]. Surface irregularities facilitate theinitial bacterial attachment on roughened surfaces because they shield thebacteriafromsalivaryflowaswellasmasticatoryfunctionandallowthem to attach to more substratum points.

Both bacterial and fungal species, particularly *Candida albicans*, exhibit a greater tendency to adhere to rougher denture base materials, leading to plaque accumulation and fungal adherence. While healthy individuals may harbor up to 10^3cfu/mL of Candida in their saliva asymptomatically, symptomatic patients often present with 10^3 to 10^6cfu/ml[2] An escalated presence of Candida species is frequently reported in cases of denture stomatitis.

This study aims to assess the proliferation of Candida Albicans on partial denture prostheses from PMMA and Polyamide denture base materials.

Material and methodology

Inclusion Criteria

1. Age group 30-50 years.
2. Maxillary tooth-supported partial edentulous (Kennedy's class III, Kennedy's class III Mod I, Kennedy's class IV patients.)
3. Willing participants

Exclusion Criteria

1. Diabetes mellitus (Type I & Type II).
2. Distal extension cases (Kennedy's Class I and Class II).

3. Mentally disabled persons.
4. Non-willing participants.
5. Betel quid chewers.
6. Tobacco chewer.
7. Smokers.
8. Poor oral hygiene.
9. History of antibiotic therapy since the last six months.

In this study, we selected forty patients from the Department of Prosthodontics at Hitkarini Dental College, Jabalpur, based on specific inclusion criteria. Twenty patients were assigned to each of two groups: one using polymethylmethacrylate (PMMA) acrylic resin for denture bases, and the other using polyamide (flexible denture base resin). Here are the details of our study:

1. Patient Selection and Consent: - Patients were informed about the study design, and their consent was obtained. - Institutional ethical clearance was obtained before commencing the study.
2. Denture Fabrication Process: - Primary impressions were made using irreversible hydrocolloid material. Master casts were created using dental stone.Temporary denture bases were fabricated using cold-cure acrylic resin. Teeth selection was based on both aesthetic and functional requirements. A try-in was performed, followed by wax-up with proper marginal gingival carving. The dentures were sealed to the master casts for final prosthesis fabrication.
3. Denture Base Processing: -
   PMMA Denture Bases: - Processed using the compression molding technique in an acrylic curing unit.
   Polyamide Denture Bases - Processed using the injection molding technique in a perfect flexi injection system. The prosthesis underwent finishing and polishing.
4. **Sample Collection for Candida Albicans Culture**: - Before prosthesis insertion, a swab was collected from the control group. After 3 months and 6 months of denture insertion, swabs were collected for Candida albicans culture.
5. Microbiology Culture Procedure -A sterile swab was rubbed on the intaglio surface of the denture (Figure 1). The swab was placed in a tube containing 50 ml phosphate-buffered saline solution and sonicated for 5 minutes at 35 kHz. - Serial decimal dilutions (100 to 10^{-2}) were prepared from the sonicated swab solution. - 100 µl of the sonicated solution was inoculated on Sabouraud's dextrose agar at 37°C for 48 hours (Figure 2). - After incubation, the agar plate was retrieved, and the number of colonies was counted (Figure 3).

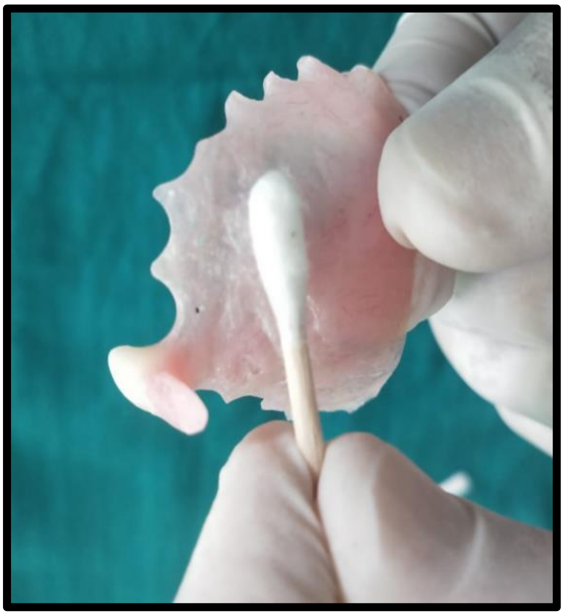
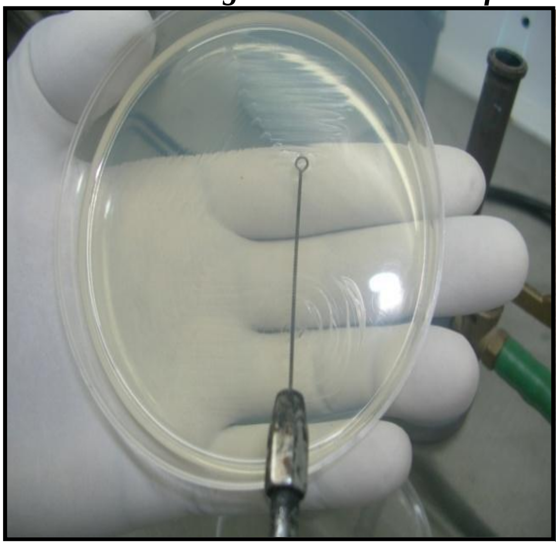


Figure 1. Swab taken for culture from prosthesis



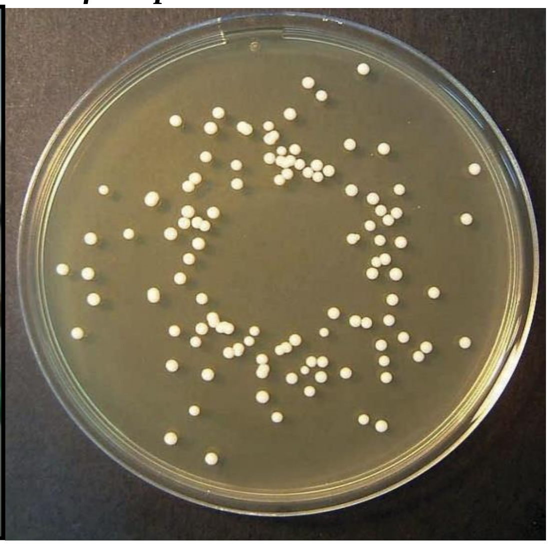


Figure 2. Preparationofcandidalculture *Figure3. Candidal colonies*

Results

The PMMA group consisted of 11 females (55%) and 9 males (45%). Likewise, the Polyamidegroup consisted of 11 females (55%) and 9 males (45%).

In the PMMA group there were 10 participants (50%) of 31 to 40 years, and 10 participants of 41 to 50 years, similarly for polyamide there were 8 participants (40%) of 31 to 40 years and 12 (60%) participants of 41 to 50 years.

Table 1shows the difference in the growth of Candida albicans in PMMA and polyamide denture base material prior to denture insertion, after 3 months of denture insertion, and after six months of denture insertion. For PMMA the p-value was found to be P≤0.001 with a mean of $0.24\times10^3\pm0.047\times10^3$ prior to denture insertion, $1.21\times10^3\pm0.47\times10^3$ after three months, and $2.26\times10^3\pm0.64\times10^3$ after six months. For polyamide, the p-value was found to be P≤0.001with mean of $0.47\times10^3\pm0.092\times10^3$ prior to denture insertion, $2.35\times10^3\pm0.60\times10^3$ after three months, and $3.50\times10^3\pm0.70\times10^3$ after six months. The mean growth of Candida albicans increased highly significantly (P≤0.001) with time in both PMMA and polyamide. It was found that polyamide shows more amount of Candida albicans growth in comparison to PMMA prior to denture insertion, after 3 months and after 6 months respectively.

Time Interval	**PMMA Mean±S.D**	**Polyamide Mean±S.D**	**t-test**	**p-value**
Prior to denture insertion	$0.24\times10^3\pm0.047\times10^3$	$0.47\times10^3\pm0.092\times10^3$	-10.084	p≤0.001*
Three months after denture insertion	$1.21\times10^3\pm0.47\times10^3$	$2.35\times10^3\pm0.60\times10^3$	- 6.629	p≤0.001*
Six months after denture insertion	$2.26\times10^3\pm0.64\times10^3$	$3.50\times10^3\pm0.70\times10^3$	-5.85	p≤0.001*

Table 1: Inter-group Comparison between PMMA (Group A) and polyamide (Group B).

Discussion

The development and intensity of denture stomatitis are influenced by various factors, including the occupancy and establishment of Candida species, the characteristics of denture base materials, the fit of dentures, and oral hygiene practices.

To date, no in vivo studies have been conducted to analyze the growth of Candida albicans on polyamide. This study aims to fill this gap by examining the in vivo proliferation of Candida albicans on both PMMA and polyamide denture bases at three intervals: before denture placement, and at three- and six-months post-insertion.

Our findings indicate a significant presence of Candida albicans on both PMMA and polyamide dentures before insertion, with a p-value of p≤0.001. This colonization could be attributed to the dentures being rinsed with tap water without undergoing a disinfection process prior to insertion. Candida albicans is normal commensal of oral cavity. The threshold for candidiasis symptoms is typically around 10,000 CFU/ml [11], suggesting that the observed Candidal growth might not immediately lead to clinical manifestations.

Surface roughness plays a pivotal role in staining resistance, patient comfort, plaque accumulation, and overall dental health. It directly affects the initial microbial adhesion, biofilm formation, and colonization by Candida species. The porous nature of these materials facilitates nutrient diffusion and water absorption, complicating cleaning efforts with standard mechanical techniques like hypochlorite and peroxide denture cleansers [16]. Surface irregularities can harbour microorganisms, protecting them from mechanical forces and acting as a reservoir. Reducing surface roughness can therefore delay plaque formation and maturation. Notably, polyamide denture bases exhibit greater surface roughness compared to PMMA, with past studies reporting average roughness values of 0.146µm for polyamide and 0.046µm for PMMA. [17]

The surface-free energy of the substrate also influences plaque development and adhesion. While surface roughness and free energy are interrelated, the former has a more pronounced impact. These findings underscore the importance of smoother surfaces with lower surface-free energy to minimize plaque formation and subsequent gingival irritation, as increased surface area promotes microbial colonization. Saliva plays a crucial part in inhibiting growth of Candida albicans. Its physical cleansing action, combined with innate Défense molecules like lysozyme, histamines, lactoferrin, calprotectin, and IgA, reduces Candidal proliferation. Other saliva elements, such as mucins, statherin, and proline-rich proteins, shown to bind to Candida albicans, enhancing adhesion. Dentures, especially upper dentures, create an environment conducive to yeast growth. Saliva's mechanical cleaning effect is absent beneath dentures, leaving yeasts colonizing these areas unaffected by salivary antimicrobial factors.

Furthermore, the pH under dentures can drop from the normal range of 6-8 to 4-5, favouring yeast adherence due to the acidophilic and aciduric nature of Candida species [18]. Candida species' ability to adhere to the oral epithelium is crucial for colonization and infection. Diabetic patients, with elevated salivary glucose levels, provide a favourable environment for Candida colonization. Studies have shown a significant association between plasma and salivary glucose levels, with higher salivary glucose associated with increased oral Candida prevalence [19,20]. Saliva is vital for oral defense, and xerostomia—often caused by poor glycaemic control or medications that reduce salivary secretion—increases the risk of Candida growth. This discussion highlights the complex interplay between denture materials, oral environment, and systemic factors in the proliferation of Candida albicans, emphasizing the need for continued research and in the current investigation, the mean proliferation of Candida on both PMMA and polyamide materials was found to be highly significant after three- and six-months post-insertion, with a p-value of $p \leq 0.001$. The data indicated an increase in Candidal colonization over time on both materials. This observation aligns with the findings of Sultana N et al. [21], who reported greater Candidal growth on polyamide due to its higher surface roughness compared to PMMA. They also noted a positive link between the presence of S. aureus and the increase in Candida albicans, although their study was limited to a 48-hour period.

The research outcomes are steady with those of Fernandez et al. [22], who observed that polyamide materials fostered more biofilm development than PMMA. They concluded that despite polyamide resin exhibiting the highest levels of Candida colonization and least surface free energy, these surface properties did not directly influence Candida biofilm formation. However, their study only considered one type of polyamide material (Flexite) and over a brief time span.

Conversely, the results diverge from the findings of Ahmad Z M et al. [23], who determined that Candida adherence was higher on acrylic resin than on Valplast. They suggested that the

surface preparation method could be a confounding factor, as their samples were not finished or polished, unlike other studies that used highly polished molds or post-construction smoothing. They proposed that the antimicrobial properties of acrylic resins, due to the release of residual monomers, might play a role in reducing Candida adherence, a property absent in nylon-based resins like Valplast. Similarly, the study contradicts the observations of Ahmed E M et al. [24], who reported a decrease in Candida albicans counts on polyamide materials compared to PMMA. They attributed this to physical properties of denture base materials affecting Candida adherence patterns. They highlighted the benefits of polyamide in prolonged use, such as tissue conditioning and stress distribution, which might reduce oral soft tissue atrophy and promote less Candida colonization due to the material's inherent flexibility. In concordance with our study, Jain V et al. [25] found a significant increase in Candida albicans growth on polyamide compared to PMMA, emphasizing that rougher surfaces facilitate microbial adhesion. Anggraini M et al. [26] and Raigar R L et al. [27] also supported these findings, showing higher Candida growth on polyamide than on PMMA and metal denture base materials. Mehendale A V et al.'s [28] study is in agreement, demonstrating a significant rise in Candida albicans growth on polyamide on the second and fourth days, with a slight increase on the eighth and twelfth days, although not statistically significant. Based on the hypothesis testing, the null hypothesis is rejected, indicating that polyamide denture base materials exhibit greater Candida albicans growth compared to PMMA. This study contributes to the understanding of material-specific colonization patterns and underscores the importance of considering surface properties and microbial interactions in the design and selection of denture base materials. The findings suggest a need for further research into the development of denture materials that minimize pathogenic colonization while maintaining patient comfort and prosthetic functionality and innovation in denture material science to enhance patient outcomes.
Hypothesis testing rejects the null hypothesis. Hence the polyamine denture base material shows more Candida albicans growth in comparison to PMMA denture base material.

Conclusion

This comprehensive study meticulously evaluated the proliferation of Candida albicans on two widely used denture base materials- PMMA and Polyamide, prior to the insertion of dentures, and subsequently at three- and six-months post-insertion. The results, while subject to the study’s inherent constraints, are summarized below:

Comparative Growth Analysis: The investigation revealed a consistent pattern where the growth rate of Candida albicans was remarkably more on Polyamide denture base material in comparison to PMMA. This trend persisted across all the time intervals examined—before denture insertion, and at three- and six-months following insertion.

Significant Growth Disparity: A pronounced and statistically significant difference was noted in the growth of Candida albicans between the PMMA and Polyamide denture base materials at each evaluated time point—prior to insertion, and at three- and six-months post-insertion.

Given these findings, PMMA is identified as the more favorable material for denture bases when considering the control of Candida albicans proliferation.

It is crucial to acknowledge the aspects not covered within the scope of this study. These unexplored factors include the assessment of residual monomer release and its potential systemic effects, the analysis of surface characteristics such as free surface energy and its correlation with microbial adhesion, the impact of various polishing techniques on surface smoothness, and the presence of surface irregularities like roughness and porosity which may contribute to microbial retention. Additionally, the interaction of these materials with human saliva, which plays a pivotal role in the oral microbiome, was not evaluated.

The study’s reliance on optical microscopy for result analysis provided valuable insights, yet the inclusion of more sophisticated imaging techniques such as electron microscopy and profilometry could offer a deeper understanding of the micro-environmental interactions at play. Furthermore, while numerous studies have explored different characteristics of denture base materials in isolation, a holistic study that integrates all these properties would provide a more comprehensive picture, aiding in the elucidation of the mechanisms behind the increased adherence observed with Polyamide resins.

In conclusion, while PMMA stands out as the superior choice in this study, the quest for the optimal denture base material necessitates ongoing research. Future studies should aim to bridge the gaps identified, employing a multidisciplinary approach.

References

1. ThumatiP, PadamjaS, RaghavendraRK.FlexibleDenturesinProsthodontics-An overview. Indian J Dent Adv. 2013;5 (4):1380-85.
2. D'SouzaD,DuaP.Rehabilitationstrategiesforpartiallyedentulous-prosthodontic principles and current trends. Med J Armed ForcesIndia.2011;67(3):296-298.
3. WostmannB,Budtz-JørgensenE,JepsonN,MushimotoE,Palmqvist S, Sofou A, Owall B. Indications for removable partialdentures:aliteraturereview.IntJProsthodont.2005;18(2):139-45.
4. Naylor WP, Manor RC. Fabrication of a flexible prosthesis for theedentulous scleroderma patient with microstomia. J Prosthet Dent.1983;50(4):536-538.
5. ThakralGK,AeranH,YadavB,ThakralR.FlexiblePartialDentures-A Hope for the Challenged Mouth. People's J Sci Res.2012;5(2):55-9.
6. Abhay PN, Karishma S. Comparative evaluation of impact andflexural strength of four commercially available flexible denturebasematerials:aninvitrostudy.JIndianProsthodontSoc.2013;13(4):499-508.
7. Takabayashi Y. Characteristics of denture thermoplastic resins fornon-metalclaspdentures.DentMaterJ.2010;29(4):353-361.
8. GoiatoMC, SantosDM, HaddadMF, PesqueiraAA.Effectof acceleratedagingonthemicrohardnessandcolorstabilityofflexibleresins fordentures.BrazOral Res.2010;24(1):114-9.
9. Vikhe DM, Saraf V, Gangadhar SA, Bhandari A, Vikhe G, TambeSD.Flexibledenture-AflexiblesubstituteforRigidDenture.PravaraMedRev.2016;8(1):30-2.
10. Vidushi M, Dhakshaini MR. Evaluation andcomparisonof theefficacyoftwocommerciallyavailabledenturecleansersinremoving stains from patients used dentures. Int J Oral Health MedRes.2015;3(4):40-44.
11. Patel M. Oral Cavity and Candida albicans: Colonisation to the Development of Infection. Pathogens. 2022;11(3):335.
12. Renner R P, Andors L, McNamara T F, Brook S. The role of C. albicans in denture stomatitis. Oral Surg.1979;47(4):323-28.
13. Singh JP, Dhiman RK, Bedi RP, Girish SH. Flexible denture base material: A viable alternative to conventional acrylic denture base material. ContempClin Dent. 2011;2(4):313-7.
14. Loster BW, Loster J, Wieczorek A, Ryniewicz W. Mycological analysis of the oral cavity of patients using acrylic removable dentures. Gastroenterol Res Pract. 2012;2012:951572.
15. Garg SK, Singh VA, Garg SK, Mittal S, Chahal GK. Effect of denture wearing on occurrence of fungal Isolates in the oral cavity: A pilot study. J ClinExp Dent. 2012;4(2):e86-90.
16. Quirynen M. The Clinical Meaning Of The Surface Roughness And Surface Free Energy Of Intra Oral Hard Substrata On The Microbiology Of The Supra And Subgingival Plaque: Results Of In Vitro And In Vivo Experiments. J Dent.1994;3(2):22-27.

17. Abuzar MA, Bellur S, Duong N, Kim BB, Lu P, Palfreyman N, et al. Evaluating surface roughness of a polyamide denture base material in comparison with poly (methyl methacrylate). J Oral Sci. 2010;52(4):577–81.
18. Narhi TO, Ainams A, Meurman JH. Salivary yeasts, saliva and oral mucosa in the elderly. J Dent Res.1993;10:14.
19. Knight L, Fletcher J. Growth of Candida albicans in saliva stimulated with antibiotics, corticosteroids and diabetes mellitus. J Infect Dis 1971;123:371– 377.
20. Malic S, Hill KE, Ralph JR et al. Characterization of Candida albicans infection of an invitro epithelial model using confocal laser scanning microscopy. Oral MicrobiolImmunol 2007;22:188–194.
21. Chaudhary N, Yadav B, Phukela S, Khandait M. Comparative Evaluation of Surface Roughness and Adhesion of Candida albicans on Conventional Heat-cured, Injection-molded Thermoplastic Resin and CAD-CAM Denture Base Resin as Affected by Denture Cleanser: An In Vitro Study. Int J ProsthodontEndod. 2023;13(3):145-53.
22. Fernandes FF. Efficacy of denture cleansers on Candida spp. biofilm formed on polyamide and polymethyl methacrylate resins. J Prosthet Dent. 2010;105:51-58.
23. Ahmad ZM, Mustafa EA, Jawad IA. Adherence of Candida albicans to Flexible Denture Base Material. Al–Rafidain Dent J. 2012;12(2): 229-235.
24. Ahmed EM, Esmat AM, Hassan HG. Candida albicans colonization on different polymeric denture base materials in controlled type II diabetic patients. Journal of The Arab Society for Medical Research.2019;14(2):95-101.
25. Jain V, Babu J, Ahuja S, Wicks R, Garcia-Godoy F. Comparison of Fungal Biofilm Formation on Three Contemporary Denture Base Materials. Int J Experiment Dent Sci.2015;4(2):104-108.
26. Anggraini M, Djais AA, Soekanto SA. Candida albicans biofilm profiles on various denture base materials. J Int Dent Med Res.;11(1):191-196.
27. Maaly T, Mohamed E. Evaluation of 2-methacryloyloxyethyl phosphorylcholine polymer coated polyamide denture base material: Surface Roughness and Candida Albicans adhesion. Egypt Dent J.2021;67(2):1391-8.
28. Mehendale AV. Adherence of candida albicans on polyamides in comparison with conventional acrylic surfaces-a short study. Int. j innov res sci stud. 2018;18(Suppl 2):262-268.