Adherence of Candida Albicans on Different Denture Base Material as Heat Cure Acrylic Denture Base Material Flexible Denture Base Material and Cobalt Chromium Alloy: An in Vitro Study

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Abstract The incidence of oral candidiasis linked denture stomatitis is very high in complete denture patients. Apart from other factors conducive to the growth of candida Albicans on dentures the choice of material employed for denture fabrication also plays an important role. The study is evaluates and compare the adherence of Candida albicans on heat cure acrylic denture base resin, flexible denture base resin and cobalt – chromium alloy which are the three most common materials used in the prosthesis. Standardized sized disc made of the three materials (30 each) were placed in a flask containing standardized cell suspension (ATCC 26555) of Candida albicans in peptone dextrose broth for 1 hour at room temperature. A loop full of the contents were then transferred on to Yeast Peptone Dextrose (YPD) Agar plate for 48 hrs.

The highest C. albicans attachment was observed on flexible denture base material followed by Heat cure acrylic resin and least in cobalt chromium alloy denture base material. Above findings suggest that the metallic denture bases are far better than acrylic denture bases in terms of incidence of denture stomatitis due to candidal infection. Also the rigid heat cure acrylic fared better compared to flexible denture bases.

In an edentulous mouth, denture-related stomatitis is the most common form of oral candidiasis .[1-11] Wearing of denture produces micro-environment conducive to the growth of Candida with low oxygen, low pH, and anaerobic environment. The essential prerequisite for successful Candida colonization and infection is the ability of the Candida albicans to adhere to the superficial epithelial cells as well as the fitting surfaces of denture, considering the latter as the reservoir of infection.[12,13] Although Candida colonization is a complex mechanism that may depend on surface free energy of materials[14], and interaction between salivary pellicle and fungal plaque accumulation, the surface roughness is known to be a factor in entrapment of microorganisms, and their protection from shear forces[15] , old age and concomitant decline of the immune defences, use of broad spectrum antibiotics[10], malnutrition, systemic diseases, smoking, wearing dentures at night, and poor oral hygiene act as supplementary factors promoting colonisation[10-14].

Material science has developed and over the years many denture base materials[16] have been used eg.

resins, metals & more recently flexible denture base materials. Adherence of C. albicans to various denture base materials has been investigated upon as 90% of the denture stomatitis is because of colonisation of candida species.[14]. Microbial adhesion on biomaterial surfaces depends on the surface structure and composition of biomaterials, and on the physicochemical properties of the microbial cell surface, again its surface charge and hydrophobicity[17-21] Also, the tissue surface of the denture is not polished for intimate mucosal contact and the resultant roughness may facilitate microbial retention and infection[19]

In contrast to the acrylic denture bases, metallic denture bases have the advantage of presenting smooth surface, impervious hydrophobic surfaces and are also considered superior in many respects. The newer flexible denture bases have not been studied for these criteria.

The purpose of this study is to evaluate and compare the **adherence of Candida albicans** on **heat cure acrylic denture base resin, flexible denture base resin and cobalt – chromium alloy**.

Material and methods

The study was undertaken to evaluate the adherence of Candida albicans on Heat cure acrylic resin (DPI Heat cure resin {Dental Products of India}, B. No. 1151) (**Fig (1)**, Cobalt-chromium alloy (Vera PDI (Cobalt-Chrome alloy for cast partial dentures, Aalba Dental Inc. California, USA) **Fig (2)** and Flexible denture base material (Valplast Flexible Material, Valplast International Corporation) **Fig (3)** at RUHS COLLEGE OF DENTAL SCIENCES

FABRICATION OF STUDY SPECIMENS

Thirty discs each of all three materials measuring 6 mm diameter and 2 mm thickness were fabricated following manufactures instructions. Silicon carbide papers were used to smooth the surfaces.

STUDY DESIGN

Each specimen was divided into 6 groups containing 5 discs in each group

Specimen Group 1 Heat Cure Acrylic (HCA) (Fig.-09)

Specimen Group 2 Flexible acrylic resin (FDB) (Fig.-10)

Specimen Group 3 Cobalt chromium Alloy (CCA) (Fig.-11)

Standard Composition (pH6.8) of yeast peptone dextrose broth

Chemical	**Composition**
Yeast	1.250gm
Peptone	1.250gm
Dextrose	3.00gm
Distilled Water	1000ml

Inoculation of Candida albicans in Yeast Peptone Dextrose Broth

Preparation of yeast inoculums

- Appropriate number of separated colonies of Candida Albicans ATCC 2655 were grown on Cystine lactose electrolyte deficient agar (CLED) media fresh culture plate and inoculated with a single colony of tested strain in yeast peptone dextrose (YPD) media and incubated at 37°C for 18h **(Fig -14)**

Attachment test of Candida albicans from denture Base Materials

All ninety disc specimens (30 from each type of denture base material i.e. heat cure acrylic, Flexible acrylic resin and cobalt chromium alloy were sterilized for 2 hours at UV wavelength of 245nm. The specimens were then placed in a flask containing standardized cell suspension (ATCC 26555) of Candida albicans in peptone dextrose broth for 1 hour at room temperature. The specimens were removed, drained and placed in 1ml phosphate buffer saline (PBS), and vortexed for 1hour. A loop full of the contents were transferred from phosphate buffer saline (PBS) on to Yeast Peptone Dextrose (YPD) Agar plate using 4mm diameter standard wire loop. These materials were spread on the plate in a lawn culture and incubated at 37°c for 48 hr. Viable count of the microorganism were determined by colony count. Results were expressed in colony forming units per square millimetre (cfu/mm^2).[31]

CFU Determination

Aliquots of 100 µl of each specimen were cultured into YPD agar media. All the YPD agar plates were cultivated at 37°C 48 hours. Number of colonies observed after 48 hours were recorded. **(Fig.-16-18)** All the specimens were tested for Candidal adherence by viable counts of the microorganism, which were determined by colony count. Digital colony counter (fig.-08) was used for colony counts. Results were expressed in cfu/mm^2.

CFU

$$\textbf{CFU/ml} = \frac{\text{No. of colonies x Dilution factor}}{\text{Volume of inoculum}} \qquad \textbf{CFU/m m}^2 = \frac{\text{CFU/ml}}{\text{Area of 5 discs}}$$

Log value = log(CFU/ml)

Area of disc = πr^2

Fig 1 - Heat Cure Acrylic Resin

Fig 2 - Co-C r Alloy Material

Fig 3 - Flexible Resin Material

Fig 4 - Armamentarium used for Sample Preparation

Fig 5 - Armamentarium used for Sample Preparation

Fig 6 - Incubator

Fig 7 - Photoelectric Calorimeter

Fig 8 - Digital Colony Counter

Fig 9 - Heat Cure Acrylic Disc

Fig 10 - Co-Cr Alloy Disc

Fig 11 - Flexible Disc

Fig 12 - Material used for Microbiological

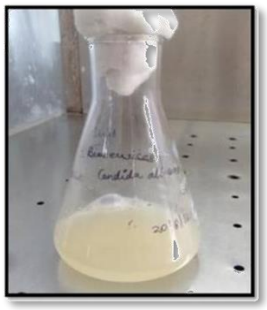


Fig 13 - Pure Culture of Candida Albicans Master Culture of Candida albicans in Yeast Peptone Dextrose Broth

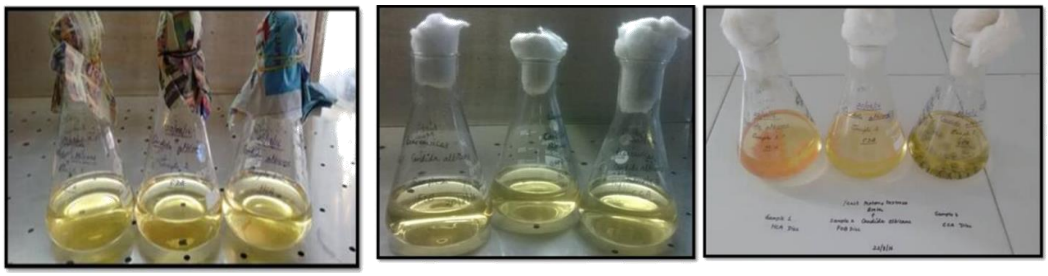


Fig 14 - Yeast Peptone Dextrose Broth Yeast Peptone Dextrose Broth + Candida Albicans + Sample Disc

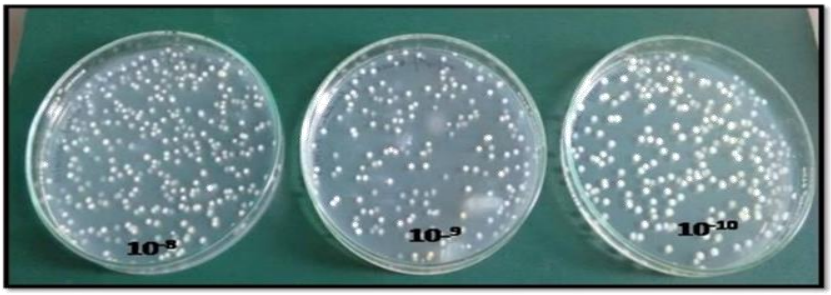


Fig 15 - Positive Control of Candida Albicans

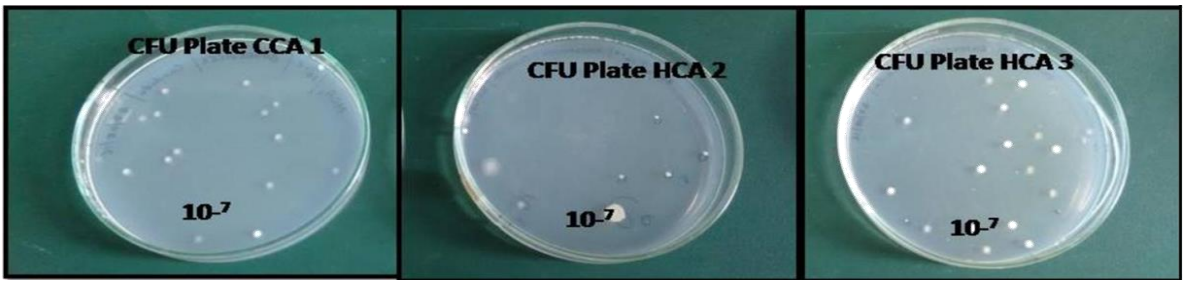


Fig 16 - Heat Cur Acrylic HCA

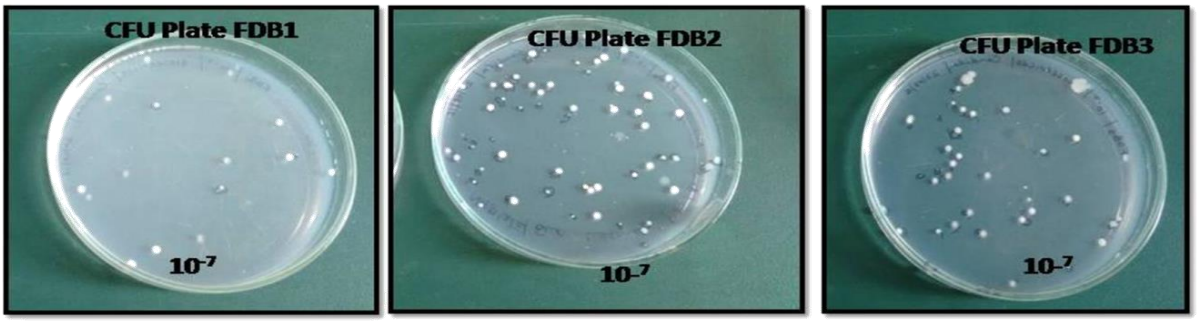


Fig 17 - Flexible Denature Base – FDB

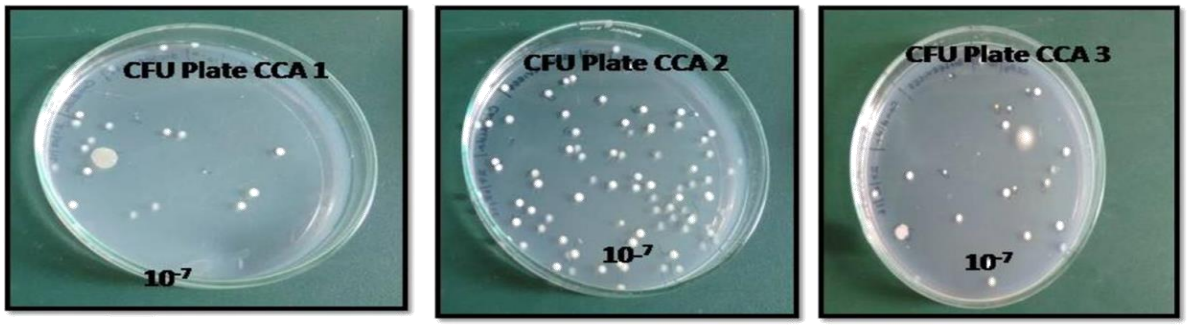


Fig 18 - Cobalt Chromium Alloy- CCA

In this present study the adherence of Candida albicans on heat cure acrylic resin cobalt-chromium alloy and flexible denture base material were measured. Viable count of the microorganism were determined by colony count. Results were expressed in cfu/mm^2.[31]

Data Analysis

The least number of CFU were seen in CCA (**9.54± 0.35) followed by HCA (10.9±1.6) and highest in FDB (09.54± 0.35)**

One way ANOVA test (Bonferroni) for number of C. albicans colony attachment on heat cure resin (**10.9x10^4**) flexible resin (**13.67x10^4**) and Co-Cr Alloy **09.54** x10^{4}i.e. The p<0.01* indicate that there are higher significant difference between groups so further multiple comparison test was carried out. Table 4

By comparing values significant difference was observed between FDB and HCA (P .003). Adherence of Candida albicans was highly significant for all other groups (p .002)

Table 1: (Heat Cure Acrylic Denture Base Material)

S.No	Specimen	No. of Colony	CFU/mm^2	10^4 CFU/mm^2
1.	**HCA1**	18	98738.34339	9.87
2.	**HCA2**	22	120680.1975	12.6
3.	**HCA3**	16	87767.41635	8.77
4.	**HCA4**	18	98738.34339	9.87
5.	**HCA5**	24	131651.1245	13.16
6.	**HCA6**	22	120680.1975	12.06

Table 2: (Flexible Denture Base Material)

S.No.	Specimen	No. of Colonies	CFU/mm^2	10^4 CFU/mm^2
1.	**FDB 1**	32	132691.9887	13.26
2.	**FDB 2**	30	124398.7394	12.43
3.	**FDB 3**	32	132691.9887	13.26
4.	**FDB 4**	34	140985.238	14.09
5.	**FDB 5**	36	149278.4873	14.92
6.	**FDB 6**	34	140985.238	14.09

Table 3: (Cobalt Chromium Alloy)

S.No.	Specimen	No of Colony	CFU/mm2	(10^4 CFU/mm2)
1.	**CCA 1**	20	103896.1039	10.38
2.	**CCA 2**	21	109090.9091	10.9
3.	**CCA 3**	21	109090.9091	10.9
4.	**CCA 4**	19	98701.2987	9.87
5.	**CCA 5**	24	124675.3247	12.46
6.	**CCA 6**	21	109090.9091	10.9

Table 4: Comparisons of Adherence of Candida Albicans on each type of Denture base Material ((10^{4}CFU/mm^2)

Variables	Mean±SD	P value
HCA	**10.9±1.6**	**0.01***
FDB	**13.67±0.87**	
CCA	**09.54± 0.35**	

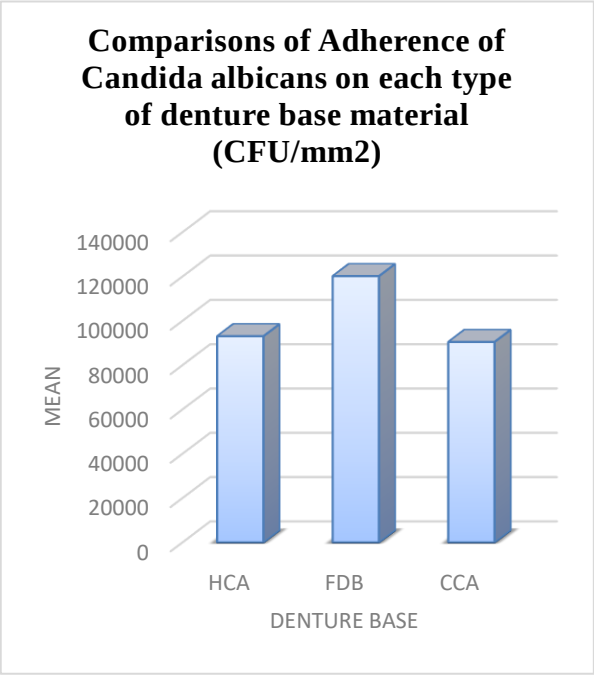
Statically significant difference at p=0.05

Table 5: Inter Group Comparisons of Adherence of Candida Albicans on each type of Denture base Material ((10^{4}CFU/mm^2)

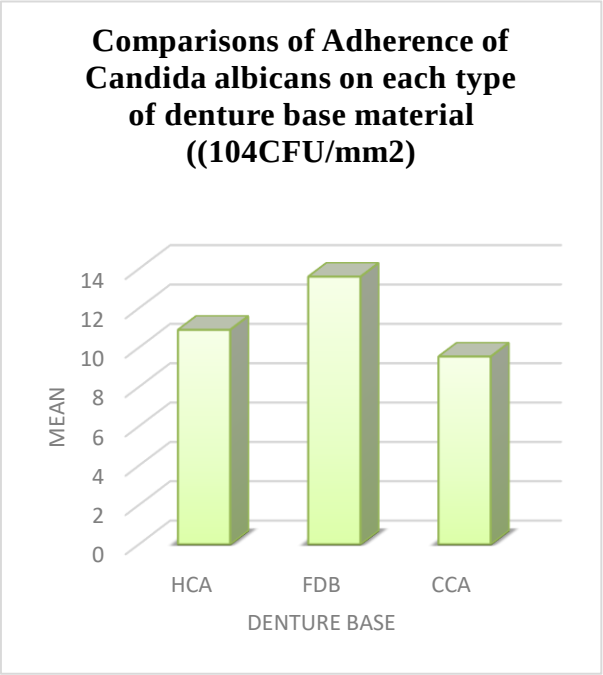
		P value
Group1 HCA	Group 2 FDB	0.003*
	Group 3 CCA	0.002*
Group 2 FDB	Group 1 HCA	00.03*
	Group 3 CCA	0.002*
Group 3 CCA	Group 1 HCA	0.002*
	Group 2 FDB	0.002*

* indicates statically significant difference at p=0.05

Test of significance - One way ANOVA with Bonferroni, SD: Standard Deviation



Graph 1: Comparisons Adherence of Candida Albicanse on each type of denture base Material (CFU/ mm2)



Graph 2: Comparisons Adherence of Candida Albicanse on each type of denture base Material (104CFU/ mm2)

Candida amongst all fungal infection is the most common cause for oral mycosis and denture stomatitis. It is a well-known fact, that removable denture bases fabricated from heat cure acrylic resin act as a reservoir for microorganism and contribute to reinfection to denture wearers.[24]

The ability to form biofilm is intimately associated with the ability to cause infection and as such should be considered an important virulence determinant during denture stomatitis. From the literature the picture emerges that many factors determine Candida harboring-biofilms. These factors include surface properties, micro-organisms interactions, biofilm architecture, and saliva. Despite therapeutic progress, opportunistic oral fungal infectious diseases have increased in prevalence, especially in denture wearers.

Candida albicans existence presents a high significance in the etiology of denture stomatitis; its incidence has been reported to occur among 11-67 % of the denture wearers[32] and is found on surfaces of hard and resilient acrylic resin materials in vivo[33]. **Miller** studied the adhesion mechanisms of C. albicans to denture base materials and factors affecting these mechanisms. Surface roughness[33,34]and type of materials are known to be two major factors for the adherence mechanism directly. The yeasts, being a part of the denture plaque, adhere and accumulate on the surface of the prosthesis that plays a storing role for them [36 37]. Studies have been done on attachment of Candida albicans on acrylic resin material. Albeit there is a lack of extensive literature on candida and its adherence on flexible and metallic denture base material. Cobalt chromium alloy is most commonly used metallic denture base and flexible denture base material is newer material in dentistry. So flexible and cobalt chromium alloy denture base material along with heat cure resin material was used for this study.

Budtz Jorgenson et al [22] in their study on clinical aspect of Candida infection in denture wearers hypothesized that the Candida induced denture stomatitis usually does not reflect on any deep-seated systemic abnormality, but the dentures are primary predisposing factors. Therefore, preventive measures must be taken against colonization of Candida on palatal mucosa and dentures.

In this study an attempt has been made to evaluate and compare the growth of Candida species (ATCC26555) in three type of denture base material (heat cure denture base material, flexible denture base material and Cobalt Chromium alloy denture base material) Candida albicans to these denture base materials was studied.

It was found that the adherence of Candida albicans on heat cure acrylic resin was significantly less compared to flexible denture base material (p value =0.003) and significantly more compared to CCA (0.002). The study by Frederico Silva et al also demonstrated significantly higher growth on polyamide resin compared with PMMA resin. Because of rougher surface.[60]

However, our results are in contrast to study by Zeina M Ahmad [35] which showed that C. albicans has lesser opportunities to adhere on Valplast than on acrylic resin denture base materials. However, in their study the colony forming units were seen on as cured and unfinished condition which could explain the difference in the results as because of the fiber content the finishing and polishing of flexible denture bases is more difficult.

It was also found that the adherence of Candida albicans on flexible denture base material was significantly higher compared to heat cure acrylic resin (p value =0.003) and significantly more compared to CCA (P value =0.002) as is corroborated by studies reporting existence of candida albicans found on surfaces of hard and resilient acrylic resin materials in vivo[33]

It was also found that the adherence of Candida albicans on Cobalt chromium alloy denture base material was significantly low compared to heat cure acrylic resin (p value =0.002) and significantly more compared to FDB (P.=0.002)

Minimum Candida attachment was found on Co-Cr alloy denture-base material because of hydrophobicity and smooth surface compare to FDB&HCC. The FDB has a rougher surface as compared to HCA and CCA, which explains the higher colony forming units in FDB. This is supported by the studies of Miller et al who showed that the adhesion mechanisms of C. albicans to denture base materials is proportional to the surface roughness and type of material [33,34]. Studies by Nikama, Waltimo, Budtzjorgenson et al have also

shown that the yeasts, being a part of the denture plaque, adhere and accumulate on the surface of the prosthesis that plays a storing role for them[33,37]

The results of the study are similar to that by Celik GY et al, to evaluate the In vitro attachment of C.albicans (ATCC 26555 and Serotype B Netherland CBS 5983) on acrylic resin and a Co-Cr alloy denture-base materials showed that there was more attachment on acrylic resin base material than to Co-Cr alloy base material.[29]

These results demonstrated that the type of denture-base material play an important role in the adherence of Candida albicans and thus the incidence of candida stomatitis.

SUMMARY

An in vitro study was done to evaluate adherence of Candida albicans on Heat cure acrylic resin, Cobalt-chromium alloy and Flexible denture base material" Total of 90 specimens were made, 30 from each type of denture base material i.e. Heat cure acrylic resin, flexible acrylic resin and cobalt chromium alloy. All disc specimens were sterilized with ultraviolet rays. For Candida albicans adherence, all group specimens were placed in standardized cell suspension (ATCC 26555) of Candida albicans for 1hour at room temperature. After the specimens were removed, drained and placed in 1ml phosphate buffer saline (PBS), and vortexed for 1h. A loop full of the content were transferred from phosphate buffer saline (PBS) on to Yeast Peptone Dextrose (YPD) Agar plate using 4mm diameter standard wire loop. This material were spread on the plate in lawn culture and incubated at 37°c for 48 hr. viable count of the microorganism were determined by colony count. Results were expressed in cfu/mm2. This study showed that the adherence of C. albicans ATCC 26555 was more on heat cure acrylic resin material compare to flexible denture base material and Co-Cr alloy denture-base material. Minimum Candidal attachment was found on Co-Cr alloy denture-base material.

CONCLUSION

Within the limitations of this study, the following conclusions were drawn.

The highest C. albicans attachment was observed on flexible denture base material followed by Heat cure acrylic resin and least in cobalt chromium alloy denture base material.

Above findings suggest that the metallic denture bases are far better than acrylic denture bases in terms of incidence of denture stomatitis due to candidal infection. Further, they are seemed to be more biocompatible with the oral tissues with minimal tissue reactions if any.

ANNEXURES

Adherence of Candida Albicans on Heat Cure Acrylic

S.No.	Specimen	No. of Colonies	10 CFU/mm	CFU/
1	**HCA 1**	18	9.87	3600000
2	**HCA 2**	22	12.06	4400000
3	**HCA 3**	16	8.77	3200000
4	**HCA 4**	18	9.87	3600000
5	**HCA 5**	24	13.16	4800000
6	**HCA 6**	22	12.06	4400000

Adherence of Candida albicans on Flexible Denture Base

S.No.	Specimen	No. of Colonies	10 4 CFU/mm	CFU/disc
1	FDB 1	32	13.26	6399734.6
2	FDB 2	30	12.43	5999751.2
3	FDB 3	32	13.26	6399734.6
4	FDB 4	34	14.09	6799718
5	FBB5	3G	14.92	7199701.4
6	FDB 6	34	14.09	6799718

Adherence of Candida albicans on Cobalt Chromium Alloy Denature Base

S.No.	Specimen	No. of Colonies	10 4 CFU/mm	CFU/disc
1	CCA 1	20	10.38	4000000
2	CCA 2	21	10.9	4200000
3	CCA 3	21	10.9	4200000
4	CCA 4	19	9.87	3800000
5	CCA 5	24	12.46	4800000
6	CCA 6	21	10.9	4200000

REFERENCES

1. Sherwood, Linda; Willey, Joanne; Woolverton, Christopher (2013). Prescott's Microbiology (9th ed.). New York: McGraw Hill. pp. 713–721. ISBN 9780073402406.OCLC 886600661.
2. Sutter, V. L. (1984). "Anaerobes as normal oral flora". Reviews of infectious diseases. 6 Suppl 1: S62–S66. doi:10.1093/clinids/6.Supplement_1.S62. PMID 6372039
3. Cui L, Morris A, Ghedin E (July 2013). "The human mycobiome in health and disease". Genome Med. 5 (7):63. doi:10.1186/gm467.PMC 3978422 PMID 23899327. : Distribution of fungal genera in different body sites
4. Trends in the epidemiology of opportunistic fungal infections: predisposing factors and the impact of antimicrobial use practices. Singh N Clin Infect Dis. 2001 Nov 15; 33(10):1692-6.
5. Aerobic and Anaerobic Bacteria in Tonsils of Children with Recurrent Tonsillitis And Oto Rhinol Laryngol May-June 1981 90:261-263,
6. Manolakaki, D.; Velmahos, G.; Kourkoumpetis, T.; Chang, Y.; Alam, H. B.; De Moya, M. M.; Mylonakis, E. (2010). "Candida infection and colonization among trauma patients". Virulence. 1 (5): 367–375.doi:10.4161/viru.1.5.12796.
7. K. Fugelsang, C. Edwards Wine Microbiology Second Edition, pp. 3–28 Springer Science and Business Media , New York (2010) ISBN 0387333495
8. Erdogan A, Rao SS (April 2015). "Small intestinal fungal overgrowth". Curr Gastroenterol Rep. 17 (4): 16. doi:10.1007/s11894-015-0436-2
9. Martins N, Ferreira IC, Barros L, Silva S, Henriques M (June 2014). "Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment".Mycopathologia. 177 (5-6): 223–240. doi:10.1007/s11046-014-9749-
10. Kourkoumpetis, Themistoklis K., et al. "The effect of cumulative length of hospital stay on the antifungal resistance of Candida strains isolated from critically ill surgical patients." Mycopathologia 171.2 (2011): 85-91.
11. Bouquot, Brad W. Neville , Douglas D. Damm, Carl M. Allen, Jerry E. (2002). Oral & maxillofacial pathology (2. ed.). Philadelphia: W.B. Saunders. pp. 192–194.

12. Epstein JB. Antifungal therapy in oropharyngeal mycotic infections. Oral Surgery Oral Med Oral Pathol 1990;69:32–41.
13. Guida RA. Candidiasis of the oropharynx and oesophagus. Ear Nose Throat J 1988;67:832–40.
14. Hydrophobic interaction in Candid aalbicans and Candida tropicalis adherence to various denture base resin materials. Minagi S, Miyake Y, Inagaki K, Tsuru H, Suginaka HInfect Immun. 1985 Jan; 47(1):11-4.
15. Prezous LF, Flaitz CM, Enelmeier RL. Colonization of Candida species in denture wearers with emphasis on HIV infection: A Literature Review. J Prosthet Dent 2005; 93:288-93.
16. Barden M. The absorption of water by acrylic resins and other materials. JProsthet Dent 1964; 14:2:307-316.
17. Hydrophobic interaction in Candida albicans and Candida tropicalis adherence to various denture base resin materials.Minagi S, Miyake Y, Inagaki K, Tsuru H, SuginakaH Infect Immun. 1985 Jan; 47(1):11-4
18. Nevzatoğlu ED, Özean M, Ozkan YK, Kadir T. Adherance of Candida albicans to denture base acrylics and silicone based resilient liner materials with different surface finishes. Clin Oral Invest 2007; 11:231-236.
19. Duggineni Chalapathi Rao1, N Kalavathy2, HS Mohammad3, A Hariprasad3, C Ravi Kumar1Evaluation of the surface roughness of three heat-cured acrylic denture base resins with different conventional lathe polishing techniques: A comparative study ear : 2015 | Volume : 15 | Issue : 4 | Page : 374-380
20. Stysiak ZD. Allergy as a cause of denture sore mouth. J Prosthet Dent 1971;25:1:16-18
21. Miner JF. The nature of denture base: A key factor in denture sore mouth. JProsthet Dent 1973; 29:3:250-255.
22. Budtz Jorgensen. Oral mucosal lesions associated with the wearing ofremovable dentures. Journal of Oral Pathology 1981; 10:65-80.
23. Samaranayake LP, Weetman DA., Geddes DAM, McFarlane TW. Carboxylic acids and pH of denture plaque in patients with denture stomatitis. J Oral Path1983; 12:84-89.
24. Radford DR1, Sweet SP, Challacombe SJ, Walter JD.Adherence of Candida albicans to denture-base materials with different surface finishes. J Dent. 1998 Sep;26(7):577-83.
25. DiTolla M. Valplast – Flexible, esthetic partial dentures.ChairsidePerspectiv Clinical tech and procedures2004; 5(1):67-73
26. Prezous LF, Flaitz CM, Enelmeier RL. Colonization of Candida species indenture wearers with emphasis on HIV infection: A Literature Review. JProsthet Dent 2005; 93:288-93.
27. Yildirim MS, Hasanreisoglu U, Hasirci N, Sultan N. Adherence of Candida albicans to glow-discharge modified acrylic denture base polymers. J Oral Rehabil 2005; 32: 518–525.
28. Perezous LF, Stevenson JC, Flaitz CM, Goldschmidt ME, Engelmeier RL,Nichols CM. The effect of complete dentures with a metal palate on Candida species growth in HIV-Infected patients. J Prosthodont 2006; 15:306-315
29. Celik GY, Aslim B, Kocabalkan V. An in vitro Assessment of the Attachment Two Oral Pathogens to Denture Base Materials. Journal of Applied Biological Sciences. 2008; 2 (2): 51-54.
30. Avon SL, Goulet JP, Deslauriers N. Removable acrylic disk as a samplingsystem for the study of denture biofilms in vivo. J Prosthet Dent 2007; 97:32-38.
31. Taylor R, Maryan C, Verran J. Retention of oral microorganisms on cobalt chromium alloy & dental acrylic resin with different surface finishes. J Prosthet Dent 1998; 80:592-97
32. Waters MG, Williams DW, Jagges RG. Adherence of Candida albicans to experimental denture soft lining materials. J Prosthet Dent 1997; 77:306-12.
33. Nikawa H, Hamada T, Yamamot T, Kumagai H. Effects of salivary or serumpellicles on the Candida albicans growth and biofilm formation on soft lining materials-in vitro. J Oral Rehabil 1997; 24:594-604.
34. Waltimo T, Vallittu P, Haapasalo M. Adherence of Candida species to newlypolymerized and water stored denture base polymers. Int J Prosthodont 2001;14:457-60.
35. Budtzjorgenson et al. Clinical aspects of Candida infection in denture wearers. J American Dental Association 1978; 96:474-9.
36. Zeina M Ahmad Eman A Mustafa DInas A Jawad Adherence of Candida albicans to Flexible Denture Base Material Al–Rafidain Dent J. 2012;12(2): 229-235.
37. Menaka A. Abuzar, Suman Bellur, Nancy Duong, Evaluating surface roughness of a polyamide denture base material in comparison with poly(methyl methacrylate)Journal of oral science vol. 52, no. 4, 577-581,2010