ISSN No:-2456-2165

Adherence of Candida Alibicans on Polyamides in Comparison with Conventional Acrylic Surfaces – A Short Study

[1]Dr. Avinash Venkatesh Mehendale, [2]Dr. Shubhratha C., [3]Dr. Eby Aluckal, [4]Dr. Glynis Anita Miranda, [5]Dr. Bharath Prabhu, [6]Dr. Mithun Upadhya, [7]Dr. Vidya K. Shenoy

Abstract:-

- ***Objective***

To compare the adherence of Candida albicans on a polyamide surface with polymethyl-methacrylate (PMMA), whose surface roughness was kept within the acceptable range after using conventional polishing techniques.

- ***Background***

Poly methyl methacrylate (PMMA) resins have been routinely used as a denture base material because of its desired properties and simple processing techniques. Polyamides developed as alternatives to PMMA, are nylon based materials, which are flexible in nature. However, the surface characteristics and especially the microbial adhesion of polyamides have not been extensively evaluated in the literature.

- ***Materials and Methods***

A polyamide material (Sunflex) and PMMA (Trevalon) were tested and compared. 10 rectangular samples of each material were processed and conventionally polished. The evaluated surface roughness values of both the materials were below the accepted threshold of 0.2µm Ra. Later these samples were tested for adherence of C albicans.

- ***Results***

The average Ra value of polyamide after polishing was 0.044 µm ± 0.2. The Student't' test, showed a significant difference in the surface roughness of the two materials, with PMMA being smoother than polyamide (p – 0.005). The average Candida colony count per microscopic field on 2nd and 4thday was significantly more on polyamide surface and it again increased on 8th and 12th day respectively but insignificantly.

- ***Conclusion***

Polyamide has a relatively rougher surface which tends to increase the adherence of Candida compared to PMMA. This might compromise the long term bio-compatibility of polyamide as a denture base material.

Keywords:- *PMMA, Polyamide, Surface Roughness, Candida Alibicans.*

I. INTRODUCTION

Poly methyl methacrylate (PMMA) resins have been the material of choice for the fabrication of dentures since its introduction in 1937[1]. This is due to PMMA's desired physical properties, availability, excellent aesthetics, low water sorption and solubility, relative low toxicity, ease of repair, and simple processing techniques[2]. Although it remains the most popular material, it is far from beingan ideal denture base material. Possible allergic reactions, hypersensitivity and cytotoxicity due to residual monomer, low impact strength are some of the drawbacks of PMMA resins reported in the literature[3-5]. Another significant disadvantage of acrylic resins is the polymerization shrinkage (especially the linear shrinkage), which can cause significant effects on the dimensionand occlusion[6]. Due to its rigidity, insertion of heat cure acrylic dentures becomes difficult and painful in presence of soft and hard tissue undercuts. In order to overcome these disadvantages, polymer chemistry has developed alternative materials to PMMA such as polyamides (nylon plastics), acetal resins, epoxy resins, polystyrene, polycarbonate resins and chlorinated polyether,all of which are suited for thermoplastic processing[7]. In general, all nylons demonstrate higher water sorption and creep than most of the dental polymers. Other drawbacks of polyamides include lack of bonding between tooth and denture base, difficult to reline and rebase and chair side polishing[8]. Over the years, there have been modifications in polyamides to overcome these disadvantages. A study by Ucar et al has shown thatpolyamide denture material produced good fracture resistance, but its modulus of elasticity was not as high as PMMA. In general, flexibility, design simplicity, minimal tooth modifications and exceedingly rare allergy response are the features supporting polyamides' popularity.[9]

Surface roughness is an important physical property for any denture base material. Rougher surfaces tend to promote development of plaque in vivo and provide a larger surface area, resulting in a more conducive environment for development of plaque associated micro-organisms[10].It is a known fact that the tissue surface of a maxillary denture is a potential reservoir for microorganisms, especially Candida species.Candida albicans in particular is an important factor associated with denture stomatitis. Adherence of Candidaspecies to denture base surface increases with an increase in surface roughness of the denture base surface[11-13].Further, surface roughness is affected by the type of material,

ISSN No:-2456-2165

polymerization method and polishing technique used. Moreover, as age advances maintenance of denture hygiene becomes less efficient. Studies have shown that surface roughness and polishing techniques of denture base materials are correlated. Mechanical polishing with conventional methods of using abrasives, pumice and lathe polishingprovides an average Ra value below the threshold of 0.2µm. Studies have concluded that the conventional polishing technique provides a smoother PMMA surface compared with other polishing techniques[14-16].

There are numerous studies in the literature which evaluates bacterial and fungal colonization of different acrylic resin surfaces. On the contrary in-vivo and in-vitro studies are necessary to investigate the same on polyamide surfaces. Thus the present study was conducted to compare the adherence of C albicans colonies on a polyamide surface with PMMA, whose surface roughness was kept within the acceptable range after using conventional polishing techniques.

II. MATERIALS AND METHODS

Two denture base materials, a polyamide material (Sunflex, Sun Dental Laboratories LTD, South Yorkshire, UK)and PMMA resin(Trevalon, Dentsply India.) were tested and compared. A microscopic slide was cut into half and glued together. A polyvinylsiloxane (PVS) putty (Aquasil, soft putty, Dentsply, Konstanz, Germany) index was made of the glued microscopic slide. This index was used to fabricate 20samples using modelling wax (Surana Modelling Wax, Mangalore, India).Using compression mould technique,10 wax samples were processed with PMMA resinin a denture polymerisation unit (Acrylizer, Confident India Pvt Ltd, India) with a long curing cycle.Another 10samples were processed with a polyamide denture base material using injection mould technique according to the manufacturer's instructions. The polyamide was processed using an injection molding machine (Thermopress 400, Bredent GmbH & Co KG, Germany) with an injection pressure of around 750kPa at 393°F with a pre-heating time of 15 min. The processed samples were retrieved and subjected to finishing and polishing procedures.

A. Polishing

Conventional polishing techniques were used to polish all the samples. The initial finishing of the samples was done with a tungsten carbide bur (Cross-cut, coarse –DFS, Germany) followed byan abrasive paper (Million flint paper 80, Million Ind, India)with light manual pressure.A slurry of medium and fine grit pumice (Vensons India, Bangalore, India) mixed in a 1:1 ratio of water was used with a felt cone and a cloth wheel on the polishing lathe. The final polishing was done with a high shine buff with polishing brown tripoli (Dento Kem, Fridabad, India) on the polishing lathe.All the samples were placed and sealed in water tight plastic containers with 10ml water. Each sample was dried under high air pressure before subjecting to surface roughness test.

B. Measuring surface roughness (Ra)

A surface profilometer (Form Talysurf Intra, Taylor Hobson Precision, and Paoli, Pennsylvania, USA) was used to measure the surface roughness (Ra) values of each sample.Asurface profilometer has a diamond stylus which moves vertically in contact with a sample and then moved horizontally across the sample for a specified distance and specified contact force. A profilometer can measure small surface variations and the vertical stylus displacement ranges in height from 10 nanometres to 1 millimetre. The movement of the diamond stylus at specified height and position generates a signal which is digitally stored, analyzed and displayed.

C. Adherence of Candida albicans to the samples

- *Procedure:-*

First the resin slabs where sterilized with 1.5% gluteraldehyde and then they wereimmersedin BHI (Brain Heart Infusion) broth with Candida albicans suspension which was equivalent to 0.5 Mac Farland standard. These slabs were then incubated at 37ºC for 2, 4, 8 and 12 days' time interval. After completing the required incubation, the slabs where inoculated in the culture plates, and incubated for 24hrs. The growth (colony) on the slabs was counted under inverted microscope (25 per microscopic field) on 2nd, 3rd, 8th, and 12th day respectively.

- *Culturing of Candida*

The standard strain was grown in BHI broth first and then subcultured on SDA (Sabouraud Dextrose Agar)medium which was incubated for 24 hours. The character of colonies were creamy and pastry in appearance. Further confirmation was done by simple staining,which under the microscope appeared as 4 to 5 µm diameter oval shaped structures. Subsequently these isolated colonies were added to BHI broth and then incubated overnight and this subculture broth was used for the test.

- *Statistical Analysis*

The obtained data was completed systematically and the analysis was done using SPSS software version 19. Student't'-test was used to determine difference among the groups at different intervals; 'p' value less than 0.05 was accepted to indicate the statistical significance.

III. RESULTS

The surface roughness of the polished surfaces of both heat cure (PMMA) and Sunflex(polyamide) materials was checked and the difference was tested using the student't' test.

The average Ra value of PMMA after polishing was 0.022 µm ± 0.01. This level of smoothness is much lower than the accepted norm of 0.2 µm Ra, and hence can be safely used in the oral cavity with less chances of microbial colonization.

The average Ra value of polyamide after polishing was 0.044 µm ± 0.2. This value is also under the threshold of the accepted norm of 0.2 µm, and hence can be satisfactorily be used in the oral cavity. The average values obtained show that the PMMA is smoother than the polyamide after polishing, which is statistically significant. (Table 1)(Graph 1)

When the individual Ra values of both PMMA and polyamide were plotted and evaluated using the student ' t' test, a statistically significant difference was obtained in the surface roughness of the two materials, with PMMA being smoother than polyamide (p – 0.005) (Table 2)

The average colony count per microscopic field on 2nd, 4th, 8th and 12th day for heat cure denture base and polyamide surface are summarised in (Table 3)(Graph 2). On comparison it was seen that the candida levels on polyamide surface was significantly higher than PMMA surface at time intervals of 2nd and 4th day and the candida levels on polyamide once again increased on 8th and 12th day respectively but insignificantly as shown in the table. (Table 4).

IV. DISCUSSION

Alterations in surface topography of denture base materials can lead to discoloration, discomfort and biofilm formation on the prostheses surfaces[17].Hence it is very important to evaluate the surface characteristics of denture base materials before its clinical application. In this study the results have shown that the nature and surface roughness of denture base resin affects the extent of colonization of resin by candida species. This is in accordance with a study done byTD Morgan and M Wilson in which they concluded that choosing an appropriate type of smooth acrylic could lead to reduced bio-film formation in vivo. Studies byRadford DR et al also have confirmed that bacterial and fungal species have an affinity to adhere to rougher denture base surfaces[18, 19]. Surface free energy (SFE) is another physical factor which influences microbial adhesion. Surfaces with greater surface areas act as protected sites for microbial colonization[20].Studies also indicate that in addition to microbial colonization, rough surfaces increase staining of the dentures resulting in unaesthetic appearance. Therefore an ideal denture base material should have a stain resistant, more aesthetic and a favourable microbiological surface[21].

Bollen CML and Quirynen et al have suggested a threshold level of Ra = 0.2 µm for surface roughness of dental materials used in the oral cavity where no further reduction in plaque accumulation occurs below that level[22, 23]. The surface roughness values obtained in this study are well below the accepted threshold (Ra = 0.2 µm).

Several in-vitro studies in the literature have evaluated different polishing techniques and their effects on surface roughness of acrylic resins. Oliveira LV et al in their study on the effect of different polishing methods on surface roughness of acrylic resins concluded that conventional polishing technique resulted in smoother surface, well below the accepted threshold ofRa = 0.2 µm. The polishing technique used in this study is in accordance with the conventional polishing techniques comprising of a rag wheel and slurry of pumice followed by polishing with a high shine buff. [15, 24]

Abuzar AM et al have shown that both polished and unpolishedpolyamides produced a rougher surface than PMMA. The high amount of temperature and pressure used for injection molding of polyamides followed by very slow cooling results in a relatively stiff but a rough surface material. Also due to its low melting temperature and heat produced during polishing of the specimens, the polyamides become rougher..But recent advances in polyamides which are claimed to be superpolyamides results in relatively less rougher surface which is also easy to polish[25, 26]. Vojdani M. and Giti R in their literature review, have statedsurface roughness and bacterial contamination as among the several drawbacks of Polyamides, as a denture base material[27].The difference in surface roughness values of polyamides and PMMA after polishing in the present study was found to be statistically significant.

BHI broth (Brain Heart Infusion) is used to culture a wide variety of fastidious organisms. When supplemented with antibiotics, varying amounts of sodium chloride and yeast, BHI broth provides a rich medium for bacteria, yeasts and pathogenic fungi[28]. Sabouraud Dextrose Agar (SDA) is also another media specifically used for cultivating pathogenic and commensal fungi and yeasts[29]. Accordingly, in the present study candida on the denture bases were grown and sub-cultured using BHI broth and SDA respectively.

The results of the present study showed that both PMMA and polyamide surfaces had significant colony count of C albicans. The difference in amounts of adherent C albicans on two different surfaces was statistically significant on the 2nd and 4th day. Subsequently as the incubation period was increased to 8 and12 days, a decrease in Candida adhesion was seen on both of the resin surfaces and the difference of adhesion between the two was not statistically significant. This observation could be attributed to C albicans reaching a point of saturation by 4th day and attaining a log phase (equilibrium phase) by 8th and 12th day. This observation is accordance to a study which evaluates adherence of C albicans surfaces to surface modified denture resin surfaces[30]. In accordance with the Ra values of PMMA and polyamide, in the present study candida growth was significantly more on polyamides than PMMA. Hence in the long run, maintenance might be an issue with dentures made of polyamides. Since this was an *in-vitro* study, further in-vivo studies are required to assess the candida growth on different denture surfaces as influenced by the oral environment.

V. LIMITATIONS OF THE STUDY

- It was an in-vitro study; hence oral conditions could not be simulated.
- All polished surfaces were flat unlike the denture surfaces placed in the mouth which affects level of polishing.
- Operator variability could be a limitation during finishing and polishing of the samples

VI. CONCLUSION

Within the limitations of the study,

- The conventional polishing technique used for polishing acrylic resins resulted in a smoother PMMA surface than the polyamide and surface roughness values for both were below the accepted threshold Ra value.
- The average Candida colony count on polyamide surface initially increased significantly compared to PMMA surface and with time the Candida adherence still increased on polyamides, but insignificantly.

Thus, even though polyamides have certain advantages over PMMA resins, its relatively rougher surface tends to increase the adherence of Candida compared to PMMA which can compromise its long term bio-compatibility as a denture base material.

REFERENCES

[1]. Rueggeberg FA. From vulcanite to vinyl, a history of resins in restorative dentistry. J Prosthet Dent 2002; 87:364-379.
[2]. Winkler S. Denture base resins. Dent Clin North Am 1984; 28:287-97.
[3]. Jagger DC, Harrison A, Jandt KD. The reinforcement of dentures.J Oral Rehabil 1999; 26:185-94.
[4]. Leggat PA, Kedjarune U. Toxicity of methyl methacrylate in dentistry. Int Dent J 2003; 53:126-31.
[5]. Faot F, Costa MA, Del BelCury AA, Rodrigues Garcia RCM. Impact strength and fracture morphology of denture acrylic resins. J Prosthet Dent2006; 96: 367-73.
[6]. Anusavice, K. J, Shen C, Rawls RH. Phillips' Science of Dental Materials. 12thed. Elsevier Inc, St Louis, Missouri, 2013; 474-98
[7]. Singh JP, Dhiman KP,GirishHS.Flexible denture base material: A viable alternative to conventional acrylic denture base material. ContempClin Dent 2011; 2:313-17.
[8]. Matthews E, Smith DC .Nylon as a denture base material. Br Dent J 1955; 98: 231-37.
[9]. Ucar Y, Akova T, Aysan I.Mechanical properties of polyamide versus different PMMA denture base materials. J Prosthodont. 2012; 21:173-76.
[10]. Yamauchi M, Yamamoto K, Wakabayashi M, Kawano J. In vitro adherence of microorganisms to denture base resin with different surface texture. Dent Mater J 1990; 9: 19-24.
[11]. Budtz-Jorgensen E. Oral mucosal lesions associated with the wearing of removable dentures. J Oral Pathol1981; 10:65-80.
[12]. Karaagaclioglu L, Can G, Yilmaz B, Ayhan N, Semiz O, Levent H. The adherence of Candida albicans to acrylic resin reinforced with different fibers. JMater Sci Mater Med 2008; 19: 959-963.
[13]. Pereira-Cenci T, CuryAA., Cenci M S, Rodrigues-Garcia R C. Invitro Candida colonization on acrylic resins and denture liners: influence of surfacefree energy, roughness, saliva, and adhering bacteria. Int J Prosthodont1981; 20:308-10.
[14]. Preston A J, Kearns A, Barber M W,Gosney M A.The knowledge of healthcare professionals regarding elderly persons' oral care.Br Dent J 2006; 201: 293-95.
[15]. Kuhar ,Funduk N. Effects of polishing techniques on the surface roughness of acrylic denture base resins. J Prosthet Dent 2005; 93:76-85
[16]. Berger JC, Driscoll CF, Romberg E, Lou Q, Thompson G. Surface roughness of denture base acrylic resins after processing and after polishing. J Prosthodont 2006; 15:180-86.
[17]. Yamauchi M, Yamamoto K, Wakabayashi M, Kawano J. In vitro adherence of microorganisms to denture base resin with differentsurface texture. Dent Mater J 1990; 9: 19-24.
[18]. Morgan DT, Wilson M. The effects of surface roughness and type of denture acrylic on biofilm formation by Streptococcus oralis in a constant depth film fermentor. J ApplMicrobiol 2001; 91:47-53.
[19]. Radford DR, Sweet SP, Challacombe SJ, Walter JD. Adherence of Candida albicans to denturebasematerials with different surface finishes. J Dent1998; 26: 577-583.
[20]. Absolom DR, Lamberti FV, Policova Z, Zingg W, van Oss CJ, Neumann AW. Surface thermodynamics of bacterial adhesion. App Environ Microbiol 1983; 46:90-97.
[21]. Boyd P T. The relationship between substratum surface roughness and microbiological and organic soiling: A review Biofouling 2001; 17: 59-71.
[22]. Bollen CML, Papaioannou W, van Eldere J, SchepersE, QuirynenM, van Steenberghe D. The influence of abutment surface roughness on plaque accumulation and peri implant mucositis. Clin Oral Implants Res 1996; 7: 201-11.
[23]. Bollen CML, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. Dent Mater 1997; 13: 258-269.
[24]. Oliveira LV, Mesquita MF, Henriques GEP, ConsaniRLX, Fragoso WSF.Effect of polishing technique and brushing on surface roughness of acrylic resins. J Prosthodont2008; 17:308-311.
[25]. Abuzar AM, Bellur S, Duong N,.Kim BB, Lu P, Palfreyman N, Surendran D and Tran TV.Evaluating surface roughness of a polyamide denture base material in comparison with poly (methyl methacrylate).J Oral Sci2010; 52:577-581.

[26]. Hargreaves AS. Nylon as a denture-base material. Dent Pract Dent Rec 1971; 22: 122-28.
[27]. Vojdani M. and Giti R.Polyamide as a Denture BaseMaterial: A Literature ReviewJ Dent Shiraz UnivMed Sci., 2015 March; 16(1 Suppl): 1-9.
[28]. Atlas R M. Handbook of microbiological media1993. CRC Press, Boca Raton, FL: 147-53
[29]. Sandven P, Lassen J. Importance of selective media for recovery of yeasts from clinical specimens. Journal of clinical microbiology 1999; 37: 3731–32.
[30]. Park ES, Blisset R, Susarla MS, Weber PH, Dent M. Candida albicans Adherence to Surface –Modified Denture Resin Surfaces. J Prosthodont 2008; 17: 365-69.

TABLES

Ra	N	Mean	Std Deviation	Std error mean
S	10	0.0444	0.01920	0.00607
H	10	0.0216	0.00784	0.00248

Table 1:- Surface roughness

Between SSA and HSA	T	df	P
	3.475	11.923	0.005

Table 2:- T-test for equality of means

	Material	N	Mean	Std Deviation	Std error mean
C2	S	10	60.00	1.491	0.471
	H	10	29.00	1.401	0.438
C4	S	10	116.0	1.349	0.219
	H	10	120.00	1.105	0.362
C8	S	10	164.00	2.006	0.434
	H	10	144.00	1.762	0.472
C12	S	10	204.00	2.010	0.464
	H	10	158.00	1.634	0.487

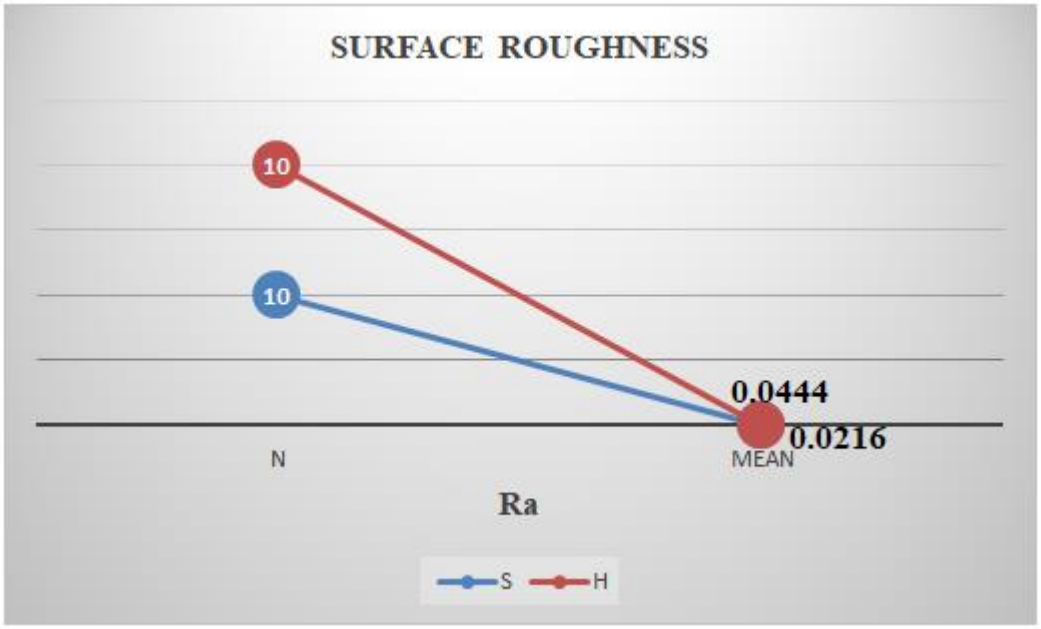
Table 3:- Candida albicans count

Between SSA and HSA at	T	df	P
C2	46.500	18.120	0.000
C4	-6.000	19.254	0.001
C8	30.000	17.497	0.086
C12	69.000	18.683	0.107

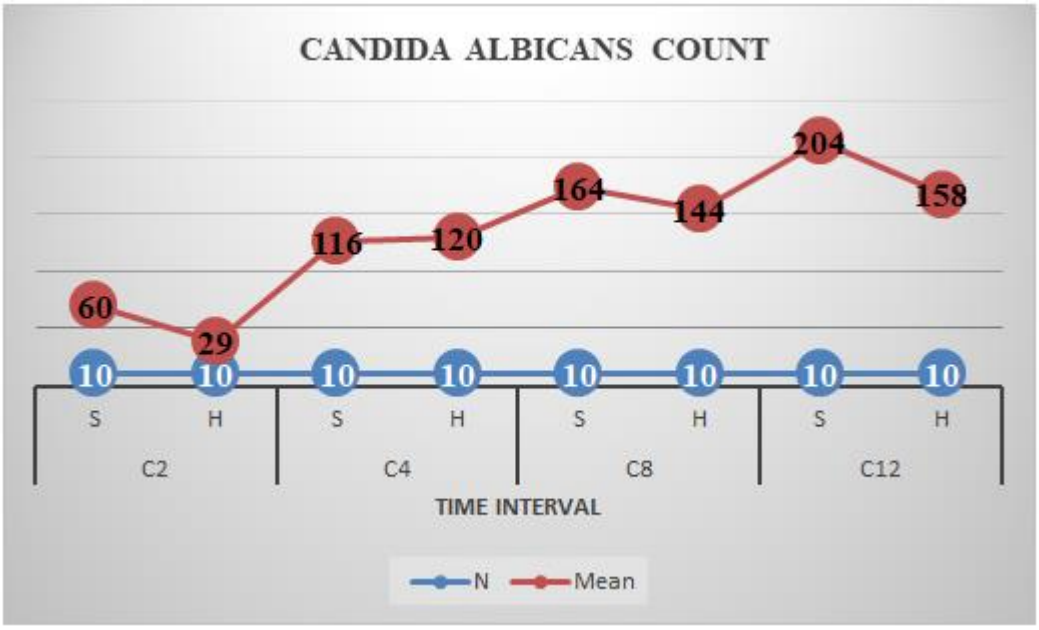
Table 4:-T-test for equality of Means

ISSN No:-2456-2165

GRAPHS



Graph 1:- Surface roughness



Graph 2:- Candida Albicans Count