ORIGINAL ARTICLE

The Effect of Type II Diabetes Mellitus, *Candida Albicans* and *Streptococcus Mutans* on the Biofilm Formation on Prosthetic Materials

[1]Shagun Gulia, [2]Vinaya Bhatt, [3]Manoj Shetty, [4]Krishna Prasad D, [5]Prateek Gupta

ABSTRACT

Aim: To investigate the biofilm formation on Prosthetic materials as affected by type II diabetes mellitus, Candida albicans and *Streptococcus mutans*.

Materials and methods: Two types of saliva, Natural saliva, and artificial saliva were collected and prepared respectively. The natural saliva was divided into diabetic and non-diabetic saliva. The Artificial saliva was further divided into two groups, one inoculated with *Streptococcus mutans* and the second with *Candida albicans*. The 150 samples of various prosthetic materials were prepared using nickel-chromium alloy, ceramic, soft liner, tooth molding powder; heat cured the acrylic resin. The samples were then immersed in natural saliva and artificial saliva and studied for biofilm formation.

Results: Diabetic saliva formed more biofilm than non-diabetic saliva. *Streptococcus mutans* were able to form more biofilm than *Candida albicans* in artificial saliva on constitutive androstane receptor (hCAR) and spinal length (SL). In Diabetic saliva, there was a significant difference in the biofilm formation seen between MC and NCA ($p < 0.05$). No biofilm was formed on hCAR in Natural Saliva (Diabetic or non-diabetic). In Artificial saliva inoculated with *Candida albicans* and *streptococcus mutans* there is a significant difference in the biofilm formation in all the materials except NCA.

Conclusion: Diabetic saliva has more potential to form biofilm than non-diabetic saliva. Also, *Candida albicans* and *Streptococcus mutans* both can form a biofilm on materials used with the maximum formation on hCAR. Smoother materials formed less biofilm than rougher surfaces like hCAR, PCM, SL.

Clinical significance: It is desirable for dental restorative materials to have a low susceptibility for accumulation and formation of biofilm as it may lead to pathologies such as dental caries, periodontal disease, peri-implantitis, etc. which are plaque-related. The most commonly used materials in prosthodontics have been used in the study to establish a direct relationship with the formation of biofilm, this, in turn, helps us to take the right call in choosing a material for a patient with an already compromised systemic condition.

Keywords: Artificial saliva, Biofim, Candida albicans, Natural saliva, Prosthetic materials, *Streptococcus mutans*, Type II diabetes mellitus.

[1]Department of Prosthodontics and Crown and Bridge and Implantology, Faculty of Dental Sciences SGT University, Haryana, India

[2,4]Department of Prosthodontics Including Crown and Bridge, AB Shetty Memorial Institute of Dental Sciences, Nitte (Deemed to be University), Mangaluru, Karnataka, India

[3]Department of Implantology, AB Shetty Memorial Institute of Dental Sciences, Nitte (Deemed to be University), Mangaluru, Karnataka, India

[5]Department of Periodontics, PDM Dental College, Bahadurgarh, Haryana, India

Corresponding Author: Shagun Gulia, Department of Prosthodontics and Crown and Bridge and Implantology, Faculty of Dental Sciences SGT University, Haryana, India, Phone:+918882687471, e-mail: shagun.gulia@gmail.com

INTRODUCTION

Biofilms are defined as structural microbial communities that are attached to a surface and encased in a matrix of exopolymeric material.[1] In the majority of natural habitats, most microorganisms grow as structured biofilm communities on surfaces rather than individually in suspension. This is important as it is known that all infections involve biofilm formation. It has been estimated that 65% of all human microbial infections involve biofilms. Biofilms have been demonstrated on the surfaces of devices such as catheters, prosthetic heart valves, joint replacements, and oral prosthesis. The role of biofilms in diseases have been investigated in detail over a number of years, and there is a considerable

amount of information available on their structure and properties.[2]

The most significant feature of a microbial biofilm is its resistance to a variety of drugs. The possible mechanisms for this could be restricted penetration of drugs through the biofilm matrix, phenotypic changes resulting from a decreased growth rate, nutrient limitation or surface induced expression of resistance gene. Alsosome believe that a small number of 'persister cells' are the cause of resistance.[3]

One of the most common and frequently found yeast in the oral cavity is *Candida albicans*. This fungi is normally found in humans, but in the presence of foreign materials like stents, pacemakers, prosthesis, and implants, they have been found to tend to support colonization and biofilm formation. This biofilm is the third leading cause of prosthesis related infections, causing mortality since they are resistant to antifungal therapy.[4] Another potential organism in the oral cavity involved most often in biofilm formation is *Streptococcus mutans*. Also, it is the primary etiological agent involved in caries and the most abundant microorganism in the oral cavity.[5]

The normal oral commensals turn virulent when the patients become immunocompromised in diseases such as diabetes etc. Patients with diabetes are known to be prone to oral infections like oral candidiasis. Higher salivary glucose concentrations are associated with increased oral candida carriage in diabetic subjects. Also, salivary glucose levels in diabetic patients favour yeast growth owing to an increased number of available receptors for candida. Reduced salivary flow in these patients may be another reason for Candida colonization.[6]

Several studies have been carried out to evaluate the formation of biofilm by various fungi and bacteria in the oral cavity. Separate work has been done on Diabetic patients to compare and assess its quality and quantity. However, there are no studies reported in the literature where in, different materials used in the oral cavity are studied for the biofilm formation by these microorganisms in diabetic patients. Hence this study was taken up to investigate the biofilm formation on prosthetic materials as affected by type II diabetes mellitus, *candida albicans* and *Streptococcus mutans*. This study was done with the following objectives in mind:

- To compare and evaluate the biofilm forming ability between diabetic and non-diabetic saliva in natural saliva
- To evaluate and compare the biofilm forming ability between *Candida albicans* and *Streptococcus mutans* in artificial saliva.
  - To quantitatively assess and compare the biofilm formation on prosthetic materials used in the oral cavity.

MATERIALS AND METHODOLOGY

The present study comprised of two groups. In one group, Natural Saliva was used to study the effect of diabetes on biofilm formation on prosthetic materials.

In the second group, artificial saliva was used to study the effect of the two microorganismsnamely *Candida albicans* and *Streptococcus mutans* on biofilm formation on prosthetic materials.

SAMPLE PREPARATION

Saliva Collection

The 200 mL of saliva was used in the study. Two types of saliva were used :
- 100 mL of natural saliva was collected from patients and
- 100 mL of artificial saliva was prepared using known ingredients.

Saliva used in the study:
- Natural Saliva (further divided into):
  (a) Diabetic saliva
  (b) Nondiabetic saliva
- Artificial Saliva (further divided into):
  (a) Artificial Saliva inoculated with *Candida albicans*
  (b) Artificial Saliva inoculated with *Streptococcus mutans*

  Distilled water was used as the control group.

  Natural saliva was used to study the effect of diabetes on biofilm formation among different prosthetic materials, and artificial saliva was used to study and compare the ability of two microorganisms to form a biofilm, on the same materials.

Natural Saliva Collection

Based on the following selection criteria patients were selected from the out patient department (OPD) of A.B. Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore. Written informed consent to participate in the study was obtained from each patient. Ethical clearance was obtained from the ethics committee of Nitte University.

Inclusion Criteria

- Age–40 to 70 years
- Patient not on medication for any systemic disease
- No known salivary gland dysfunction

Exclusion Criteria

- If on medication for type II diabetes mellitus
- Age below 40 years and above 70 years
- Patients with any other systemic disease
- Smoker/alcoholic/tobacco chewer

A questionnaire was prepared to record the subjects medical, dental and surgical history. Fasting blood was drawn from each patient to segregate them into diabetic and non-diabetic patients. Blood glucose level of 80–100 mg/dL was taken as normal.

A total of 100 mL of natural saliva was collected from 20 patient (10 patients were diabetic, and 10 were nondiabetic). They were called on a certain selected day, together. Then, stimulated saliva was collected from each patient using the spit method and pooled together in a sterile vial. This was done separately for diabetic and non-diabetic patients to make sure that the natural saliva was further grouped for diabetic and nondiabetic saliva. From the saliva collected, 50 mL of saliva, diabetic and nondiabetic each, was used for the study.

This way, 50 mL of diabetic and 50 mL of nondiabetic saliva was collected. The Natural saliva was not stored, the study was carried out, immediately.

Artificial Saliva Preparation

Ingredients

1. Sodium hydrogen phosphate (Na_2HPO_4) – 0.26 g/L
2. Sodium chloride (NaCl) – 6.70 g/L
3. Potassium/Sodium phosphate KH_2PO_4 – 0.20 g/L (NaH_2PO_4)
4. Potassium chloride (KCL) – 1.20 g/L
5. Sodium bicarbonate $NaHCO_3$ – 1.50 g/L
6. Bovine serum albumin – 100 mg

All the above components were mixed in the given concentration and made into 100 mL using distilled water. The pH was maintained between 5.6 and 6.6. It was stored in steel airtight bottles till use.

Procuring and Inoculation of Microorganisms in Artificial Saliva

Isolates of *Candida albicans* and *Streptococcus mutans* were obtained from the microbiological lab, K S Hegde Medical Academy, Mangalore, with following specifications

Candida albicans – ATCC90028
Streptococcus Mutans – MTCC 890

Fabrication of Prosthetic Material samples

The dental materials used in the study were as follows:
• Heat polymerised acrylic resin (HPAR) *(Trevalon, Gurgaon)*
• Metal ceramic (MC) *(Wiron 99, Germany)*
• Ni-Chr Alloy (NCA) or all metal *(Wirolloy, Germany)*
• Soft Liner (SL) *(GC, Hyderabad)*
• Provisional crown material (PCM) or Tooth Mould (TM) *(DPI Self Cure Tooth Moulding Powder, Mumbai)*

A total of 150 samples were fabricated using a metal die, in each of the above mentioned five materials (30 each) and they were stored in distilled water till use.

The metal dies of known specifications was machined using stainless steel (Fig 1). It had a flat rectangular top with 14 depressions each of 5.1 mm diameter and 2.1 mm depth. For HPAR, TM, and SL the materials were mixed according to the manufacturer's directions and were placed into the depressions after applying to separate medium. The pressure was applied to the materials using a clamp to ensure uniform and smooth samples. Once the material was hard, the die was separated to release the samples. HPAR samples were placed in hot water (60 degrees) for 1 and a 1/2 hours to further complete the polymerization, as recommended for the material.

For MC and NCA samples, auto polymerised resin samples were polished and were later cast using an appropriate alloy. MC samples were further sandblasted, and ceramic was applied and fired.

MATERIALS AND METHODS

Exposure of Samples to Saliva for Biofilm Formation

The collected natural salivary sample (Diabetic and Non-Diabetic, 50 mL each) was poured into two separate uricol bottles and were labeled.

Artificial saliva was divided into two samples of 50 mL each and poured into two separate uricol bottles. In one bottle of 50 mL of artificial saliva, 40 µl of *Candida albicans* per 10 mL was inoculated, and to the other, 40 µl of *Streptococcus mutans* per 10 mL was inoculated. No storage of the natural and artificial saliva was done, and all the prosthetic material samples were immediately exposed.

A control group was added using only 50 mL of distilled water.

Thus there were 5 groups as follows:
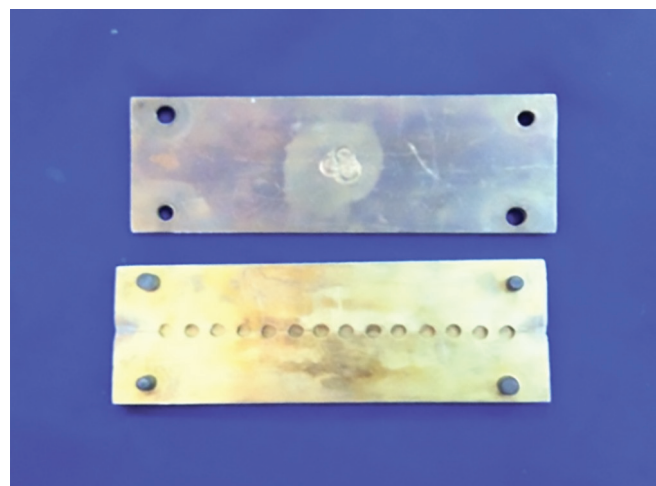• *Group 1*: Natural saliva (diabetic saliva) 50 mL

Fig. 1: Metal die for fabrication of samples

- *Group 2*: Natural saliva (non-diabetic saliva) 50 mL
- *Group 3*: Artificial saliva with *Candida albicans* 50 mL
- *Group 4*: Artificial saliva with *Streptococcus mutans* 50 mL
- *Group 5*: Control group (distilled water) 50 mL

Then, six samples from each prosthetic material were placed into the bottles, including the control group.

Preparation of Samples for ELISA

Staining of Samples

After 48 hours, samples were removed from bottles, washed in water, to remove the non adherent microorganisms. Then they were dried and stained using Methylene Blue (Himedia, Mumbai) for 15 minutes. After staining, the samples were again washed to remove the excess stain and were dried.

Destainingofthe Samples

The samples were then placed in microtitre plates (Fig. 2). In each plate, 33 % glacial acetic acid was poured with the help of micro titrepipettes (Fig. 3). The samples were allowed to stand for 15 min and were later removed carefully with the help of tweezers. The ELISA re ader read the microtitre plates at 560 to 630 nm. (Fig. 4)

Statistical Analysis

The values for the control group were deducted from the study group uniformly for all the materials from all the groups.

The data obtained were tabulated and subjected to statistical analysis using t-Test and one way analysis of variance (ANOVA) test.

RESULTS

Table 1 shows that there is significant difference seen in the formation of biofilm in NCA and MC groups in diabetic and nondiabetic saliva. No significant difference was seen in any other group. No biofi lm was formed on HCAR in diabetic as well as nondiabetic saliva.

Table 2 shows that in artificial saliva, when Candida Albicans and Streptococcus mutans, was compared, there is a significant difference in the biofilm formation in all the materials except NCA.

Table 3 shows that there is a significant difference in the biofilm formation between the materials in the diabetic saliva, artificial saliva with Candida albicans and Streptococcus mutans. No significant difference was seen in the formation of biofilm between the materials in the
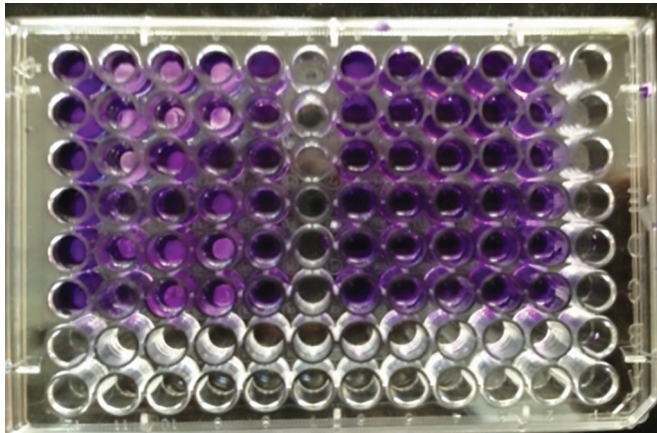

**Fig. 2:** Microtitre plates

Fig. 3: Microtitre pipettes

Fig. 4: Fully automated ELISA machine

Table 1: Comparison of biofilm formation in natural saliva: diabetic *vs* nondiabetic Saliva; t-test

		Group	*N*	*Mean*	*Std. deviation*	*t*	*df*	*Sig. (2-tailed)*
Ceramic	Biofilm	Diabetics	6	0.047017	0.016183	4.75	10	0.001
		Nondiabetics	6	0.011967	0.008053			
Heat cured	Biofilm	Diabetics	6	−0.0364	0.11739	−0.152	10	0.883
		Nondiabetics	6	−0.02597	0.120984			
Soft liner	Biofilm	Diabetics	6	0.10675	0.124341	0.149	10	0.885
		Nondiabetics	6	0.0988	0.041425			
All metal	Biofilm	Diabetics	6	0.0725	0.023759	−5.507	10	<u><0.001</u>
		Nondiabetics	6	0.179067	0.041013			
Tooth mould	Biofilm	Diabetics	6	0.0791	0.073326	−1.936	10	0.082
		Nondiabetics	6	0.236333	0.184981			

Table 2: Comparison of biofilm formation in artificial saliva by candida albicans and *streptococcus mutans* in each material

		Group	*N*	*Mean*	*Std. deviation*	*t*	*df*	*Sig. (2-tailed)*
Ceramic	Biofilm	Candida	6	0.10295	0.044836	2.499	10	0.031
		Streptococcus	6	0.05315	0.019286			
Heat Cured	Biofilm	Candida	6	0.343718	0.23657	−3.101	10	0.011
		Streptococcus	6	0.713435	0.17126			
Soft Liner	Biofilm	Candida	6	0.255633	0.089697	−3.331	6.065	0.016
		Streptococcus	6	0.646817	0.273334			
All Metal	Biofilm	Candida	6	0.118783	0.042098	1.416	10	0.187
		Streptococcus	6	0.093233	0.013441			
Tooth Mould	Biofilm	Candida	6	0.256017	0.079858	5.087	10	<0.001
		Streptococcus	6	0.036583	0.06918			
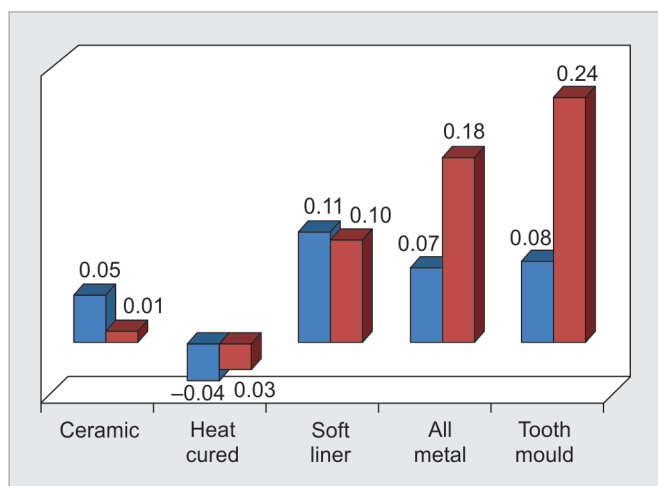
Graph 1: Biofilm formation In natural saliva: diabetics and nondiabetic saliva

has been produced by the organisms themselves which help them to adhere to a bioprosthetic device. In the oral cavity, biofilm can be formed on the acrylic denture surfaces, implants, metal, and ceramic surfaces and the mucosa. It is desirable for dental restorative materials to have a low susceptibility for the accumulation of microorganisms as it may lead to pathologies such as dental caries, periodontal disease and peri-implantitis which are plaque-related.[3-6]

Dental plaque is also a microbial biofilm. The mechanisms controlling the formation and development of biofilms can be helpful in understanding the emergence and progression of such pathologies which will aid in effective treatment.[7] Therefore it becomes imperative to understand the formation of biofilm in the oral cavity and the complications it can pose. This in vitro study aims at comparing the biofilm formed in the saliva of diabetic and non-diabetic patients and by Candida albicans and Streptococcus mutans. This study was taken up to evaluate the effect of diabetes on the formation of biofilm on five materials most routinely used in prosthodontics to rehabilitate the dentition, namely, metal, ceramic, soft liner, heat cured acrylic resin and tooth molding Material (auto polymerising resin).

In the present study in natural saliva, more biofilm was formed in diabetic saliva than in non-diabetic saliva. Diabetic patients have shown to form more biofilm than non-diabetic patients as these patients are more

non-diabetic saliva. Natural saliva and artificial saliva was assessed in this table.

Graph 1 shows that in natural saliva, diabetic saliva has maximum biofilm formation in PCM followed by NCA and then SL. Least amount of biofilm was formed on hCAR and MC. In non-diabetic saliva, maximum biofilm was formed in SL, followed by PCM, and NCA and MC. No biofilm was formed in HCAR.

DISCUSSION

Biofilm is a colony of microorganisms (bacteria and fungi) enclosed in an organic polymeric matrix that

Table 3: Comparison of the 5 materials: One way ANOVA

		N	Mean	Std. deviation	F/Statistics	Mean square/Df2	p-value
Natural Saliva (Non-diabetic Saliva)	Ceramic	6	0.047017	0.016183	2.534	0.018	0.065
	Heat cured	6	−0.0364	0.11739			
	Soft liner	6	0.10675	0.124341			
	All metal	6	0.0725	0.023759			
	Tooth mould	6	0.0791	0.073326			
	Total	30	0.053794	0.092675			
Natural Saliva (Diabetic Saliva)	Ceramic	6	0.011967	0.008053	26.591	10.374	<0.001
	Heat cured	6	−0.02597	0.120984			
	Soft liner	6	0.0988	0.041425			
	All metal	6	0.179067	0.041013			
	Tooth mould	6	0.236333	0.184981			
	Total	30	0.10004	0.138007			
Artificial saliva inoculated with candida albicans	Ceramic	6	0.10295	0.044836	6.978	12.021	0.004
	Heat cured	6	0.343718	0.23657			
	Soft liner	6	0.255633	0.089697			
	All metal	6	0.118783	0.042098			
	Tooth mould	6	0.256017	0.079858			
	Total	30	0.21542	0.146351			
Artificial Saliva Inoculated With Streptococcus Mutans	Ceramic	6	0.05315	0.019286	27.069	11.34	<0.001
	Heat cured	6	0.713435	0.17126			
	Soft liner	6	0.646817	0.273334			
	All metal	6	0.093233	0.013441			
	Tooth mould	6	0.036583	0.06918			
	Total	30	0.308644	0.338881			

susceptible to fungal infections (Table 1). Salivary glucose levels in diabetic patients favour yeast growth owing to increased numbers of available receptors.[8] Soysa et al. also concluded that the reason for more biofilm formation in diabetic patients is the poor glycaemic control that exists in these patients.[9]

In diabetic saliva, provisional crown material and nickel-chromium alloy formed maximum biofilm, and least biofilm was formed on heat cured acrylic resin. whereas in non-diabetic saliva, soft liner and provisional crown material formed maximum biofilm whereas least amount of biofilm was formed on heat cured acrylic resin. Therefore, the provisional crown material formed maximum biofilm in diabetic and non-diabetic saliva (Graph 1). Our study is in accordance with a study done by Morgan et al., which suggested that the type of acrylic used and surface roughness affects the biofilm formed. They concluded that more biofilm was formed on cold cure acrylic than heat cured acrylic resin, as a cold cure has a rougher surface than heat cure even after polishing the two to the same amount.[10]

In the present study, more biofilm was formed on soft liners when compared to ceramic and heat cured acrylic resin in diabetic saliva, and maximum biofilm on soft liner was seen in non-diabetic saliva (Graph 1). Also in 2004 Khaled et al. suggested that initial attachment of yeast penetration to soft liner materials is comparable irrespective of surface roughness, though, over a period, smoother surfaces retain fewer cells.[11] Nikawa et al. studied the biofilm formation on soft denture lining materials, immersed in denture cleansers for 180 days. They concluded that biofilm formation on soft liners was formed significantly, and the combination of soft liners with denture cleansers showed more biofilm.[12] Nikawa has further established this in another study in where more biofilm was formed on tissue condi tioners than

acrylic resin.[13] Chen et al. also said that more biofilm is formed on soft liner when compared to cold cure acrylic.[14] The reason for this as suggested by Nikawa et al., may be the reduction of the antifungal effects of soft liner, facilitation of biofilm formation, firm colonization by the microorganisms and hyphal invasion of the yeast present in the oral cavity.[15]

When the biofilm forming ability of *Candida albicans* and *Streptococcus mutants* was compared, it was seen that *Streptococcus mutans* formed more biofilm on HCAR and SL compared to *Candida albicans* (Table 2). This is in accordance with a study done by Pereira et el., where he said that candida biofilms are significantly affected by saliva, substratum type and presence of other microorganisms. The yeast alone may not be able to form a substan tial biofilm on materials.[16]

Candida albicans formed more biofilm on provisional crown material and metal ceramic. On metal, both the organisms formed almost the same amount of biofilm with no substantial difference (Table 2). The adherence of the microorganisms is affected by the surface roughness, as suggested by Rebecca et al. in their study.[17] Therefore if the surface roughness is same, it won't have too much of an effect on the adherence of Streptococcus mutans or *Candida albicans*, as suggested by the present study. Less biofilm was formed on metal ceramic, in both natural and artificial saliva, as compared to other materials. This can be explained on the surface roughness, the ceramic is glazed, and the surface becomes absolutely smooth when compared to any other material used in this study. Therefore, the adherence of microorganisms on metal ceramic was least when compared to heat cured acrylic resin and soft liner, which exhibit rougher surfaces.

Heat cured acrylic resin showed the maximum amount of biofilm formation by both the organisms, followed by the soft liner. This is in accordance with a study done by Radford et al., which strongly suggested the adherence of *Candida albicans* to the denture base materials.[18]

Among the materials tested, there is significant difference in biofilm formation amongst the materials in diabetic saliva ($p < 0.001$), *Candida albicans* ($p = 0.004$) and *Streptococcus mutans* ($p < 0.001$) (Table3). In Diabetic saliva, maximum biofilm was formed in Provisional crown material and least biofilm was seen in heat cured acrylic resin. In Non-diabetic saliva, maximum biofilm was formed on SL and least on HCAR. In artificial saliva inoculated with *Candida albicans*, maximum biofilm was formed in heat cured acrylic resin, and least biofilm formation was on metal ceramic. In artificial saliva inoculated with *Streptococcus mutans*, maximum biofilm was seen in heat cured acrylic resin and least was formed on provisional crown material (Table 2).

In the artificial saliva, however, maximum biofilm was formed by hCAR by both the organisms inoculated. MC and NCA showed less biofilm formation. Amongst the two organisms, *Streptococcus mutans* formed more biofilm on hCAR when compared to *Candida albicans* and the difference was clinically significant (Table 3).

According to the literature, however, there is a conflicting result in this study, where heat cured acrylic resin, has formed more biofilm than soft liner (Table 2). This could be because of as mentioned by Doron and Bussscher et al. in their study, where they concluded that results of biofilm formation could be conflicting when in-vitro studies are done. In the oral cavity, the biofilm is affected continuously and refreshed because of the presence of saliva and antibacterial properties of agents used. Whereas in *in vitro* studies this does not happen. Also, there is a constant flushing of organisms not involved in the biofilm, in the oral cavity. In *in vitro* studies, the organisms present in the saliva are not shaken and left undisturbed to adhere to the surface of the materials.[18,19]

Therefore, according to this study, diabetic saliva has more potential to form biofilm than non-diabetic saliva. Also, *Candida albicans* and *Streptococcus mutans* both are able to form the biofilm on materials used with the maximum formation on heat cured acrylic resin. Smoother materials formed less biofilm than rougher surfaces like heat cured acrylic resin, provisional crown material, soft liner. However, the limitations of this study are that it is an *in vitro* study, the conditions present in the oral cavity cannot be duplicated. Further studies are needed to compare the materials used in Prosthodontics, to establish facts on biofilm formation and reasons for its ability to adhere strongly to only certain materials in the same environment, apart from surface roughness.

CONCLUSION

In natural saliva, diabetic saliva has ability to form more biofilm as compared to non-diabetic saliva.

The bacteria *Streptococcus mutans* was found to be more effective in forming biofilm as compared to the fungi *Candida albicans.*

Amongst all the materials used maximum biofilm was seen on heat cured acrylic resin (HCAR), followed by tooth molding (TM) and soft liner (SL). Ceramic and nickel-chromium alloy formed a mi nimum biofilm.

REFERENCES

1. Ramage G, Saville S, Thomas D, López-Ribot J. Candida Biofilms: An Update. Eukaryotic Cell. 2005;4(4):633-638.

2. Douglas LJ. Medical importance of biofilms in Candida infections. Rev Iberoam Micol. 2002;19(3):139-143.

3. Donlan RM. Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Ricrobial Rev. 2002;15:167-193.

4. Bhat V, Sharma SM. Role of candida biofilms on denture stomatitis. J Ind Acad dent spec research. 2013;2(1):27-35.

5. Nyvad B, Kilian M. Comparison of the initial streptococcal microflora on dental enamel in caries- active and in caries inactive individuals. Caries res.1990;24(4):267-272.

6. SashikumarR ,Ranganathan K. Salivary glucose levels and oral candidal carriage in type II diabetics.OralSurg Oral Med Oral Pathol Oral RadiolEndod. 2010;109(5):706-711.

7. Sbordone L , Bortolaia C. Oral Microbial Biofilms And Plaque-Related Diseases: Microbial Communities And Their Role In The Shift From Oral Health To Disease Clinical Oral Investigations 2003;7(4):181-188.

8. Volpato P, Claudia A. Candida prevelance in well controlled type II diabetes patients with denture stomatitis. Oral Surg Oral Med Oral Pathol Oral RadiolEndod2011;111:726-733.

9. Soysa NS. Diabetes mellitus as a contributory factor in oral candidosis. Diabetic Medicine. 2006;23(5):455-459.

10. Morgan TD, 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(1):47-53.

11. Bulad K, Taylor RL, Verran J, McCord JF.Colonization and penetration of denture soft lining materials by *Candida albicans*. Dent Mat 2004;20(2):167-175.

12. Nikawa H, Jin C, Makihira S, Egusa H, Hamada T, Kumagai H. Biofilm formation of Candida albicans on the surfaces of deteriorated soft denture lining materials caused by denture cleansers in vitro. J Oral Rehab 2003;30(3):243-250.

13. Nikawa H, Yamamoto T, Hamada T, Rahardjo MB, Murata H. Commercial denture cleansers--cleansing efficacy against Candida albicans biofilm and compatibility with soft denture-lining materials. Int JProsthodont1995;8(5):434-444.

14. Nikawa H, Chen J, Hamada T, Nishimura M, Polyzois G. Candida albicans colonization on thermal cycled maxillo-facial polymeric materials in vitro. J Oral Rehab2001;28(6): 526-533.

15. Nikawa H, Hamada T, Yamamoto T, Kumagai H. Effects of salivary or serum pellicles on the C.albicans growth and biofilm formation on soft lining materialsin vitro. J Oral Rehab1997; 24: 594-604.

16. Pereira-Cenci T , Deng DM, Kraneveld EA, MandersEM,Del Bel Cury AA, Ten Cate JM, Crielaard W. The effect of *Streptococcus mutans* and *Candida glabrata* on *Candida albicans*biofilms formed on different surfaces. Archives of Oral Biology. 2008; 53(8):755-764.

17. Taylor R1, Maryan C, Verran J. Retention of oral microorganisms on cobalt chromium alloy and dental acrylic resin with different surface finishes. J Prosthet Dent 1998;80(5):592-597

18. Busscher HJ1, Rinastiti M, Siswomihardjo W, van der Mei HC. Biofilm Formation on Dental Restorative and Implant Materials.J Dent Res. 2010;89(7):657-665.

19. Steinberg D. Studying Plaque Biofilms on Various Dental Surfaces. Handbook of Bacterial Adhesion: 2000, pp 353-370.