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ORIGINAL ARTICLE

Evaluation of Subgingival Microflora in All Ceramic Restorations with Subgingival Heavy Chamfer Finish Lines

M. Dhanraj · S. Anand · Padma Ariga

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Abstract Microbial colonization in the gingival sulci of abutment teeth receiving all ceramic retainers with subgingival margins need to be studied to assess the prognosis of periodontal health, which determine the eventual success of fixed partial dentures. This prospective observational study was done to evaluate the quantitative alteration in the microbial flora in the gingival sulci of abutment teeth adjacent to the edentulous space prior and after receiving all ceramic retainers over varying time intervals of 1 week, 1 month and 2 months respectively. Twenty, healthy partially edentulous patients, aged 20–50 years with single missing central incisor were selected for this prospective observational study and their microbial samples were collected from the gingival sulci of abutments adjacent to edentulous space with sterile paper points and cultured and the estimated values for microflora served as controls. The same abutments were prepared to receive all ceramic retainers with subgingival heavy chamfer marginal finish lines. The patients were recalled after 1 week, 1 month, 2 months intervals during which the collected subgingival microbial samples were cultured and the corresponding quantitative microbial alteration in the restored gingival sulci was recorded. The obtained data was statistically analysed using the student *t* test and repeated analysis of variance test. The results of the study inferred student *t* test expressed a statistically significant ($p < 0.001$) progressive increase in gingival sulcular microbial colonisation in the abutment teeth before [M = 2.52 ± SD 1.21(10^6) CFU/ml] and after receiving all ceramic retainers over varying time intervals of 1 week [M = 3.25 ± SD 1.21(10^6) CFU/ml], 1 month [M = 4.64 ± SD 1.13(10^6) CFU/ml] and 2 months [M = 4.75 ± SD 1.16(10^6) CFU/ml] respectively. The result of repeated analysis of variance test inferred that there was a statistically significant difference ($p < 0.001$) in the subgingival microfloral count between the pre operative and post operative samples at 1 week, 1 month and 2 months. Subgingivally placed all-ceramic retainers with heavy chamfer finish lines in the abutment teeth demonstrated a statistically significant increase in sulcular microbial colonization over varying time intervals of 1 week, 1 month and 2 months respectively and this may affect periodontal health of abutment teeth progressively.

Keywords Subgingival microflora · All-ceramic restorations · Subgingival margins

M. Dhanraj (✉) · S. Anand · P. Ariga
Department of Prosthodontics, Saveetha Dental College, Chennai 600077, India
e-mail: dhanrajmganapathy@yahoo.co.in

Introduction

The oral cavity is an abode of multiple species of microbiota and the gingival sulcular chamber harbors aerobic and anaerobic microbial flora [1–3]. Prosthodontics has enabled the effective management of partial and complete edentulism. Fixed partial dentures used in rehabilitation of partially edentulous patients can be fabricated from various types of materials like metal and alloys, resins, metal ceramic, and all ceramic restorative materials. Among these materials all ceramic restorations are the most esthetic and bio compatible comparable with noble metals [4–6].

Certain clinical conditions like short clinical crowns, over expression of gingival margins during smile, root caries and esthetics, may indicate placement of the restoration margins in the subgingival zone.

Subgingivally placed restoration margins can alter the microbial environment in the corresponding gingival sulci [7, 8] the type of restoration, restorative material, marginal finish line, nature of luting agent, micro leakage can contribute to fluctuation of pathogenic microflora and hence can induce periodontal disease [9–13]. Hence this study was attempted to evaluate all ceramic restorations with subgingival marginal finish lines in the abutments and the corresponding microbial alteration in gingival sulci over varying time intervals. The null hypothesis formulated for the study was that there would be no significant variation in the gingival sulcular microfloral colonization in the abutments before and after receiving all ceramic retainers with heavy chamfer subgingival marginal finish lines over varying time intervals.

Method

Study Design

Type of study	Prospective observational study
No of samples	Twenty healthy partially edentulous patients with single missing maxillary central incisor
Inclusion criteria	Age: 20–50 years, both genders, short clinical crownheight $\leq$7 mm, OHI score of 0–1.2, willingness to participate in the study
Exclusion criteria	Poor oral hygiene status, severe gingival recession, severe attrition, mobile, carious and filled teeth, multiple edentulous spaces, endodontically treated tooth, teeth with periodontal disease, patients under antibiotic therapy, patients under medication for any other purposes.

Sample Collection

Twenty partially edentulous patients satisfying the inclusion criteria with desire to undergo fixed partial denture treatment for replacement of single missing maxillary central incisor were selected after obtaining informed consent to participate in the study. After suitable mouth preparation the microbial samples from the deepest gingival sulci in labial/buccal surface of the abutments on either side of the edentulous space were isolated using sterile paper points [14, 15].

(ABSORBENT PAPER POINTS, ISO COLORCODED, Ref A 022R, DENTSPLY). The paper points were placed in the deepest gingival sulci of labial/buccal sulcular surfaces of the abutments for a period of 60 s and transferred in a tube containing a nutritive liquid medium tryptic-glucose-yeast extract transport medium (TGY) and transported in icepack containers and processed in the laboratory immediately. The interval between collection and processing of specimen was less than an hour.

The samples were serially diluted in sterile tryptic soy broth (TSB) and 200 µl from 10^{-3}, 10^{-4}, 10^{-5} were spread on to the surface of blood agar plates. Undiluted samples were inoculated on to blood agar, chocolate agar and macconkey agar plates. All these plates were incubated at 37 °C. Chocolate agar plates were incubated at 10 % CO_2 atmosphere. After 24–48 h incubation the total numbers of colonies were counted and the viable count determined. The isolated colonies were further processed and identified microscopically with their biochemical characteristics.

The abutments were prepared to receive all-ceramic retainers for (IPS EMAX, IVOCLAR VIVADENT, LIECHTENSTEIN) fixed partial denture with subgingival heavy chamfer finish line. Dual cord technique was used to achieve gingival retraction (ULTRAPAK). Impressions were made with additional poly vinyl siloxane (AQUASIL, SOFT PUTTY/REGULAR SET, AQUASIL ULTRA LV, TYPE III LIGHT BODY, REGULAR SET, DENTSPLY, GERMANY) using double mix, double impression technique and casts were poured with TYPE IV die stone (ULTRA ROCK, KALABHAI, MUMBAI).

The fixed partial dentures were subsequently fabricated, tried and luted with Variolink N (IVOCLAR VIVADENT, LIECHTENSTEIN) and post insertion adjustments done. The patients were recalled after 1 week, 1 month, 2 months during which, the subgingival bacterial samples were isolated and the corresponding microbial alteration in the restored gingival sulci were evaluated and data recorded and expressed as CFU/ml.

Statistical Analysis

All the data were entered in Microsoft excel sheets; statistical analysis was done using SPSS SOFTWARE SYSTAT VERSION 7.0. The data were analyzed by Student '*t*' test and repeated analysis of variance.

Results

Table 1, describes the comparison between the pre operative subgingival microflora and post operative subgingival microflora in the abutments after the placement of all ceramic restorations at a time interval of 1 week, 1 month and 2 months respectively.

The results of the study inferred there was a statistically significant ($p < 0.001$) progressive increase in gingival

Table 1 Comparison between the pre operative subgingival microflora and post operative subgingival microflora in the primary abutments after the placement of all ceramic restorations at a time interval of 1 week, 1 month and 2 months

	Mean	N	SD	SEM	*p*-value
Pair 1 pre op	2.5240	20	1.21612	0.27193	$p < 0.001$
Post op week 1	3.2575	20	1.21244	0.27111	
Pair 2 pre op	2.5240	20	1.21612	0.27193	$p < 0.001$
Post op month 1	4.6425	20	1.13763	0.25438	
Pair 3 pre op	2.5240	20	1.21612	0.27193	$p < 0.001$
Post op month 2	4.7590	20	1.16586	0.26070	

sulcular microbial colonization before [M = 2.52 ± SD 1.21(10^6 CFU/ml] and after receiving all ceramic restorations over varying time intervals of 1 week [M = 3.25 ± SD 1.21(10^6) CFU/ml], 1 month [M = 4.64 ± SD 1.13(10^6) CFU/ml] and 2 months [M = 4.75 ± SD 1.16(10^6) CFU/ml] respectively.

The result of repeated analysis of variance test in table 2 inferred that there was a statistically significant difference ($p < 0.001$) in the subgingival microfloral count between the pre operative and post operative samples at 1 week, 1 month and 2 months.

Discussion

The role of the oral microflora in the oral cavity is extremely complex and their behavior is influenced by several factors. The microfloras express varying levels of commensalism, symbiotism and parasitic behavior with the host. Age, gender, oral hygiene, plaque deposition, fluctuation in salivary flow rates, systemic diseases, and local lesions can greatly modify microbial behavior in human subjects. Flores de jacoby et al. [16] have reported the influence of fixed dental restoration and prosthesis over microbial behavior in humans and concluded microbial colonization increases around fixed restorations.

The commonly used materials for restorative and prosthetic needs can be grouped predominantly into resins, metals, ceramics and synthetic polymers. Each material used to fabricate complete veneer retainer and its corresponding luting cement [17, 18] can evoke a specific and complex microbial response owing to its surface charges, surface topography, redox potential, tarnish and corrosion, and galvanism. Among the choice of restorative dental materials ceramics occupy a contemptuous position with regards to esthetics and bio compatibility compared to other dental materials. The ceramic restorations can be broadly categorized into metal ceramics and all ceramics restorations.

Esthetics and biocompatibility concerns of the partially edentulous patient can be efficiently addressed by fabricating all-ceramic fixed partial dentures. In all-ceramic restorations placement of marginal finish lines assumes great clinical significance. The finish lines can be terminated in three locations namely supra gingival, crestal gingival and subgingival positions [19]. Subgingival marginal finish lines are indicated in short clinical crowns, root hypersensitivity, mild root caries and the demanding esthetic concern of the patient.

Several factors contribute to the therapeutic success of subgingivally place all ceramic restorations. The various factors include type of ceramic selected, type of finish line selected, marginal fit of the restorations and contour of the restorations and the choice of luting agent selected. The commonly used subgingival finish line includes shoulder and heavy chamfer margins. Placement of subgingival margins can modify the microbial response in a significant manner, which can be of great clinical importance [20]. Hence, this study was formulated to evaluate the predominant species of microbial flora inhabiting the gingival sulci in the primary abutments adjacent to edentulous space in healthy partially edentulous patients and to evaluate the qualitative and quantitative differences in the microbial flora in the gingival sulci following restoration with all ceramic restorations, over varying time intervals.

This study demonstrated alpha hemolytic *Streptococci*, *S. mutans*, *S. sanguis*, *S. salivarius*, *S. epidermidis*, *S. mitis* were the predominant subgingival microbial flora in all the experimental human subjects. The other gram negative cocci, rods, spirocheates, and opportunistic yeasts were

Table 2 Repeated analysis of variance for pre operative and post operative microbial samples

	Paired differences					*t*	df	*p*-value
	Mean	SD	SEM	95 % confidence interval of the difference				
				Lower	Upper			
Pair 1 pre op-post op (week 1)	−0.73350	0.37014	0.08277	−0.90673	−0.56027	−8.862	19	$p < .0001$
Pair 2 pre op-post op (month 1)	−2.11850	0.88411	0.19769	−2.53227	−1.70473	−10.716	19	$p < .0001$
Pair 3 pre op-post op (month 2)	−2.23500	0.86756	0.19399	−2.64103	−1.82897	−11.521	19	$p < .0001$

present in less significant quantities in all the test human subjects. Cuesta et al. reported similar inferences in their study. The results of the study support the rejection of null hypothesis that there would be no significant variation in the gingival sulcular microfloral colonization in the abutments before and after receiving all ceramic retainers with heavy chamfer subgingival marginal finish lines over varying time intervals. The results of the study inferred (Table 1) that there was a statistically significant ($p < 0.001$) progressive increase in gingival sulcular microbial colonisation before [M = 2.52 ± SD 1.21(10^6) CFU/ml] and after receiving all ceramic restorations over varying time intervals of 1 week [M = 3.25 ± SD 1.21(10^6) CFU/ml], 1 month [M = 4.64 ± SD 1.13(10^6) CFU/ml] and 2 months [M = 4.75 ± SD 1.16(10^6) CFU/ml] respectively.

The repeated variance of analysis test (Table 2) also shows that there was a statistically significance rise in the subgingival microbial population between the pre operative samples and the post operative samples at varying time intervals.

The possible reason for increase in microbial population includes distension of gingiva by the subgingivally placed all ceramic restoration with mild possible over contouring in the cervical area. The variation in gingival crevicular fluid flow to newly placed retainers could have facilitated increased microbial colonization. The subsequent increase in microbial population can be attributed to gingival remodeling due to occlusal forces, partial dissolution of luting cement due to salivary ingress, brushing pattern of the patients, redox potential and surface topography of the restoration which could have induced a mild inflammatory response facilitating increase microbial colonization.

A significant increase in the sulcular microbial colonization warrant increased pathogenic activity. Similar findings were reported in the literature with regards to non ceramic subgingival restorations [21–24]. Niklaus et al. studied the clinical and microbiological effects of subgingival restorations with over hanging and clinically perfect margins and concluded subgingival margins appear to induce increased microbial growth. Tarnow et al. and Sorensen and Flores de Jacoby et al. studied the effect of crown margin location on plaque and periodontal health and concluded subgingival margins facilitate increased plaque deposition and subsequent increase in microbial growth which could affect periodontal health.

Possible variation in microbial habitation can be present in subjects with underlying systemic diseases, metabolic disorders and local lesions. Habits like smoking [25, 26], alcoholism, pan chewing, can significantly alter the microbial population and response. The brushing pattern and abstinence from interdental cleansing aids could alter sulcular microbial growth.

Antibiotic therapy and immune suppressive drug therapy, gingival enlargement inducing phenytoin and nifedipine therapy for prolonged periods and usage of antiseptic mouth rinses may modify sulcular microbial inhabitation and colonization. Periodontal response due to primary and secondary trauma from occlusion could modify microbial response. The duration, frequency and technique of tooth brushing and the choice of medicated dentifrices [27] could alter the microbial response and colonization.

Owing to the increased susceptibility of microbial colonization with subgingival margins despite the superior biocompatibility of all ceramic biomaterial, whenever subgingival margins are indicated, suitable mouth preparation with gingivoplasty and other crown lengthening procedures and orthodontic extrusion may be considered to increase the clinical crown height to facilitate placement of supragingival marginal finish lines.

Conclusion

Within the limitations of this study it can be concluded that

1. Alpha hemolytic *Streptococci* (*S. mutans*, *S. sanguis*, *S. salivarius*, *S. epidermidis*, *S. mitis*) were the most predominant microbial species inhabiting the abutment gingival sulci in all healthy partially edentulous test human subjects.
2. Subgingivally placed all-ceramic retainers demonstrated a statistically significant, progressive increase in microbial colonization in the abutment teeth over varying time intervals of 1 week, 1 month and 2 months respectively and this may affect periodontal health of the abutment teeth progressively.

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