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Evaluation of Effect of 2% Chlorhexidine on Antibacterial Activity of Resin Cement.

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Abstract
Aim: *To evaluate the effect of 2% Chlorhexidine on antibacterial activity on Resin cement.*
Methods: *A total of 100 patients of 29-56 years of age who required Fixed dental Prosthesis were selected for the study. 2 teeth were prepared in each patient for All Ceramic crowns. In every patient, the 2 teeth prepared were divided in to 2 groups: Control(no antimicrobial agent applied after etching of tooth) , Test (2% Chlorhexidine applied after etching of the tooth) , .Bacteriologic samples were collected at 5 different sample* ***times:*** *Baseline visit,at the time of cementation,1 ,3 and 6 month after cementation.Microbiogical processing of all samples were done and the results were statistically analysed.*
Result: *There was significant shift in Control group towards Gram negative,anaerobic,rod atmosphere from Baseline till 6 month postcementaion. In Test group,atmosphere shifts towards Aerobic ,Gram positive ,cocci till 3 months post cementation but becomes Anaerobic ,Gram negative,cocci/rod in 6 month postcementation.*
Conclusion: *This study shows that application of 2% Chlorhexidine on prepared tooth surface after etching definitely increases antibacterial activity of Resin cement*.

I. Introduction

Fixed dental prosthesis is one of the mainstay in restoring missing teeth.Maintenance of periodontal health is essential for long term success of Fixed Prosthesis.Poor crown margins,rough surfaces,faulty impression procedure,inadequate lab support are most common reasons for poor periodontal health around fixed prosthesis.[1-4] Good luting agent is detrimental for developing and maintaining optimal periodontal health around fixed prosthesis.Microleakage,solubility and disintegration are common issues related to most of luting agents.[5,6] Streptococcus mutans has been most commonly associated with microbial infection developed underneath fixed prosthesis causing periodontal issues. Apart from other properties,ideal luting agent should also possess antibacterial and anticariogenic properties.Luting agents like Zinc Phosphate,Zinc Polycarboxylate and Glass ionomer cement have antibacterial properties because of low ph and/or release of flouride but Resin cement does not exhibit significant antibacterial action.[5-9,13]

Role of Chlorhexidine gluconate as antibacterial agent has been well documented. Chlorhexidine has been used in past in various concentrations to improve antimicrobial activity of Glass ionomer cements,Zinc Poycarboxylate cement ,Resin cement,Bonding agents and root canal irrigating solutions.[17-20,22-25,28-29] There are also a few studies on positive effect of Chlorhexidine on bond strength of dentin and retention of fixed prosthesis without interfering in other physical properties of the cement.[14-16] Antimicorbial substantivity of Chlorhexidine has also been proven in past.[31-32]Considering past studies, 2% Chlorhexidine is expected to improve antibacterial activity of Resin cement.

The aim of this present clinical study was to evaluate the effect of 2% Chlorhexidine on antibacterial activity of Resin cement.

II. Material and Method

A total of 100 patients of 29-56 years of age who required Fixed dental Prosthesis were selected for the study.The Procedure was explained to the patients before starting any procedure and informed consent was taken.The patients with systemic disease or taking medications that can affect gingival health were excluded from the study.Silness Loe plaque index and Loe Silness gingival index of less than 2 and Probing sulcus depth of less than 4 mm of abutment teeth was maintained for every patient before the beginning of the study.Abutment teeth were evaluated for Preparation.2 teeth were prepared in each patient for All Ceramic crowns(IPS emax CAD,Ivoclar,Mumbai,India) with minimal trauma and shoulder finish line was given in every preparation by same clinician.Finish lines were located at the gingival margin.Patients were given oral prophylaxis treatment after bacteriologic sample were collected at the baseline visit.In every patient, the 2 teeth prepared were divided in to 2 groups: Control(no antimicrobial agent was applied after etching of tooth) , Test (2% Chlorhexidine was applied after etching of the tooth) .Bacteriologic samples were collected at 5 different

sample times: Baseline visit(as Patient enters the OPD),At the time of cementation,1 month after cementation,3 month after cementation and 6 month after cementation. Sterile standardized endodontic paper points(Diadent,south korea) were used to collect bactriologic samples.The paper points were placed 30s in to gingival sulcus at 4 locations(mesibuccal,distibuccal,midbuccal and mid lingual or palatal regions) on each abutment tooth.A single broth was obtained by putting all four paper points in one pool providing one broth sample per tooth.Every patient provided 10 bacteriolgic samples(2x5) and a total of 1000 samples were collected(10x100).

After preparation of the tooth,etching was done using Total Etch(Ivoclar,Mumbai,India) for 15s.In control group ,nothing was applied after etching,whereas in Test 1 group ,after etching, 2% Chlorhexidine gluconate(Hexidine,ICPA,India) was applied through cotton pellet for 60s and then dried for 10s.Resin cement (Multilink automix,Ivoclar,vivadent,Mumbai,India) was used as Luting agent.Primer A and B were mixed in 1:1 ratio and was applied to prepared tooth for 30s(as per manufacturer instruction).The All ceramic crown were thoroughly rinsed with water and dried.Monobond plus was applied to inner surface of the crown for 60s and dispersed with strong stream of air.Then,Multilink automix luting cement was applied to inner surface of crown and cement was luted.

Microbiologic Processing

All microbiological samples were inserted in to Robertson cooked media and were sent to Microbiological department for anaerobic and aerobic culture procedures.The samples were cultured on Brucella blood agar,Kanakmycin-Vancomycin laked blood agar, and Bacteroides bile esculin agar(Hi media laboratories pvt. Ltd,Mumbai,India) for Anaerobic bacteria.The plates were placed in an anaerobic chamber.(Fig.1).Aerotolerance test was done for each different colony prior to gram staining to determine purities,spore formation and morphologies.Catalase and Pigment activities were also observed.Identification of anaerobes was done using API 20A and ID 32A strips(Biomerieux,SA,France) were used.Bacterial Pathogenicity was cateogorised according to whether the oraganism was associated with Periodontally suspected bacteria and not Periodontally suspected bacteria.5% blood agar(Figure 2),Mcconkey agar (Figure 3) and Chocolate agar(with vancomhycin,clindamycin and bacitracin) in laminar flow were used for culturing aerobic bacteria(Labine instruments,Kochi,India).Standard microbiological methods and API automated systems were used to identify isolated bacteria.

The statistical evaluation was done with help of SPSS version 2016 using X^2 and P value.

III. Results

A total of 1000 broth samples were collected during the study and 3375 different bacterial colonies were observed .

In Control group,at Baseline level,Predominantly Hemophilus spp.(13.8%),Neisseria spp.(10.9%) and Streptococci spp. (33.4%) were found with Aerobic/Facultative gram positive cocci atmosphere.At cementation,predominantly Clostridiumspp(12%), Hemophilusspp.(10%) and Streptococc spp. (30%) were found with Aerobic/Facultative gram positive cocci atmosphere.After 1 month post cementation,predominantly Fusobacteriumspp.(15%),Prevotellaintermedia spp.(14.2%),Veillonellaparvula spp.(16.8%) and Streptococci spp (17.6%) were found with Anaerobic gram negative rod atmosphere.After 3 month post cementation,predominantly Fusobacterium nucleatum spp.(17%),Prevotellaintermedia spp.(14.5%),Veillonellaparvula spp.(18.8%) and Streptococci spp. (16.6%) were found with Anaerobic gram negative rod atmosphere.After 6 month postcementation,predominantly Fusobacteriumnucleatum(15%),Veillonellaparvula spp.(14.8%) and Streptococci spp. (20.6%) were found with Anaerobic gram negative rod atmosphere. In Control group, there is Aerobic atmosphere at Baseline(51%) and at cementation (53%),that becomes Anaerobic at 1 month post cementation(56%) and remains Anaerobic 3 months (59%) and 6 month post cementation(58%).There is Gram positive atmosphere at Baseline(64%) and at Cementation(66%) that becomes Gram negative at 1 month post cementation(66%) and remains Gram negative 3 months (62%) and 6 months (55%) Post cementation.There are more number of Cocci at Baseline level (63%)and at time of cementation(61%) but number of Rods increase at 1 month after cementation(62%) and remains increased at 3 months post cementation(55%) and 6 months post cementation(51%) .Thus there was Aerobic gram positive cocci atmosphere in control group till time of cementation which became Anaerobic gram negative atmosphere after 1 month post cementation and continued till 6 months of post cementation.[Table 2,3,4]

In Test group,at Baseline level,Predominantly Diptheroid bacilli spp.(9.8%),Campylobacter rectus spp.(9.6 %) and Streptococci spp. (35.6%) were found with Aerobic/Facultative gram positive cocci atmosphere.At cementation,predominantly Clostridiumspp(11.5%), Staphylococcus aureus spp.(13.5%) and Streptococc spp. (36%) were found with Aerobic/Facultative gram positive cocci atmosphere.After 1 month post

cementation,predominantly Bifidobacterium spp.(5.2%),Coagulase negative Staphlococci spp.(6.9%),Streptococci spp (64.1%) were found with Aerobic gram positive cocci atmosphere.After 3 month post cementation,predominantly Bifidobacterium spp.(5.2%),Coagulase negative Staphylococccus spp.(6.5%),and Streptococci spp. (65.2%) were found with Aerobic gram positive cocci atmosphere.After 6 month postcementation,predominantly Hemophilus spp.(7.5%),Filifactoralocis spp.(7.3%) and Streptococci spp. (38.6%) were found with Anaerobic gram negative cocci/rod atmosphere. There is Aerobic atmosphere at Baseline(57%) and at cementation (58%),that remains Aerobic at 1 month post cementation(66%) ,and at 3 months (64%) but becomes Anaerobic at 6 month post cementation(54%).There is Gram positive atmosphere at Baseline(58%) and at Cementation(62%) that becomes more Gram positive at 1 month post cementation(71%) and at 3 months (69%) and becomes Gram negative at 6 months (56%) Post cementation.There are more number of Cocci at Baseline level (57%)and at time of cementation(54%) and number of Cocci increase at 1 month after cementation(63%) and remains increased at 3 months post cementation(67%) and becomes slightly lesser at 6 months post cementation(51%) Thus there was Aerobic gram positive cocci atmosphere in Test group till 3 months postcementation which became Anaerobic gram negative cocci/rod atmosphere after 6 month post cementation .[Table 2,3,5]

IV. Discussion

Fixed dental prosthesis are frequently associated with development of periodontal problems in patients. Development of caries within the restoration,faulty crown margin design,improper embrasure design are one of the most common reasons for this.[1-4] Use of luting cement with good antibacterial activity is always preferred to reduce or control periodontal diseases due to fixed dental prosthesis.Cements like Zinc phosphate,Glass ionomer cement,Zinc polycarboxylate have good antibacterial activity but Resin cement shows poor antibacterial activity.[19,20,24-25] Different antibacterial agents have been used with Dentin bonding agents,Root canal irrigating solutions,Luting cement to affect antibacterial activity.Chlorhexidine gluconate is proven antibacterial agent[13,26-27].They have been used in different concentration to study their influence on antibacterial activity and other physical properties of luting cements.[28,29,33-34]

Chlorhexidine is associated with promotion of Hybrid layer and improvement of physical properties of Resin cement.[26-27] It also diminishes the loss of bonding effectiveness over time associated with etch and rinse and self etch cements and also reduces microleakage at gingival margin after storage[2329,30].

Gram positive facultative rods and cocci are found in periodontally healthy site with predominance of Capnocytophaga,Neisseria and Veillonella spp.In chronic gingivitis sites,there are equal proportions of gram positive species(56%) and gram negative species(44%) with facultative anaerobic microorganisms with predominance of Fusobacterium nucleatum,P intermedia,.[1,2,19,20]

There was significant shift in Control group towards Gram negative,anaerobic,rod atmosphere from Baseline till 6 month postcementaion.It is evident as percentage of Fusobacterium nucleatum spp. Increased from 2.3% at baseline level to 15% after 6 months postcementation.Porphyromonas gingivalis was missing in control group at baseline level and reach to 3.2% till 6 months post cementation.Prevotella intermedia increased from 1.3% at baseline level to 12.5% after 6 month postcementation.Veillonella parvula increased from 1.2% at baseline to 14.8% after 6 month of postcementation.

In Test group,atmosphere shifts towards Aerobic ,Gram positive ,cocci till 3 months post cementation but becomes Anaerobic ,Gram negative,cocci/rod in 6 month postcementation.Streptococci % increased from 35.6% to 65.2% till 3 month postcementation and then falls back to 38.6% in 6 month postcementation.Neisseria spp was 4.3% at baseline level but was missing till 3 months postcementation to reappear again in 6 month postcemetation (4.8%).This can be co-related with antibacterial property of Chlorhexidine and substantivity of the effect till 3 months postcementation ,which fades out after that.[19-20,31-32].

V. Conclusion

This study shows that application of 2% Chlorhexidine on prepared tooth surface after etching definitely increases antibacterial activity of Resin cement and promotes development of Gram positive,Aerobic, Cocci atmosphere.

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Table 1: Overall Distribution of Bacteria Isolated in Control and 2% CHX

Bacteria type	Control n=1800		2% CHX n=1575	
	PSB	N-PSB	PSB	N-PSB
Facultative GNB	39(2.2)	153(8.5)	66(4.2)	213(13.5)
Aerobic GPB	61(3.4)	105(5.84)		197(12.5)
Aerobic GNC		116(6.46)		82(5.2)
Facultative GPC	48(2.7)	647(35.9)	83(5.3)	708(45)
Anaerobic GNB	155(8.64)	194(10.8)	30(1.9)	
Anaerobic GPB		135(7.5)		155(9.8)
Anaerobic GNC	102(5.56)		41(2.6)	
Anaerobic GPC		45(2.5)		
Totals	405(22.5%)	1395(77.5%)	220(14%)	1355(86%)

Table 2: Distribution of Bacteria(%) in Control and Test group n(%) for Gram Stain,Atmosphere of Growth,Morphological Properties and Pathogenicity

Type of Bacteria	Control	2% CHX	X2	P value
Baseline				
N-PSB	75	77	2.284	0.319
PSB	25	23		
Aerobic/facultative	51	57	2.06	0.357
Anaerobic	49	43		
Gram-positive	64	58	1.73	0.419
Gram-negative	36	42		
Cocci	63	57	0.776	0.678
Rods	37	43		
At Cementation				
N-PSB	80	73	1.635	0.441
PSB	20	27		
Aerobic/facultative	53	58	2.496	0.287
Anaerobic	47	42		
Gram-positive	66	62	1.051	.591
Gram-negative	34	38		
Cocci	61	54	1.061	0.588
Rods	39	46		
1 month Post Cementation				
N-PSB	65	88	26.654	0.0000
PSB	35	12		
Aerobic/facultative	44	66	15.485	0.0000
Anaerobic	56	34		
Gram-positive	34	71	16.103	0.0004
Gram-negative	66	29		
Cocci	38	63	25.424	0.0000
Rods	62	37		
3 month Post Cementation				
N-PSB	68	85	9.207	0.0100
PSB	32	15		
Aerobic/facultative	41	64	12.651	0.0017
Anaerobic	59	36		
Gram-positive	38	69	22.603	0.0000
Gram-negative	62	31		
Cocci	45	67	10.118	0.0063
Rod	55	33		
6 months Post Cementation				
N-PSB	70	74	1.924	0.3821
PSB	30	26		
Aerobic/facultative	42	46	0.763	0.6828
Anaerobic	58	54		
Gram-positive	45	44	0.19	0.9093
Gram-negative	55	56		
Cocci	49	52	1.308	0.5199
Rod	51	48		

Table 3: Distribution of Bacteria(%) isolated in Control and Test group at all sample times

Type of Bacteria	Control	2% CHX	X^2	P value
Baseline			3.979	0.679
PSB	25	23		
Anaerobic	49	43		
Gram-negative	36	42		
Rods	37	43		
At Cementation			3.353	0.763
PSB	20	27	3.979	0.679
Anaerobic	47	42		
Gram-negative	34	38		
Rods	39	46		
1 month Post Cementation				
PSB	35	12	5.894	0.435
Anaerobic	56	34		
Gram-negative	66	29		
Rods	62	37		
3 month Post Cementation				
PSB	32	15	1.355	0.968
Anaerobic	59	36		
Gram-negative	62	31		
Rod	55	33		
6 months Post Cementation				
PSB	30	26	0.765	0.999
Anaerobic	58	54		
Gram-negative	55	56		
Rod	51	48		

Table 4: Distribution and Bacterial isolated in Control group at all sample times

Type of Bacteria	Control(n=1800)				
	Baseline 432(24%)	At Cementation 378(21% of 1800)	1 month Post cementation 324(18%)	3 month Post cementation 270(15%)	6 month Post cementation 396(22%)
Actinomycesnaeslundii FG+veR	14(3.2%)		7(2%)	8(3%)	21(5%)
Actinomycesviscosus FG+veR	13(2.9%)		5(1.5%)	5(2.5%)	14(3.5%)
Bifidobacterium spp FG+veR	23(5.6%)	23(6%)	5(1.5%)	7(1.5%)	7(2.5%)
Clostridium spp FG+veR		45(12%)			
Diphtheroid bacilli AG+veR	11(2.6%)	19(5%)	24(7.3%)	12(4.3%)	29(7.3%)
Escherichia coli FG-veR		11(3%)			
Eubacterium spp FG+veR[1]	8(1.8%)				
Fusobacteriumnucleatum AnG-veR[1]	10(2.3)	15(4%)	48(15%)	45(17%)	60(15%)
Haemophilus spp FG-veR	59(13.8%)	38(10%)	25(8.1%)	22(8.1%)	24(6.1%)
Coagulase-negative *Staphylococci* FG+veC	9(2.2%)	19(5%)			
Neisserria spp AG-veC	47(10.9)	8(2%)	6(1.7%)	5(1.7%)	11(2.7%)
Peptostreptococcus AnG+veC	29(6.7%)				
Porphyromonasgingivalis AnG-veR[1]			17(5.2%)	14(5.2%)	13(3.2%)
Prevotellaintermedia AnG-veR[1]	6(1.3%)	11(3%)	46(14.2%)	38(14.5%)	49(12.5%)
Propionibacteriumgranulosum FG+veR					
Staphylococcus aureus FG+veC	8(2.1%)	22(6%)			
Veillonellaparvula AnG-veC[1]	5(1.2%)	22(6%)	54(16.8%)	51(18.8%)	59(14.8%)
Streptococci AG-veC	143(33.4%)	115 (30%)	57(17.6%)	44(16.6%)	82(20.6%)
Campylobacter rectus FG-veR	34(7.8%)	15(4%)	24(7.4%)	14(5.1%)	12(3.1%)
Treponemadenticola AnG-veC[1]	7(1.7%)	11(3%)			
Gemella spp FG+veC					
Filifactoralocis FG+veR	6(1.3%)	4(1%)	6(1.7%)	5(1.7%)	15(3.7%)

Total (*n*)	432	378	324	270	396
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 5: Distribution and Bacterial isolated in Test group 2% CHX group at all sample times

Type of Bacteria	2%CHX (n=1575)				
	Baseline 236(15 %)	At Cementation 284(18 % of 1575)	1 month Post cementation 315(20 %)	3 month Post cementation 346(22%)	6 month Post cementation 394(25%)
Actinomycesnaeslundii FG+veR		-	4(1.4%)	5(1.5%)	
Actinomycesviscosus FG+veR		-	6(1.8%)	7(1.9%)	
Bifidobacterium spp FG+veR	18(7.5%)	10(3.4%)	16(5.2%)	18(5.2%)	25(6.3%)
Clostridium spp FG+veR		33(11.5%)	-	-	
Diphtheroid bacilli AG+veR	23(9.8%)	11(3.8%)	5(1.5%)	7(2%)	27(6.8%)
Escherichia coli FG-veR		10(3.6%)	-	-	
Eubacterium spp FG+veR[1]		11(3.9%)	-	-	
Fusobacteriumnucleatum AnG-veR[1]		20(7%)	-	-	
Haemophilus spp FG-veR	17(7.3%)	10(3.6%)	15(4.9%)	13(3.9%)	30(7.5%)
Coagulase-negative *Staphylococci* FG+veC	11(4.5%)	8(2.8%)	22(6.9%)	22(6.5%)	16(4%)
Neisserria spp AG-veC	10(4.3%)	10(3.6%)	-	-	19(4.8%)
Peptostreptococcus AnG+veC		-	-	-	
Porphyromonasgingivalis AnG-veR[1]		-	-	-	
Prevotellaintermedia AnG-veR[1]	6(2.5%)	5(1.9%)	-	-	12(3%)
Propionibacteriumgranulosum FG+veR	5(2.1%)	-	-	-	8(2.1%)
Staphylococcus aureus FG+veC	7(2.9%)	38(13.5%)	10(3.2%)	13(3.8%)	7(1.9%)
Veillonellaparvula AnG-veC[1]	5(2.1%)	6(2.2%)	4(1.4%)	8(2.4%)	9(2.3%)
Streptococci AG-veC	83(35.6%)	103(36%)	203(64.1%)	226(65.2%)	151(38.6%)
Campylobacter rectus FG-veR	23(9.6%)	-	10(3.2%)	15(4.2%)	42(10.6%)
Treponemadenticola AnG-veC[1]	11(4.5%)	6(2%)	-	-	19(4.8%)
Gemella spp FG+veC		-	11(3.6%)	6(1.6%)	
Filifactoralocis FG+veR	17(7.3%)	3(1.2%)	9(2.8%)	6(1.8%)	29(7.3%)
Total (*n*)	236	284	315	346	394
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001