

## Evaluation of Effect of 0.2% Cetrimide on Antibacterial Activity of Resin Cement.

Yashpal Singh<sup>1</sup>,YS, Monika Saini<sup>2</sup>,MS , Suraj Suvarna<sup>3</sup>,SS

<sup>1</sup>associate Professor, Al Qassim Pvt. College, Buraydah, Ksa

<sup>2</sup>associate Professor, Al Qassim Pvt. College, Buraydah, Ksa

<sup>3</sup>professor, Sbb Dental College, Ghaziabad, U.P., India.

### Abstract

**Aim:** To evaluate the effect of 0.2% Cetrimide 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 posterior 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 (0.2% Cetrimide 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.Microbiological 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 post cementation. In Test group,there is shift towards aerobic ,gram positive cocci from baseline till 1 month post cementation which persists but becomes till 3 month post cementation and becomes Gram negative,anaerobic,rod after 6 month postcementation.

**Conclusion:** This study shows that application of 0.2% Cetrimide on prepared tooth surface after etching definitely increases antibacterial activity of Resin cement. Substantivity of 0.2% Certrimide persists but keeps on decreasing from 1 month till 3<sup>rd</sup> month of postcementation.

### 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.<sup>1-4</sup> 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.<sup>5,6</sup> 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 cements have antibacterial properties because of low ph and/or release of flouride but Resin cement does not exhibit significant antibacterial action.<sup>5-9,13</sup>

Role of Cetrimide as antibacterial agent has been well documented. Cetrimide have been used in past in various concentrations to improve antimicrobial activity of Glass ionomer cements,Zinc Poycarboxylate cement ,Bonding agents and root canal irrigating solutions.<sup>17-20,22-25,28-29</sup> There are also a few studies on positive effect of Cetrimide on bond strength of dentin and retention of fixed prosthesis without interfering in other physical properties of the cement.<sup>14-16</sup> Antimicorbial substantivity of Cetrimide has also been proven in past.<sup>31-32</sup> Considering past studies, 0.2% Cetrimide is expected to improve antibacterial activity of Resin cement. The aim of this present clinical study was to evaluate of the effect of 0.2% Cetrimide on antibacterial activity of Resin cement.

### II. Material and Method

A total of 100 patients of 29-56 years of age who required Posterior 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 posterior 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.In every patient, the 2 teeth prepared were divided in to 2 groups:Control,Test . 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 group 0.2% Cetrимide(Sigma-Aldrich Chemie, Steinheim, Germany) was applied through cotton pellet for 60s and then dried for 10s after etching .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. The crown was etched with 5% hydrofluoric acid ( IPS Ceramic etching gel,Ivoclar,Mumbai, India) for 20 seconds.Then crown was rinsed with water spray and dry it with oil free air. The bonding surface of the crown was coated with Ivoclean using a microbrush or brush for 20s. The crown was thoroughly rinsed with water Monobond plus was applied to inner surface of the crown for 60s and dispersed with strong stream of air.Multilink automix luting cement was applied to inner surface of crown for luting the crown.

Bacteriologic samples were collected at 5 different sample times: Baseline visit,at the time of cementation,1 ,3 and 6 month after cementation. .Patients were given oral prophylaxis treatment after bacteriologic sample were collected at the baseline visit .Sterile standardized endodontic paper points(Diadent,south korea) were used to collect bacteriologic samples.The paper points were placed 30s in to gingival sulcus at 4 locations(mesibuccal,distribuccal,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 bacteriologic samples(2x5) and a total of 1000 samples were collected(10x100).

### 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 categorized according to whether the organism was associated with periodontally suspected bacteria and not periodontally suspected bacteria.5% blood agar(Figure 2),Mcconkey agar (Figure 3) and Chocolate agar(with vancomycin,clindamycin and bacitracin)(Figure 4) 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 2925 different bacterial colonies were observed .Total percentage of Pathogenic periodontally suspected bacteria present was 22.5% and 12% in Control group, and Test group respectively.Pathogenic anaerobic gram negative bacilli were highest in Control group (8.64% ) followed by Test group(1.3%).Pathogenic anaerobic gram negative cocci were highest in Control group (5.56%) followed by Test group(1.2%).[Table 1]

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 *Clostridium spp.*(12%), *Hemophilus spp.*(10%) and *Streptococci spp.* (30%) were found with Aerobic/Facultative gram positive cocci atmosphere.After 1 month post cementation,predominantly *Fusobacterium spp.*(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.(10.3%),Campylobacter rectus spp.(9 % ) ,Hemophilus spp(7.9%) and Streptococci spp. (35%) were found with Aerobic/Facultative gram positive cocci atmosphere.At cementation,predominantly Clostridium spp(11.9%), Staphylococcus aureus spp.(10.5%) ,Neisseriaspp(5.6%) and Streptococci spp. (33%) were found with Aerobic/Facultative gram positive cocci atmosphere.After 1 month post cementation,predominantly Bifidobacterium spp.(7.2%),Coagulase negative Staphylococci spp.(6.9%),Streptococci spp (54.1%) were found with Aerobic gram positive cocci atmosphere.After 3 month post cementation,predominantly Coagulase negative Staphylococcus spp.(5.5%), Campylobacterrectus spp(6.2%) and Streptococci spp. (65.7%) were found with Aerobic gram positive cocci atmosphere.After 6 month postcementation,predominantly Hemophilus spp.(7.5%), Treponemadenticolaspp.(10.4%) and Streptococci spp. (53.6%) were found with Anaerobic gram negative cocci/rod atmosphere. There is Aerobic atmosphere at Baseline(61%) and at cementation (64%),that remains Aerobic at 1 month post cementation(69%) ,and at 3 months (61%) but becomes Anaerobic at 6 month post cementation(60%).There is Gram positive atmosphere at Baseline(55%) and at Cementation(59%) that becomes more Gram positive at 1 month post cementation(78%) and at 3 months (64%) and becomes Gram negative at 6 months (58%) Post cementation.There are more number of Cocci at Baseline level (59%)and at time of cementation(56%) and number of Cocci increase at 1 month after cementation(72%) and decreases slightly at 3 months post cementation(59%) and even lesser at 6 months post cementation(44%).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.<sup>1-4</sup> 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.<sup>19,20,24-25</sup> Different antibacterial agents have been used with Dentin bonding agents,Root canal irrigating solutions,Luting cement to affect antibacterial activity. Cetrimide is proven antibacterial agents<sup>13,26-27</sup>.It has been used in different concentration to study their influence on antibacterial activity and other physical properties of luting cements.<sup>28,29,33-34</sup> Cetrimide has reported to have positive influence on flexural strength and antibacterial activity of conventional luting cement.<sup>25</sup> 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,<sup>1,2,19,20</sup>

There was significant shift in Control group towards Gram negative,anaerobic,rod atmosphere from Baseline till 6 month postcementation.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,there is stronger shift towards aerobic ,gram positive cocci from baseline till 1 month postcementation but becomes lesser in 3 month post cementation and becomes Gram negative,anaerobic,rod (even more than Control group) after 6 month postcementation.Hemophilus spp was 7.9% at baseline which dropped till 4.9% after 3 month postcementation to rise again till 7.5% after 6 month post cementation.Similarly Treponemadenticola spp was 5.4% % at baseline which disappeared after 3 month postcementation to appear again after 6 month post cementation(4.8%). This can be co-related by the fact that 0.2% Cetrimide is effective antibacterial agent but its substantivity declines after 30 days and remains till 90 days after which it has no effect on antibacterial activity.<sup>17-18,22,31-32</sup>

#### **V. Conclusion**

This study shows that application of 0.2% Cetrimide on prepared tooth surface after etching definitely increases antibacterial activity of Resin cement and promotes development of Gram positive,Aerobic, Cocci atmosphere.The effect of 0.2% Cetrimide decreases after 1 month postcementation but it persists till 3 months.

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**Table 1:** Overall Distribution of Bacteria Isolated in Control and Test group n(%)

Bacteria type	Control n=1800(40%)		0.2% Cetrimide n=1125(25%)	
	PSB	N-PSB	PSB	N-PSB
Facultative GNB	39(2.2)	153(8.5)	47(4.2)	118(10.5)
Aerobic GPB	61(3.4)	105(5.84)		152(13.5)
Aerobic GNC		116(6.46)		92(8.2)
Facultative GPC	48(2.7)	647(35.9)	60(5.3)	540(48)
Anaerobic GNB	155(8.64)	194(10.8)	15(1.3)	
Anaerobic GPB		135(7.5)		88(7.8)

Anaerobic GNC	102(5.56)		13(1.2)	
Anaerobic GPC		45(2.5)		
Totals	405(22.5%)	1395(77.5%)	135(12%)	990(88%)

**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	0.2% Cetrimide	X <sup>2</sup>	P value
<b>Baseline</b>				
N-PSB	75	68	2.284	0.319
PSB	25	32		
Aerobic/facultative	51	61	2.06	0.357
Anaerobic	49	39		
Gram-positive	64	55	1.73	0.419
Gram-negative	36	45		
Cocci	63	59	0.776	0.678
Rods	37	41		
<b>At Cementation</b>				
N-PSB	80	79	1.635	0.441
PSB	20	21		
Aerobic/facultative	53	64	2.496	0.287
Anaerobic	47	36		
Gram-positive	66	59	1.051	.591
Gram-negative	34	41		
Cocci	61	56	1.061	0.588
Rods	39	44		
<b>1 month Post Cementation</b>				
N-PSB	65	91	26.654	0.0000
PSB	35	9		
Aerobic/facultative	44	69	15.485	0.0000
Anaerobic	56	31		
Gram-positive	34	78	16.103	0.0004
Gram-negative	66	22		
Cocci	38	72	25.424	0.0000
Rods	62	28		
<b>3 month Post Cementation</b>				
N-PSB	68	81	9.207	0.0100
PSB	32	19		
Aerobic/facultative	41	61	12.651	0.0017
Anaerobic	59	39		
Gram-positive	38	64	22.603	0.0000
Gram-negative	62	36		
Cocci	45	59	10.118	0.0063
Rod	55	41		
<b>6 months Post Cementation</b>				
N-PSB	70	65	1.924	0.3821
PSB	30	35		
Aerobic/facultative	42	40	0.763	0.6828
Anaerobic	58	60		
Gram-positive	45	42	0.19	0.9093
Gram-negative	55	58		
Cocci	49	44	1.308	0.5199
Rod	51	56		

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

Type of Bacteria	Control	0.2% Cetrimide	X <sup>2</sup>	P value
<b>Baseline</b>				
PSB	25	32	3.979	0.679
Anaerobic	49	39		
Gram-negative	36	45		
Rods	37	41		
<b>At Cementation</b>				
PSB	20	21	3.353	0.763
Anaerobic	47	36		
Gram-negative	34	41		

Rods	39	44		
<b>1 month Post Cementation</b>				
PSB	35	9	5.894	0.435
Anaerobic	56	31		
Gram-negative	66	22		
Rods	62	28		
<b>3 month Post Cementation</b>				
PSB	32	19	1.355	0.968
Anaerobic	59	39		
Gram-negative	62	36		
Rod	55	41		
<b>6 months Post Cementation</b>				
PSB	30	35	0.765	0.999
Anaerobic	58	60		
Gram-negative	55	58		
Rod	51	56		

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

Type of Bacteria	Control(n=1800)				
	Baseline	At Cementation	1 month Post cementation	3 month Post cementation	6 month Post cementation
<i>Actinomycesnaeslundii</i> FG+veR	14(3.2%)		7(2%)	8(3%)	21(5%)
<i>Actinomycesviscosus</i> FG+veR	13(2.9%)		5(1.5%)	5(2.5%)	14(3.5%)
<i>Bifidobacterium</i> spp FG+veR	23(5.6%)	23(6%)	5(1.5%)	7(1.5%)	7(2.5%)
<i>Clostridium</i> spp FG+veR		45(12%)			
Diphtheroid bacilli AG+veR	11(2.6%)	19(5%)	24(7.3%)	12(4.3%)	29(7.3%)
<i>Escherichia coli</i> FG-veR		11(3%)			
<i>Eubacterium</i> spp FG+veR <sup>1</sup>	8(1.8%)				
<i>Fusobacteriumnucleatum</i> AnG-veR <sup>1</sup>	10(2.3)	15(4%)	48(15%)	45(17%)	60(15%)
<i>Haemophilus</i> 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%)			
<i>Neisseria</i> spp AG-veC	47(10.9)	8(2%)	6(1.7%)	5(1.7%)	11(2.7%)
<i>Peptostreptococcus</i> AnG+veC	29(6.7%)				
<i>Porphyromonasgingivalis</i> AnG-veR <sup>1</sup>			17(5.2%)	14(5.2%)	13(3.2%)
<i>Prevotellaintermedia</i> AnG-veR <sup>1</sup>	6(1.3%)	11(3%)	46(14.2%)	38(14.5%)	49(12.5%)
<i>Propionibacteriumgranulosum</i> FG+veR					
<i>Staphylococcus aureus</i> FG+veC	8(2.1%)	22(6%)			
<i>Veillonellaparvula</i> AnG-veC <sup>1</sup>	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%)
<i>Campylobacter rectus</i> FG-veR	34(7.8%)	15(4%)	24(7.4%)	14(5.1%)	12(3.1%)
<i>Treponemadenticola</i> AnG-veC <sup>1</sup>	7(1.7%)	11(3%)			
<i>Gemella</i> spp FG+veC					
<i>Filifactoralocis</i> 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 0.2% Cetrимide group at all sample times

Type of Bacteria	0.2% Cetrимide (n=1125)				
	Baseline	At Cementation	1 month Post cementation	3 month Post cementation	6 month Post cementation
<i>Actinomycesnaeslundii</i> FG+veR		-	8(3.4%)	4(1.8%)	
<i>Actinomycesviscosus</i> FG+veR		-	11(4.8%)	4(1.6%)	
<i>Bifidobacterium</i> spp	13(7%)	7(3.2%)	16(7.2%)	5(2.2%)	9(3.3%)

Evaluation Of Effect Of 0.2% Cetrimide On Antibacterial Activity Of Resin Cement.

FG+veR					
<i>Clostridium</i> spp FG+veR		25(11.9%)	-	-	
<i>Diphtheroid bacilli</i> AG+veR	19(10.3%)	9(4%)	6(2.5%)	5(2%)	8(3%)
<i>Escherichia coli</i> FG-veR		9(4.2%)	-	-	
<i>Eubacterium</i> spp FG+veR <sup>1</sup>		6(2.9%)	-	-	
<i>Fusobacteriumnucleatum</i> AnG-veR <sup>1</sup>		19(9%)	-	-	
<i>Haemophilus</i> spp FG-veR	14(7.9%)	12(5.6%)	13(5.9%)	12(4.9%)	19(7.5%)
Coagulase-negative <i>Staphylococci</i> FG+veC	9(5.1%)	6(2.8%)	16(6.9%)	14(5.5%)	5(2%)
<i>Neisseria</i> spp AG-veC	7(4%)	12(5.6%)	-	-	12(4.8%)
<i>Peptostreptococcus</i> AnG+veC		-	-	-	
<i>Porphyromonasgingivalis</i> AnG-veR <sup>1</sup>		-	-	-	
<i>Prevotellaintermedia</i> AnG-veR <sup>1</sup>	5(2.8%)	4(1.9%)	-	-	5(2%)
<i>Propionibacteriumgranulosum</i> FG+veR	4(2.1%)	-	-	-	6(2.4%)
<i>Staphylococcus aureus</i> FG+veC	4(2%)	22(10.5%)	15(6.7%)	4(1.8%)	3(1.6%)
<i>Veillonellaparvula</i> AnG-veC <sup>1</sup>	5(3%)	5(2.2%)	3(1.4%)	12(4.9%)	6(2.3%)
<i>Streptococci</i> AG-veC	63(35%)	71(33%)	122(54.1%)	163(65.7%)	141(53.6%)
<i>Campylobacter rectus</i> FG-veR	16(9%)	-	5(2.2%)	15(6.2%)	27(10.4%)
<i>Treponemadenticola</i> AnG-veC <sup>1</sup>	10(5.4%)	4(2%)	-	-	12(4.8%)
<i>Gemella</i> spp FG+veC		-	6(2.6%)	5(1.9%)	
<i>Filifactoralocis</i> FG+veR	13(6.4%)	3(1.2%)	5(2.3%)	4(1.5%)	6(2.3%)
Total (n)	180	214	225	247	259
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001