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Research Article

Antibacterial Efficacy of Different Luting Cements on Streptococcus Mutans, *Lactobacillus acidophilus* and *Porphyromonas gingivalis*

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Abstract

Aims and Background: Leading contributing factor to failure of crown is frequently due to dental caries and adhesion of microorganisms mainly by Streptococcus mutans (S.M.), Lactobacillus Acidophilus (L.A.) and Porphyromonas Gingivalis (P.G.) in over cavity (O.C.). Therefore the goal of our study was to evaluate and compare the effect of antibacterial property (ABP) of 5 different luting cements (i.e. ZOE, NON- Eugenol (N-E), Dual Cure (D.C.), Polycarboxylate (P.C.) and Self Adhesive Resin (SAR) Cement) on L.A., P.G. and S.M. with zone of inhibition (ZOI) method.

Materials and Methods: A total of 100 patients were included. A saliva sample was collected from each patient, and three different oral bacterial species were extracted and analyzed using the MALDI-TOFMS method. Further 50 patients were treated with neem bark (N.B.) concentrations of 20mg, 40 mg, and 60 mg in 5 different dental luting cements (D.L.C.), and the remaining 50 were treated with 5 different D.L.C. only. Result: Group1, ZOE cement, N-E and SAR cement with N.B. for S.M., and P.G. showed concentration differences after anova testing. Whereas, D.C., P.C. cement for S.M. showed no difference. In Group 2, all three bacteria showed high differences in interaction with various D.L.C.

Conclusion: We come to conclude that there was a significant increase in AB efficacy against 3 bacterias on addition of neem bark at different concentration to D.L.C.

Clinical significance: In order to enhance the A.B. effectiveness against the three predominant intraoral bacteria, it is recommended to incorporate N.B. concentrations which could be 20mg to 60mg into our luting cements for good routine dental practice.

Keywords: *Streptococcus mutans; Lactobacillus acidophilus* and *Porphyromonas gingivalis*. Zone of Inhibition, Antibacterial Property

Abbreviation

MALDI-TOFMS: Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry; RCM broth: Robertson's Cooked Meat Broth; PG: *Prophyromonas gingivalis*; ZOE Cement: Zinc oxide Eugenol; ZOI: Zone of Inhibition; DC: Dual Cure; NE: Non-Eugenol; NB: Neem Bark; SM: *Streptococcus mutans*; LA: *Lactobacillus Acidophilus*; SAR: Self Adhesive Resin; PC: Polycarboxylate

Introduction

The research's discoveries indicate that a significant contributing factor to the failure of traditional crowns (TC) and fixed partial dentures (FPD) is the loss of crown retention (CR) [1]. Studies also concluded that cariogenic bacteria are able to turn fermentable carbohydrates into acids, which could cause tooth tissue and DC, bridges, inlays, onlays, or veneers to lose their mineral content. DC exhibit a high tendency to dissolve in oral fluids, leading to marginal leakage and the formation of a rough surface that attracts food debris and bacteria. The utilization of cement with antibacterial properties can be an effective measure to prevent bacterial migration, as it mitigates the risk of leakage. Previous studies have examined the antimicrobial properties of DC in laboratory settings and have consistently found that all tested materials exhibited a bacteriostatic effect [2]. Furthermore, studies from past have also been prooving that, "DLC serve as the crucial intermediary which is connecting a stationary prosthesis to the meticulously prepared tooth structure that provides support. It is an ideal way to establish a robust connection between materials that are dissimilar in nature. Additionally, it should exhibit desirable compressive and tensile strengths while also demonstrating sufficient fracture toughness to prevent any dislodgement caused by failures at the interface or within the material itself. Furthermore, it is crucial for the cement to display appropriate film thickness and viscosity to ensure complete and proper placement. Moreover, it should pos-

sess resistance against disintegration within the oral cavity and be compatible with the surrounding tissues" [3, 4]. Researchers have also concluded that, the primary cause of FPD failure is commonly attributed to caries. This condition is primarily associated with two prevalent bacteria, namely SM and LA [5].

Research conducted over an extended period of time consistently demonstrates that SM is one of the bacteria most commonly associated with DC [6-11]. Research has shown that cariogenic bacteria, specifically S. mutans, possess the ability to efficiently convert fermentable carbohydrates into acids. These acids, in turn, have the potential to cause demineralization of tooth tissue [7, 8, 11]. Studies have also proved that, "oral fluids, ions, molecules, and bacteria can leak through the space between a tooth and a dental restoration and reach the dentinal tubules and pulp. This is called microleakage". Reseachers through their studies have proved that the S.M. bacteria grow unhindered in the area between the tooth that has been prepared and the restoration that is put on it. This makes it easy for them to spread. This growth is helped by a gradual drop in oxygen levels, which in turn helps mutans streptococci, which can grow well in both oxygen-rich and oxygen-poor environments, take over. Consequently, the survival of aerobic bacteria, which rely on sufficient oxygen availability, is compromised [12]. Studies have shown that it may cause an abnormally high number of S. mutans colonies to grow beneath the restoration, causing secondary caries and especially reducing the lifetime of the restoration. For the determination of the total removal of caries during preparation, there are no clear criteria available. It has been demonstrated that the residual bacteria of a carious lesion may cause an increase in pulp sensitivity as well as inflammation and secondary caries [13].

Based on an alternative investigation, it has been determined that uncemented restorations rank as the third most prevalent cause of prosthetic replacement, succumbing to failure within a mere 5.8 years of utilization [14]. While it is of utmost importance to establish optimal resistance and retention forms during tooth preparation as because studies have proved that it is equally imperative to employ DC as a means of fortifying the interface between the tooth and restoration. By effectively sealing this region and facilitating surface attachment, dental cement serves as a protective barrier against microbial leakage [15]. In addition to this, a number of studies have shown that an ideal dental adhesive should be able to wet both the tooth and the restoration, have sufficient fracture toughness to prevent dislodgement due to interfacial or cohesive failures, exhibit adequate film thickness and viscosity to ensure complete seating, be resistant to disintegration in the OC, be compatible with tissue, and exhibit adequate working and working anatomical properties. All of these characteristics are necessary for an ideal dental adhesive [16-18].

Additionally, based on research findings, it is understood that prostheses serve the purpose of facilitating prognoses and providing patients with functional abilities, phonation, and aesthetically pleasing outcomes. These prostheses are designed to ensure tissue compatibility until a permanent restoration is achieved [19-21]. In order for provisional restorations (PRs) to achieve success, it is imperative that they possess the ability to withstand the attachment of microorganisms. This resistance is crucial as it hinders the colonization of surfaces and the maturation of plaque, subsequently reducing the likelihood of periodontal infections. This observation has been made by multiple researchers [22].

In vitro studies have been showing since ages that, FR with a mean surface roughness (MSR) exceeding 0.2 m exhibits an elevated level of bacterial adhesion [23, 24]. Further, in this context studies have also shown that the physical and chemical effects of bacterial adhesion, like leukotoxins, high levels of protease activity, and tissue invasion, may contribute to the gradual loss of gingival attachment seen in periodontitis [25]. Thus, researchers, through their studies, have also evaluated the anaerobic species *Porphyromonas Gingivalis* (PG), which is commonly associated with periodontal disease, in relation to bacterial adhesion for loss of CR, which resulted in a positive relationship with the same [26-28]. Additionally, studies revealed that, these cements need to be anticariogenic, biocompatible, translucent, radiopque, adhere to tooth structure, restoratie type, highly compressive, tensile, low soluble, long working time, rapid onset in oral temperature, low viscosity and film type of thickness [12]. Yet we have evidence from our past studies that adhesion of microorganisms leads to colonization and plaque maturation, which finally increase the risk of periodontal infection, followed by the diluting of DLC and the loosing of dental prostheses [29]. But, in spite of an extensive range on the market, studies proved that, there is no one ideal DLC as each type is physically and chemically distinct [22]. Henceforth, as per our knowledge there is no literature available yet, focusing on AB-P of 5 different DLC (i.e. ZOEt, N-E, DC, PC and SAR cement) without or with NB concentration (20mg, 40mg and 60mg) added against SM, LA and PG bacteria by using ZOI. Thus, the goal of our study was to evaluate the efficacy of all five DLCs without NB in the three different species listed.

Aim

The aim of this study is to evaluate and compare the effect of antibacterial property (AB-P) of 5 different luting cements (i.e. ZOE, NE, DC, PC and SAR Cement) on *Lactobacillus Acidophilus*, *Porphyromonas Gingivalis* and Streptococcus mutans.

Objective

- To isolate and identify the anaerobic isolates from Prosthodontic patients.
- To demonstrate the AB-P of 5 different luting cements that are routinely in the clinics.
- Comparative analysis of luting cements for group 1 and group 2.

Materials and Method

Material

- Discs of 4mm Whatmann filter paper 1
- Strains - SM, LA and PG.
- NB
- RCM broth
- (MALDI-TOFMS) analysis
- Culture media - Brucella agar
- Horizontal laminar air flow bench
- Mc Farland 0.5 opacity.
- 5 different luting cements- ZOE, NE, DC, PC and SAR Cement.

Method

The neem bark extraction was conducted using the soxhlet method at PAC Pharmacy College. The semi-solid extract was stored at a temperature of 4 °C in plastic tubes until it was ready for subsequent utilization. The unstimulated saliva of 100 patients was collected and reported to the department of Prosthodontics. The saliva samples were subsequently placed in the unidentified organisms were subjected to analysis using Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOFMS) and subsequently stored at a temperature of -80°C. In the present study, the bacteria SM, LA, and PG were investigated. Subsequently, the suspensions of three distinct bacterial strains were appropriately adjusted to the Mc Farland standard. A 0.5 opacity was subsequently achieved on Brucella blood agar by utilizing a metallic loop on the Horizontal laminar air flow bench. Moreover, the antimicrobial susceptibility testing was conducted utilizing the disc diffusion technique. The Whatman filter paper is a type of filter paper commonly used in scientific research and laboratory settings. 1 Circular specimens with a diameter of 6mm were fabricated, subjected to sterilization, and subsequently impregnated with DC cement. Robertson's Cooked Meat Broth (RCM broth) and stored in a refrigerator. Further, group 1, 50 patients received NB extract (in three strengths: 20mg, 40mg, and 60mg) on DLC (precisely weighed on a Sartorius scale), whereas group 2 remaining 50 patients received only DLC without NB. ZOI surrounding the discs were measured. All test were carried out with strains of SM (ATCC 25173), LA (ATCC 314), and PG (ATCC 33277).

Statistical analysis

Following incubation, measurements were taken, and statistical analysis was performed using ANOVA.

Result

Intra-group comparison

Intra-group for different NB concentration on 3 different bacteria.

With C1 dental cement we found that at 20 mg - 40 mg and 20mg - 60mg, P value for SM was stastically significant whereas the difference was not significant for 40mg - 60mg respectively. Hence, on anova test it was stastically significant. Further, p value at 20mg- 40mg, 20mg- 60mg and 40mg- 60mg for LA was found to be stastically insignificant. Hence on anova test, it was statistically insignificant. Additionally, at 20mg- 40mg and 20mg- 60mg it was found to be statistically significant whereas at 40mg- 60mg it was statistically insignificant. Hence, on anova test, it was statically significant difference.

Group 1 + C1= ZOE Cement

		SM	LA	PG
20Mg + C1/40mg	P value	0.006	0.178	0.010
20Mg + C1/60Mg + C1	P value	0.019	0.549	0.041
40Mg + C1/60Mg + C1	P value	0.630	0.468	0.647
ANOVA	P value	0.017	0.409	0.037

Table a

Group 1 + C2 = NE Cement

C2 dental cement we found that there was statistically significant difference in impact of different NB concentration on addition to cement against listed 3 bacteria in our study.

		SM	LA	PG
20Mg + C2/40mg	P value	-	0.444	0.481
20Mg + C2/60Mg + C2	P value	-	0.586	0.559
40Mg + C2/60Mg + C2	P value	-	0.175	0.214
ANOVA	P value	-	0.407	0.442

Table b

Group 1+ C3 = DC Cement

		SM	LA	PG
20Mg + C3/40mg	P value	0.751	<0.001	0.012
20Mg + C3/60Mg	P value	0.139	0.048	0.135
40Mg + C3/60Mg	P value	0.076	<0.001	0.430
ANOVA	P value	0.114	<0.001	0.067

Table c

For C3 dental cement, we found that at 20mg- 40mg, 20mg- 60mg for SM it was statistically non significant in difference whereas for 40mg - 60mg it was statistically significant. Hence, on anova test, we found that p value was statically non-significant for all 3 different concentration. At 20mg - 40mg and 40mg- 60mg it was found that difference was statistically highly significant for LA whereas at 20mg- 60mg it was statistically significant. Hence, on anova test it was found that difference was statistically highly significant. At 20mg- 60mg and 40mg- 60mg it was found that difference was stastically non-significant for PG whereas for 20mg - 40mg it was statistically significant. Hence, on anova test, it was statistically significant.

Group 1 + C4 = PC Cement

For C4 dental cement, we found that at 20mg - 40mg, 20mg - 60mg and 40mg - 60mg it was statistically non-significant for all the 3 bacteria's. Hence, on anova test it was found that statistically non-significant for all the 3 bacteria.

		SM	LA	PG
20Mg + C4/40mg	P value	0.946	0.920	0.396
20Mg + C4/60Mg	P value	0.865	0.263	0.933
40Mg + C4/60Mg	P value	0.907	0.302	0.352
ANOVA	P value	0.984	0.448	0.584

Table d

Group 1 + C5 = SAR Cement

		SM	LA	PG
20Mg + C5/40mg	P value	<0.001	0.032	<0.001
20Mg + C5/60Mg	P value	<0.001	0.294	<0.001
40Mg + C5/60Mg	P value	<0.001	0.007	<0.001
ANOVA	P value	<0.001	0.020	<0.001

Table e

In C5 dental cement, we found that 20mg - 40mg, 20mg- 60mg and 40mg - 60mg showed statistically highly significant for SM and PG. Hence, on anova test, it was found to be statistically highly significant for both the bacteria. Whereas at 20mg- 40mg and 40mg-60mg it was statistically significant and statistically non- significant at 20mg- 60mg for LA. Hence, on anova test, it was found statistically significant difference.

Group 2

		SM	LA	PG
Group 2 + C1/C2	P value	<0.001	<0.001	<0.001
Group 2 + C1/C3	P value	<0.001	0.196	<0.001
Group 2 + C1/C4	P value	0.004	0.110	<0.001
Group 2 + C1/C5	P value	0.002	<0.001	<0.001
Group 2 + C2/C3	P value	0.235	<0.001	1.000
Group 2 + C2/C4	P value	<0.001	<0.001	<0.001
Group 2 + C2/C5	P value	<0.001	0.003	<0.001
Group 2 + C3/C4	P value	<0.001	0.836	<0.001
Group 2 + C3/C5	P value	<0.001	<0.001	<0.001
Group 2 + C4/C5	P value	0.716	<0.001	0.463
ANOVA	P value	<0.001	<0.001	<0.001

Table f

Intra-group comparison between the 2 cements in group 2

For C1 /C2, it was found that P value was statistically highly significant for all the 3 bacteria. For C1/C3, P value was statistically highly significant in difference for 2 bacteria i.e. SM and P.G whereas it was statistically non-significant for LA. For C1/C4, the P value was statistically significant for SM, statistically highly significant for PG but statistically non-significant for LA. For C1/C5, P value was statistically highly significant for LA and PG whereas it was statistically significant for SM. For C2/C3, P value was statistically highly significant for LA, statistically significant for *P. gingivalis* but statistically non-significant for SM. For C2/C4, it was found that statistically highly significant for all the 3 bacteria's. For C2/C5, P value was statistically highly significant for SM and PG whereas it was statistically significant for LA. At C3/C4 it was statistically highly significant for SM and PG whereas it was statistically non-significant for LA. At C3/C5, P value was statistically highly significant for all the 3 bacteria's. At C4/C5, P value was statistically non- significant for SM and PG whereas it was statistically highly significant for LA. Hence, on anova test, it was found that all the 3 bacteria's showed statistically highly significant difference with different cement group interactions.

Intra-group comparison

SM

	SM	Group 1 (n = 50)	Group 2 (n = 50)	P value
C1	Mean	12.03	9.16	<0.001
	SD	0.97	3.51	
C2	Mean	0.00	1.64	<0.001
	SD	0.00	2.24	
C3	Mean	0.36	1.18	<0.001
	SD	1.25	1.55	
C4	Mean	9.15	10.98	0.006
	SD	4.43	2.59	
C5	Mean	7.37	11.18	<0.001
	SD	5.79	2.88	

Table g

We found cements (C1, C2, C3 and C5) actions showed statistically highly significant difference between the 2 groups for AB-P. On the other hand, C4 cement action showed no statistically significant difference between the groups against the bacteria.

LA

	LA	Group 1 (n = 50)	Group 2 (n = 50)	P value
C1	Mean	13.25	12.92	0.353
	SD	1.49	3.50	
C2	Mean	0.13	1.74	<0.001
	SD	0.37	1.95	
C3	Mean	3.81	12.1	<0.001
	SD	5.40	2.76	
C4	Mean	9.88	12	<0.001
	SD	4.10	2.01	
C5	Mean	0.87	3.22	<0.001
	SD	2.35	2.87	

Table h

We found that cements (C2, C3, C4 and C5) actions showed statistically highly significant difference for AB-P. On the other hand, C1 did not show any statistically significant difference against the bacteria on comparison with 2 groups.

PG

We found that C2 and C5 showed statistically highly significant AB-P on comparison of 2 groups whereas C3 and C4 showed statistically significant difference. On the other hand, C1 showed statistically non-significant difference between the 2 groups on comparison.

	PG	Group 1 (n = 50)	Group 2 (n= 50)	P value
C1	Mean	16.62	16.98	0.396
	SD	1.31	4.68	
C2	Mean	11.69	1.52	<0.001
	SD	1.56	2.04	
C3	Mean	0.74	1.52	0.006
	SD	1.70	1.72	
C4	Mean	9.87	12.06	0.002
	SD	4.78	2.81	
C5	Mean	7.34	11.68	<0.001
	SD	5.96	2.33	

Table i

Discussion

Studies revealed that sufficient control of dental plaque is necessary for caries prevention. Still, studies revealed that not all patients can take care of their oral hygiene perfectly. Therefore, the antibacterial properties of luting cements are desirable [12]. Extensive research has been conducted on the correlation between surface roughness and bacterial adhesion [29]. Researchers have provided evidence for the presence of a roughness threshold (0.2 μm) at which no additional influence on bacterial adhesion can be anticipated [23,24]. Studies have demonstrated for a very long time that LA ssp can synthesize antimicrobial substances like hydrogen peroxide, lactate, teichoic acid, and bacteriocins. Through the production of these chemicals, these species were able to efficiently inhibit the growth of a wide variety of bacteria, including PG [30,31]. Additionally, studies also found that treatment with LA can reduce the expression of inflammatory cytokines in gingival epithelial cells (GECs) [32,33]. and human macrophages (32), both of which were used to model the oral microbiota [34]. Previous studies have also proved that the AB effects of SAR cements have long-term exposure to a low pH, which can have a negative impact on the adhesion of the cement to dentin, despite the fact that a low pH value initially plays a significant role in antibacterial effects and etching of enamel and dentin [35,36]. Magalhaes., *et al*. showed that RelyX ARC, a conventional resin cement, and RelyX U200, a SAR cement, exhibit significant AB-P against S. mutans for 24 hours [37]. Research studies have also demonstrated a correlation between post-treatment periodontal disease activity and the presence of *P. gingivalis*. These studies have found a significant positive association between the presence of *P. gingivalis* and the loss of periodontal attachment [38-40]. Henceforth, in our study we have decided to evaluate and compare the effect of AB-P of 5 different DLC on LA, PG and SM.

Therefore, in our study, we found cements (C1, C2, C3 and C5) actions showed statistically highly significant difference between the 2 groups for AB efficacy. Whereas only C4 cement action showed no statistically significant difference between the groups against the SM bacteria. Furthermore, for LA, cements (C2, C3, C4 and C5) actions showed statistically highly significant difference for AB efficacy. Whereas only C1 did not showed any statistically significant difference against the bacteria on comparison with 2 groups. For PG, C2 and C5 cement ahowed statistically highly significant antibacterial efficacy on comparison of 2 groups whereas C3 and C4 showed statistically significant difference. Only C1 showed statistically non-significant difference between the 2 groups on comparison.

Conclusion

Although DLC have good properties yet crown retention is the most important deciding factor for the postoperative success of the treatment. Loss of which can lead failure of crowns which is mainly due to caries resulting by marginal leakage and a roughened surface that accumulates food debris and bacteria. Hence, we come to conclusion that significant increase in AB efficacy against 3 bacteria's on addition of neem bark at different concentration to DLC.

Clinical Significance

Based on the findings of our study, it was observed that the inclusion of NB at various concentrations in dental luting cement resulted in enhanced AB effectiveness, as determined through the ZOI method. Additionally, our findings indicate that there is a significant variation in the intra-group comparison of three different concentrations (20mg, 40mg, and 60mg) across three distinct bacterial strains. Thus, in a bid to enhance the AB effectiveness against the three predominant intraoral bacteria, it is recommended to incorporate NB concentrations which could be 20mg to 60mg into our DLC for good routine dental practice.

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