Increased Antibacterial Activity of Zinc Polycarboxylate Cement by the Addition of Chlorhexidine Gluconate in Fixed Prosthodontics

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Purpose: This study evaluated the antibacterial activity of water-activated zinc polycarboxylate cement with adjunctive 0.12% chlorhexidine gluconate on the subgingival microbiota in fixed partial dentures. ***Materials and Methods:*** Thirty-six teeth prepared as fixed partial denture abutments in 9 patients were cemented randomly using water-activated zinc polycarboxylate cement (control group) or water-activated zinc polycarboxylate cement, including 0.12% chlorhexidine gluconate (test group). A total of 108 subgingival plaque samples were analyzed at baseline, immediately before permanent cementation (5 weeks), and 8 weeks later (at 13 weeks). ***Results:*** In the control group, the subgingival microbiota altered to closely resemble the flora of chronic gingivitis (increased proportions of gram-negative anaerobes such as *Prevotella intermedia, Fusobacterium nucleatum*) by 13 weeks. In contrast, the microflora at test sites comprised predominantly gram-positive facultative cocci and rods at 13 weeks. ***Conclusion:*** This study demonstrated that the addition of 0.12% chlorhexidine gluconate may enhance the antimicrobial action of polycarboxylate cements to ensure the maintenance of a microflora compatible with periodontal health, at least up to 13 weeks post-cementation. *Int J Prosthodont 2005;18:377–382.*

The use of metal-ceramic denture restorations in partially dentate patients is the mainstay of fixed prosthodontic reconstruction. Recently, the biologic aspects of fixed reconstructions have received attention because dental materials possess a greater capacity to accumulate and retain plaque than either enamel or dentin.[1] Additionally, rough surfaces may enhance the development of gingivitis by facilitating plaque accumulation.[1] Therefore, insertion of fixed prostheses may have a negative impact on periodontal tissues. Various clinical observations have reported that pocket formation and gingival recession may be more prevalent around prosthodontically treated teeth.[2–4] In particular, subgingival margin placement is associated with deleterious effects on the periodontium and is regarded as one of the main reasons for crown and fixed partial denture (FPD) failure.[1,3–6]

Despite improvements in impression materials, alloys, and waxing techniques, cast restorations cannot be fabricated to adapt perfectly to the margins of prepared teeth. Dental cements are used as adhesive materials that protect, seal, and insulate the teeth in fixed prosthodontics. Luting cement helps maintain the marginal integrity of fixed prostheses by sealing the interface between the restoration and the tooth structure. The cement lute is of prognostic significance to the periodontal condition of crowned abutment teeth and must be as stable and insoluble as possible.[7] Because cement degrades over time, this interface becomes susceptible to colonization by plaque microorganisms. Therefore, the "transition zone," which includes the crown margin, the cement, and the prepared tooth, rep-

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resents a favorable site for plaque accumulation in fixed prostheses.[1,4] Several studies have demonstrated significant microleakage, solubility, and disintegration among a number of common luting agents (zinc oxide–eugenol, silicophosphate, zinc phosphate, conventional polycarboxylate, water-activated polycarboxylate cement, glass ionomer with varnish, and glass ionomer without varnish).[7–9] From a microbiologic perspective, the large area of exposed luting agent surface and the associated microleakeage are assumed to foster microbiologic growth and gingival tissue irritation because of the presence of microbial exotoxins, endotoxins, and metabolic byproducts. For this reason, cement with antibacterial properties may impart plaque-inhibitory properties and act as a secondary line of defense against bacterial migration.

Locally delivered antibacterial agents have become an important part of periodontal therapy.[10] The effectiveness of chlorhexidine gluconate (CHX) in reducing supragingival plaque and subsequent gingival inflammation has been well documented.[11,12] Several studies have demonstrated a reduction of putative periodontal pathogens and gingival inflammation below fixed prosthodontic restorations with the adjunctive use of this antimicrobial mouth rinse during the treatment phase.[12,13] In view of the high efficacy of the CHX mouthrinse, the use of CHX in conjunction with an adhesive material may be expected to contribute to short-term and longer-term gingival health. Furthermore, the addition of CHX has been shown to increase the antimicrobial action of polycarboxylate cement without interfering with its physical properties.[14]

The aim of this clinical study was to evaluate the antibacterial activity of water-activated zinc polycarboxylate cement with adjunctive 0.12% CHX on the subgingival microbiota around FPD abutments.

Materials and Methods

Selection of Subjects

Patients (29 to 56 years of age) who required symmetric FPD restorations for the treatment of edentulous spaces participated in this study. Nine patients were referred for replacement of symmetric second premolar teeth and gave their informed consent to participate in the study. All patients were systemically healthy and had received neither antibiotics nor antibacterial agents for at least 6 months prior to the study. At the beginning of the study, all subjects demonstrated a mean Silness-Löe Plaque Index[15] and Löe-Silness Gingival Index[16] of less than 2, and probing sulcus depths of less than 4 mm at the abutment teeth. Clinical measurements were made at 6 points around each tooth.

The FPD abutment teeth were evaluated for microbiologic parameters. After bacteriologic samples were collected at the baseline visit, all patients received periodontal treatment, including oral hygiene instruction and scaling. FPDs were fabricated from porcelain, and preparations were completed with a chamfer finish line. The margins were located at the gingival margin. A minimally traumatic tooth preparation was performed by same clinician.

Thirty-six teeth prepared for FPDs were randomly assigned to either the test group or the control group. In the control group, the FPDs were cemented with conventional water-activated zinc-polycarboxylate cement (4 scoops powder + 4 drops water). In the test group, 0.12% CHX solution was used instead of distilled water according to the manufacturers' instructions.

Collection of Data

A total of 108 subgingival plaque specimens were collected at baseline prior to initial therapy and tooth preparation, at 5 weeks prior to permanent cementation of the FPD, and at 13 weeks (8 weeks after final cementation). Subgingival microbiologic samples were obtained by inserting sterile standardized endodontic paper points for 30 s into the gingival sulcus subjacent to the restoration. Paper points were gently placed at 4 locations (mesiobuccal, distobuccal, midbuccal, and midlingual/palatal regions) on each abutment tooth after isolating the quadrant from saliva contamination with cotton wool rolls and a saliva ejector. All 4 samples per tooth were pooled into a single broth, providing 1 broth sample per tooth per patient. Therefore, at each of the 3 sample times, there were 2 test samples and 2 control samples per subject (total of 6 test and 6 control samples per subject $\times$ 9 subjects = total 108 samples).

Microbiologic Processing

The microbiologic samples obtained from all subgingival sites were inserted into a thioglycolate broth and dispatched immediately to the Microbiology Department for aerobic and anaerobic culture procedures. Samples were cultured for aerobic bacteria on *(1)* 5% blood, *(2)* eosin methylene blue (EMB), and *(3)* chocolate (with vancomycin, clindamycin, and bacitracin) agars. Blood and EMB agars were incubated aerobically and the chocolate agar was incubated in air + 5% carbon dioxide (CO_2) at 37°C for 24 hours. Isolated bacteria were identified by using both standard microbiologic methods and API automated systems. For anaerobic bacteria, samples were cultured on *(1) Brucella* blood agar, *(2)* kanamycin-vancomycin laked blood agar, and *(3) Bacteroides* bile esculin agar

Table 1 Distribution of Bacteria Isolated in Control and Test Groups on the Basis of Pathogenicities at All Sample Times

Bacteria type	Control (n = 153)		Test (n = 139)		Total (n = 292)
	PSB	N-PSB	PSB	N-PSB	
Facultative GNB	2 (1.3%)	18 (11.8%)	2 (1.4%)	24 (17.3%)	46 (15.8%)
Aerobic GPB	–	6 (3.9%)	–	11 (7.9%)	17 (5.8%)
Aerobic GNC	–	9 (5.8%)	–	8 (5.8%)	17 (5.8%)
Facultative GPC	1 (0.7%)	63 (41.2%)	–	73 (52.5%)	137 (46.9%)
Anaerobic GNB	12 (7.8)	15 (9.8%)	3 (2.2%)	–	30 (10.3%)
Anaerobic GPB	–	15 (9.8%)	–	15 (10.8%)	30 (10.3%)
Anaerobic GNC	11 (7.2%)	–	3 (2.2%)	–	14 (4.8%)
Anaerobic GPC	–	1 (0.7%)	–	–	1 (0.3%)
Totals	26	127	8	131	292

PSB = periodontally suspected bacteria; N-PSB = not periodontally suspected bacteria; GNB = gram-negative bacilli; GPB = gram-positive bacilli; GNC = gram-negative cocci; GPC = gram-positive cocci.

(Difco). The plates were placed into an anaerobic chamber containing a gas mixture of 80% to 90% nitrogen, 5% to 10% hydrogen, and 5% to 10% CO_2 and incubated at 37°C for 48 to 72 hours. Later, aerotolerance tests were performed for each different colony, prior to gram-staining, to determine the purity, spore formation, and morphologies. Catalase and pigment activities were also evaluated. API 20A and ID32A strips (BioMerieux) were used for identification of anaerobes. Categorization of bacterial pathogenicity was done according to whether the organism was associated with progressive periodontal disease (periodontally suspected bacteria [PSB]) or not (not periodontally suspected bacteria [N-PSB]).

Statistical Analysis

The data collected were analyzed by using SPSS for Windows (version 10.01, 1999, SPSS) on a personal computer. Differences within the groups were evaluated using Pearson Chi-square and Fisher's exact tests. $P < .05$ was accepted as statistically significant.

Results

A total of 108 samples (54 tests, 54 controls) were collected (2 test and 2 control samples per subject at baseline, 5 weeks, and 13 weeks). Table 1 describes the composition of the microflora of the test and control sites with respect to morphology, atmosphere of growth, and Gram stain in all sample times. A total of 292 different bacterial colonies were observed: 15.8% facultative gram-negative bacilli, 5.8% aerobic gram-positive bacilli, 5.8% aerobic gram-negative cocci, 46.9% facultative gram-positive cocci, 10.3% anaerobic gram-negative bacilli, 10.3% anaerobic gram-positive bacilli, 4.8% anaerobic gram-negative cocci, and 0.3% anaerobic gram-positive cocci. In the control group, a total of 153 different bacterial colonies were observed: 55.6% cocci and 44.4% rods. In the test group, a total of 139 different bacterial colonies were observed: 60.4% cocci and 39.6% rods. There was no significant difference between the proportions of cocci and rods ($P = .4$). The test group showed a significantly lower distribution of pathogenic bacteria than the control group ($P = .01$). In this group, the proportion of cultivable anaerobic bacteria was significantly lower than for the control group ($P = .001$). In the control group, the distribution of pathogenic anaerobic gram-negative bacteria was significantly higher than the test group ($P = .008$).

The cultivable subgingival microflora in test and control sites at baseline, 5 weeks, and 13 weeks are shown in Tables 2 and 3. At the beginning of the experimental period (baseline), the predominant cultivable subgingival microflora at control and test sites was composed of facultative/aerobic gram-positive cocci and rods. In the control group, a total of 51 cultivable bacteria were isolated and consisted of predominantly *Haemophilus* spp (15.7%), *Streptococci* (39.2%), and *Neisseria* spp (13.7%). In the test group, a total of 49 cultivable bacteria were isolated and consisted mostly of *Haemophilus* spp (19.6%), *Streptococci* (37%), *Neisseria* spp (15.2%), and diphtheroid rods (15.2%). There was no statistical difference between control and test groups ($P = .556$).

At 5 weeks, a total of 44 cultivable bacteria were isolated in the control group and consisted of predominantly *Haemophilus* spp (11.4%) and *Streptococci* (38.6%), whereas in the test group a total of 49 bacteria were isolated and consisted of predominantly *Haemophilus* spp (17.6%), and *Streptococci* (35.2%). At this time, there was no statistical difference between control and test groups ($P = .948$).

At 13 weeks, there were a total of 58 cultivable bacteria in the control group, consisting predominantly of *Fusobacterium nucleatum* (19.0%), *Prevotella intermedia* (13.8%), *Veillonella parvula* (15.5%), and *Streptococci*

Table 2 Proportion (%) and No. of Bacteria Isolated at Control and Test Sites at Baseline, 5 Weeks, and 13 Weeks

	Baseline		5 weeks		13 weeks	
	Control	Test	Control	Test	Control	Test
Actinomyces naeslundii D2	2.0% (1)	–	–	–	1.7% (1)	2.4% (1)
Actinomyces viscosus D2	3.9% (2)	–	–	–	1.7% (1)	2.4% (1)
Bifidobacterium spp D1	3.9% (2)	2.2% (1)	4.5% (2)	3.9% (2)	1.7% (1)	4.8% (2)
Clostridium spp D1	–	–	9.1% (4)	11.8% (6)	–	–
Diphtheroid bacilli D3	2.0% (1)	15.2% (7)	4.5% (2)	3.9% (2)	5.2% (3)	4.8% (2)
Escherichia coli B2	–	–	4.5% (2)	3.9% (2)	–	–
Eubacterium spp D1*	2.0% (1)	–	–	2.0% (1)	–	–
Fusobacterium nucleatum B1*	2.0% (1)	–	6.8% (3)	5.9% (3)	19% (11)	–
Haemophilus spp B2	15.7% (8)	19.6% (9)	11.4% (5)	17.6% (9)	8.6% (5)	14.3% (6)
Coagulase-negative staphyloccoci C2	3.9% (2)	4.3% (2)	6.8% (3)	2% (1)	–	4.8% (2)
Micrococcus spp C2	–	–	2.3% (1)	2% (1)	–	–
Neisserria spp A2	13.7% (7)	15.2% (7)	4.5% (2)	2% (1)	–	–
Peptostreptococcus C1	2.0% (1)	–	–	–	–	–
Porphyromonas gingivalis B1*	–	–	–	–	3.4% (2)	–
Prevotella intermedia B1*	2.0% (1)	–	2.3% (1)	–	13.8% (8)	–
Propionibacterium granulosum D1	–	2.2% (1)	–	–	–	–
Staphylococcus aureus C2	3.9% (2)	2.2% (1)	4.5% (2)	9.8% (5)	–	–
Veillonella parvula A1*	3.9% (2)	2.2% (1)	–	–	15.5% (9)	4.8% (2)
Streptococci A2	39.2% (20)	37.0% (17)	38.6% (17)	35.2% (18)	29.3% (17)	61.9% (26)
Total (n)	51	46	44	51	58	42
	$P = .556$		$P = .948$		$P = .003$**	

A1 = anaerobic gram-negative cocci; A2 = aerobic gram-negative cocci; B1 = anaerobic gram-negative rods; B2 = facultative gram-negative rods; C1 = anaerobic gram-positive cocci; C2 = facultative gram-positive cocci ; D1 = anaerobic gram-positive rods; D2 = facultative gram-positive rods; D3 = aerobic gram-positive rods.
*Periodontally suspected bacteria.
**Differences are significant by Chi-square analysis ($P < .05$).

Table 3 Statistical Data on the Distribution of Bacteria Isolated in Control and Test Groups for Gram Stain, Atmosphere of Growth, Morphologic Properties, and Pathogenicity at Baseline, 5 Weeks, and 13 Weeks

Type of bacteria	Control (%)	Test (%)	χ^2	*P*
Baseline				
N-PSB	78	82.6	0.321	.571
PSB	22	17.4		
Aerobic/facultative	60	76.0	2.836	.092
Anaerobic	40	23.9		
Gram-positive	62	63	0.11	.916
Gram-negative	38	37		
Cocci	66	60.9	0.272	.673
Rods	34	39.1		
5 weeks				
N-PSB	80	80.4	0.002	.962
PSB	20	19.6		
Aerobic/facultative	62.2	58.8	0.115	.734
Anaerobic	37.8	41.2		
Gram-positive	71.1	70.6	0.003	.955
Gram-negative	28.9	29.4		
Cocci	57.8	51	0.445	.543
Rods	42.2	49		
13 weeks				
N-PSB	65.5	95.2	12.540	.00*
PSB	34.5	4.8		
Aerobic/facultative	43.1	85.7	18.592	.00*
Anaerobic	56.9	14.3		
Gram-positive	39.7	81	16.950	.00*
Gram-negative	60.3	19		
Cocci	44.8	55.2	6.996	.014*
Rods	71.4	28.6		

*Differences are significant by Chi-square analysis ($P < .05$).

(29.3%), whereas in the test group, a total of 41 cultivable bacteria consisted predominantly of *Streptococci* (61.9%) and *V parvula* (4.8%). There was a statistical difference between these groups ($P = .003$). At 13 weeks, the control group showed an increase from baseline values in the proportions of gram-negative anaerobic rods and cocci, which consisted of *F nucleatum* (from 2% to 19%), *P intermedia* (from 2% to 13.8%), and *V parvula* (from 3.9% to 15.5%). *Porphyromonas gingivalis* represented 3.4% of the total cultivable flora at 13 weeks in the control group, whereas this organism was not detected at baseline or at 5 weeks. The test group provided no cultivable gram-negative anaerobic rods at 13 weeks. There were no statistically significant differences in the proportions of either aerobic/facultative cocci or rods compared to baseline in the test group. At 13 weeks the flora in the test group resembled that of baseline, except that were increased levels of gram-negative anaerobic cocci. A small increase in the level of *V parvula* was observed when compared to the baseline data in the test group (from 2.2% to 4.8%).

The proportions of putative periodontal pathogens increased from 22.0% (baseline) to 34.5% (13 weeks) in the control group ($\chi^2 = 3.413$; $P = .181$). The proportions of putative periodontal pathogens decreased from 17.4% (baseline) to 4.8% (13 weeks) in the test group ($\chi^2 = 4.624$; $P = .099$) (Table 3). Although statistically not significant, in the control group there was

Table 4 Proportions of Bacteria Isolated in the Control Groups for Gram Stain, Atmosphere of Growth, Morphologic Properties, and Pathogenicity at Baseline, 5 Weeks, and 13 Weeks

	Bacteria			
Time	PSB	Anaerobic	Gram-negative	Rod-shaped
Baseline	22.0%	40.0%	38.0%	34.0%
5 weeks	20.0%	37.8%	28.9%	42.2%
13 weeks	34.5%	56.9%	60.3%	55.2%
χ^2	3.413	4.722	3.413	3.413
P	.181	.094	.004*	.82

*Differences are significant by Chi-square analysis ($P < .05$).

Table 5 Proportions of Bacteria Isolated in the Test Groups for Gram Stain, Atmosphere of Growth, Morphologic Properties, and Pathogenicity at Baseline, 5 Weeks, and 13 Weeks

	Bacteria			
Time	PSB	Anaerobic	Gram-negative	Rod-shaped
Baseline	17.4%	23.9%	37.0%	39.1%
5 weeks	19.6%	41.2%	29.4%	49.0%
13 weeks	4.8%	14.3%	19.0%	28.6%
χ^2	4.624	8.790	3.451	4.033
P	0.099	0.012*	0.178	.133

*Differences are significant by Chi-square analysis ($P < .05$).

a trend toward increased proportions of putative periodontal pathogens, anaerobic, and rod-shaped organisms from baseline to 13 weeks. In this group, the proportions of gram-negative organisms increased significantly over time (Table 4). Although statistically not significant, in the test group there was a trend toward decreased proportions of putative periodontal pathogens and gram-negative and rod-shaped organisms from baseline to 13 weeks. In this group, the proportions of anaerobic organisms decreased significantly ($P < .05$) over time (Table 5).

Discussion

The stability and esthetic success of restorative dentistry is closely related to maintenance of a healthy periodontium. In the present study, changes in subgingival microflora were detected following the cementation of FPDs with both water-activated polycarboxylate cement and polycarboxylate cement that included CHX.

The bacteria cultivated from periodontally healthy sites consist predominantly of gram-positive facultative rods and cocci (approximately 75%). Small proportions of gram-negative species are also found, most frequently *P intermedia, F nucleatum, Capnocytophaga, Neisseria* spp, and *Veillonella* spp. The bacteria found in chronic gingivitis comprise roughly equal proportions of gram-positive species (56%) (predominantly *Streptococcus sanguis, Streptococcus mitis, Actinomyces viscosus, Actinomyces naeslundii*, and *Peptostreptococcus micros*) and gram-negative species (44%) (predominantly *F nucleatum, P intermedia, V parvula,* and *Haemophilus* and *Campylobacter* spp) as well as facultative (59%) and anaerobic (41%) microorganisms.[17] In our study, at 13 weeks, the subgingival microflora of control sites had a composition that was similar to the microflora of chronic gingivitis. Increased proportions of gram-negative anaerobic bacteria (*F nucleatum, P intermedia, P gingivalis,* and *V parvula*) were identified (51.7%) at 13 weeks. In contrast to control sites, the microflora at test sites was more characteristic of periodontal health and comprised predominantly gram-positive facultative cocci and rods at that time.

In the present study, the tooth preparation margins were completed with a chamfer finish line and crown margins placed at the gingival margin. Müller[5] reported that at teeth with supragingival crown margins and crown margins at the gingival margin, the associated subgingival microflora showed a very similar composition after cementation, as well as after 1 year of observation. Subgingival crown margins possibly allow the colonization of pathogenic organisms, eg, gram-negative anaerobes or spirochetes.[6] Even a high level of oral hygiene cannot prevent an increased loss of attachment associated with subgingivally placed restorations compared to margins placed supragingivally or gingivally. In a human clinical trial, Lang et al[18] indicated that subgingival restorations, especially those with imperfect margins, enhanced plaque accumulation and altered the composition of the subgingival microflora to one similar to that associated with destructive periodontal disease. Following placement of the restorations with clinically "perfect" margins, a microbiota characteristic of gingival health or initial gingivitis was observed. They found a small and statistically nonsignificant increase in black pigmented *Bacterioides* spp, even at "perfect" subgingival restorations. Clinically "perfect" subgingival restorations may create an environment where periodontopathic microorganisms are able to survive and multiply. The results of the present study, which resemble those of Flores-de-Jacoby et al,[6] revealed that the composition of subgingival plaque around teeth with crown margins located at the gingival margin is altered. The microflora at control sites resembled a chronic gingivitis flora, in that it comprised a more pathogenic microbiota with increased proportions of gram-negative anaerobes (eg, *P intermedia, F nucleatum*).

In vitro model studies[8] have demonstrated that several commonly used luting agents (zinc oxide–eugenol, silicophosphate, zinc phosphate) have a bacteriostatic and/or bactericidal action, whereas polycarboxylate and

resin composite do not exhibit such antimicrobial activity. Myers et al[7] revealed that the inherent antimicrobial activity of these cements might diminish as a result of the dilution factor contributed by microleakage. Water activation in particular has an adverse effect on solubility. For water activation, the polyacrylic acid is dehydrated and is added in crystalline form to the powder portion of the cement. To reactivate these crystals, water is added to the powder-crystal combination. This hydration phase evidently leaves an excess of uncombined water, which has a detrimental effect on the solubility of the cement. Schwartzman and Caputo[14] showed that the addition of CHX to polycarboxylate cements increased the antimicrobial power without interfering with its physical properties, and significantly inhibited periodontally suspected bacteria in vitro. Brackett and Rosen[19] examined the antimicrobial activity of zinc phosphate cement by the addition of the antiseptic CHX in vitro. Neither the physical properties (consistency, setting time, and compressive strength) of the cement nor the chemical properties of the CHX altered the modified mix. Despite favorable laboratory results, any presumed reduction in bacterial colonization at the fixed margins should be confirmed in a clinical setting. The reviewed studies were carried out in vitro and reported that further research was required—particularly in vivo. In the present study, the use of 0.12% CHX instead of distilled water resulted in significant changes in the associated subgingival microflora at 13 weeks relative to pre-experimental findings. CHX was chosen because of its remarkable effectiveness, substantivity, safety, long-term antimicrobial properties in preventing plaque formation, and high therapeutic index. At 13 weeks, control group results demonstrated an increase the proportion of periodontally suspected bacteria comprising *F nucleatum* (from 2% to 19%), *P intermedia* (from 2% to 13.8%), and *V parvula* (from 3.9% to 15.5%) compared to baseline values. *P gingivalis* represented 3.4% of the total cultivable flora at this time, whereas this organism was not present at baseline. The test samples did not demonstrate a periodontally suspected gram-negative anaerobic flora at 13 weeks, but a flora typically associated with periodontal health.

This study has demonstrated that the addition of 0.12% CHX to polycarboxylate cement substantially increased the cement's antimicrobial action in vivo and suggests that the placement of FPDs with a CHX-associated adhesive agent resulted in significant changes in the subgingival microflora relative to results for the adhesive agent alone.

This study has shown that the addition of 0.12% CHX to water-activated zinc polycarboxylate cement significantly increased its inhibition of periodontally suspected bacteria up to 8 weeks post-cementation. CHX may enhance the antibacterial potential of water-activated zinc polycarboxylate cement in clinical use. Further studies are needed to determine the longevity of this initially favorable clinical response.

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