# The Interaction of Implant Luting Cements and Oral Bacteria Linked to Peri-Implant Disease: An In Vitro Analysis of Planktonic and Biofilm Growth – A Preliminary Study

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## ABSTRACT

*Background:* There is little consensus on the most appropriate cement to use when restoring a cement-retained, implant-supported restoration. One consideration should be the interaction of pathogenic oral bacteria with restorative cements.

*Purpose:* To determine how oral bacteria associated with peri-implant disease grow in the presence of implant cements.

*Materials and Methods:* Five test cements with varying composition (zinc oxide–eugenol [TBO], eugenol-free zinc oxide [TBNE], zinc orthophosphate [FL], and two resin cements [PIC and ML]) were used to fabricate specimen disks. The disks were submerged in bacterial suspensions of either *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum*, or *Porphyromonas gingivalis*. Planktonic bacterial growth within the test media was measured by determining the optical density of the cultures ($OD_{600}$). Positive controls (media and bacteria without cement disks) and negative controls (media alone) were similarly evaluated. The mean and standard deviations (SD) were calculated for planktonic growth from three separate experiments. ANOVA statistical analysis with post hoc Tukey tests was performed where differences existed ($p < .05$). Selected cement disks (TBO and ML) were further examined for bacterial biofilm growth. Surface bacteria were removed and grown on agar media, and colony-forming units (CFUs) were quantified.

*Results:* Planktonic growth for both *A. actinomycetemcomitans* and *P. gingivalis* was significantly inhibited ($p < .05$) when grown in the presence of cement disks consisting of TBNE, PIC, FL, and TBO. In contrast, neither of these bacteria displayed growth inhibition in the presence of ML cement disks. *F. nucleatum* growth was also significantly inhibited by PIC, FL. and TBO ($p < .05$), but not by ML and TBNE cement disks. CFU counts for the biofilm study for TBO gave minimal and, in some instances, no bacterial adherence and growth, in contrast to ML, which supported substantially greater bacterial biofilm growth.

*Conclusion:* Cements display differing abilities to inhibit both planktonic and biofilm bacterial growth. Cements with the ability to reduce planktonic or biofilm growth of the test bacteria may be advantageous in reducing peri-implant disease. Understanding the microbial growth–inhibiting characteristics of different cement types should be considered important in the selection criteria.

KEY WORDS: *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, implant cements, peri-implantitis, *Porphyromonas gingivalis*

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## INTRODUCTION

As of 2013, the American Academy of Periodontology included excess cement around dental implants as a risk factor for peri-implant disease.[1] Peri-implant diseases are complex, multifactorial conditions associated with inflammatory processes that may affect the soft tissues (perimucositis) and/or hard tissues (peri-implantitis) associated with dental implants.[2] It is well recognized that periodontitis, which has a similar pathogenesis to peri-implantitis, is caused by an over-reactive immune response to a consortium of subgingival, largely anaerobic Gram-negative bacteria.[3–9]

Implant restorations are commonly screw-retained or cement-retained. Cement-retained restorations are often preferred over screw-retained ones because if their greater ease of fabrication, superior aesthetics, and lower cost; because their use avoids the high degree of precision required for optimal fit of screw-retained implants[10]; and because dentists are also more familiar with cementation procedures.[11] However, when comparing the health of cement- versus screw- retained implant restorations, two multicenter studies demonstrated that peri-implant soft tissues responded more favorably to the screw-retained crowns.[12,13] Case reports as early as 1999 documented implant complications as a result of cement extrusion into the subgingival peri-implant tissues,[14–16] and more recently, a link was established between residual excess cement and peri-implant diseases.[17] The author suggested the issue may be due to the cement being a mechanical irritant or a repository of bacteria or both.

Many dental cements have been tested for antimicrobial properties[18–20]; however, these specific microbes relate to dental caries, which is not a disease of titanium implants. Cement selection for implant restorations appears to be arbitrary,[21] and no standard cementation protocol exists for implant restorations, with cements designed for natural tooth restorations commonly chosen.[22] With implant collar sites often approaching 5–7 mm subgingival to adjacent papillae[23] and studies suggesting that implant restoration margins as shallow as 1 mm below the free gingival margin are at risk from residual excess cement,[24] how Gram-negative microbes may affect these sites should be a consideration for the implant-restoring clinician.

The purpose of this study was to evaluate the influence of cement composition on bacterial growth and colonization of specific subgingival Gram-negative bacteria: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*. It is hoped that this study will help provide a rationale for cement selection based on the relative abilities of luting cements to restrict or support the growth of potentially pathogenic bacteria found in the peri-implant environment. The hypothesis tested was that there would be no difference in bacterial growth (planktonic or biofilm) between the tested cement specimens.

## MATERIALS AND METHODS

### Cements

Five dental luting cements were tested: zinc oxide–eugenol (Temp-Bond Original, TBO; Kerr, Orange, CA, USA), eugenol-free zinc oxide (Temp-Bond Non-Eugenol, TBNE; Kerr), zinc orthophosphate (Fleck's, FL; Keystone Industries, Cherry Hill, NJ, USA), and methacrylate-based resin (Premier Implant Cement [PIC; Premier, Plymouth Meeting, PA, USA] and Multilink Implant [ML; Ivoclar Vivadent Inc., Amherst, NY, USA]). Disks 5 mm in diameter and 2 mm thick were made by filling nylon washers with the test cements and placing the assembly between two sterile glass slabs. The cement materials were obtained from sealed packages, and mixing of each cement was done in accordance with the manufacturer's instructions under aseptic conditions.

### Bacterial Strains and Growth Conditions

The three bacterial species examined in this study were *A. actinomycetemcomitans* (strain 43717 from the American Type Culture Collection [ATCC]), *F. nucleatum* (strain 25586 from the ATCC), and *P. gingivalis* (strain 33277 from our culture collection). *A. actinomycetemcomitans* and *F. nucleatum* were grown in TYK broth (30 g/L trypticase soy broth, 5 g/L yeast extract, 1 mg/L vitamin $K_3$). *P. gingivalis* was grown in TYK broth supplemented with filter-sterilized hemin (TYHK broth); hemin was added post-sterilization just prior to inoculation, to a final concentration of 1 μg/mL. The bacterial cultures were grown overnight at 37°C in an anaerobic chamber (5% $H_2$, 5% $CO_2$, 90% $N_2$). Concentration of bacteria within the TYK broth was estimated by measuring the optical density (OD) at 600 nm in a spectrophotometer (Eppendorf, Hamburg, Germany). $OD_{600}$ measurement directly correlates with the concentration of bacteria in liquid culture.

### Measurement of Planktonic Growth

Specimen cement disks were placed in individual wells of a 24-well plate and submerged in 1 mL liquid bacterial culture containing $10^7$ test bacteria ($OD_{600}$ of 1.0 approximately corresponding to $10^9$ bacteria/mL). A well containing 1 mL bacterial culture in the absence of any cement disk served as the positive control. Wells containing sterile liquid broth alone served as the negative control, and wells with cement disks in sterile liquid broth served as a control to confirm that the cement specimens were not contaminated during disk fabrication. Following incubation under anaerobic conditions at 37°C for 48 h, bacterial planktonic growth was determined by measuring the $OD_{600}$ of the bacterial culture from each test well. The bacterial planktonic growth study was conducted three times, each time with newly made media, freshly grown bacterial strains, and freshly made cement disks. The significance of all described comparisons was established using one-way ANOVA on the triplicate samples. A significant difference of $p < .05$ was followed by TSD.

### Measurement of Biofilm Growth

Cement disks were analyzed for presence of biofilm mode of bacterial growth after exposure to bacterial culture for 48 hours. Each disk was removed and washed by swirling in a well containing TYK media alone to remove loosely adherent bacteria. More tightly adherent bacteria were dislodged by placing the cement disk in an Eppendorf tube containing 200 µL TYHK broth and mechanically agitating vigorously for 2 minutes. The 200 µL broth, now containing bacteria that previously constituted the biofilm, was placed on TYHK agar plates and incubated anaerobically for 4 days. Colony-forming units (CFUs) were recorded for each bacterium–cement combination. This experiment was repeated three times.

## RESULTS

### Planktonic Growth

Initially, the ability of the different test cements to inhibit planktonic bacterial growth was examined. A typical test illustrating variations in bacterial growth with test cement disks as well as positive and negative controls is shown in Figure 1. The final bacterial density obtained for each bacterial species as determined by the $OD_{600}$ of each sample well is shown in Figures 2–4. Cement types demonstrated significant differences in their ability to inhibit bacterial growth. For example, TBO, in contrast to ML cement, displayed potent bacterial growth inhibition against all three bacterial species (Figures 2–4). In fact, ML was least able to affect bacterial growth of each of the bacteria tested. There was no significant difference in bacterial growth between ML disks and the positive control, where no cement disk was added to the test well. Both *A. actinomycetemcomitans* and *P. gingivalis* displayed similar levels of growth inhibition for the other cements examined as well. In contrast, cement disks containing TBNE inhibited growth of *A. actinomycetemcomitans* and *P. gingivalis* but did not inhibit the growth of *F. nucleatum*, demonstrating a difference in the susceptibility of this bacterial species from *A. actinomycetemcomitans* and *P. gingivalis*.

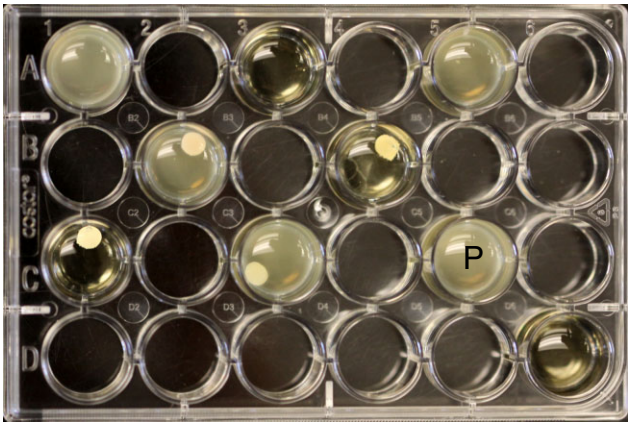


**Figure 1** Example of test wells following 2 days of anaerobic incubation for determining planktonic growth of *Fusobacterium nucleatum*. Positive and negative controls are highlighted (P and N, respectively).

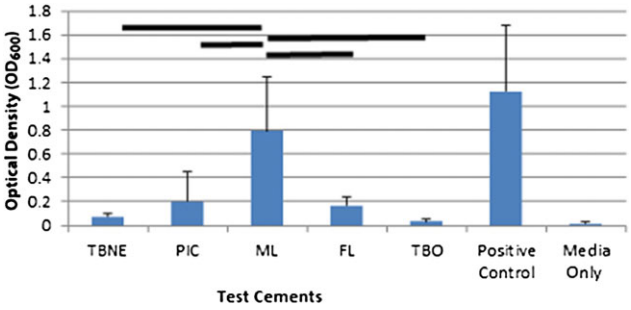


**Figure 2** Planktonic growth measurement by $OD_{600}$ test values for *Aggregatibacter actinomycetemcomitans* in the presence of different cement disks; means (SDs) from three independent experiments. Solid horizontal lines denote a significant difference in growth between cements ($p < .05$). FL = Fleck's Cement; ML = Multilink Implant; PIC = Premier Implant Cement; TBNE = Temp-Bond Non-Eugenol; TBO = Temp-Bond Original.

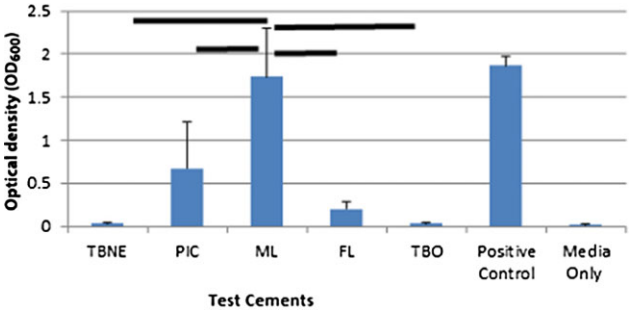


**Figure 3** Planktonic growth measurement by $OD_{600}$ test values for *Porphyromonas gingivalis*. Solid horizontal lines indicate differences between cements ($p < .05$). Means (SD) indicated. Note positive control not significantly different to ML ($p > .05$). FL = Fleck's Cement; ML = Multilink Implant; PIC = Premier Implant Cement; TBNE = Temp-Bond Non-Eugenol; TBO = Temp-Bond Original.

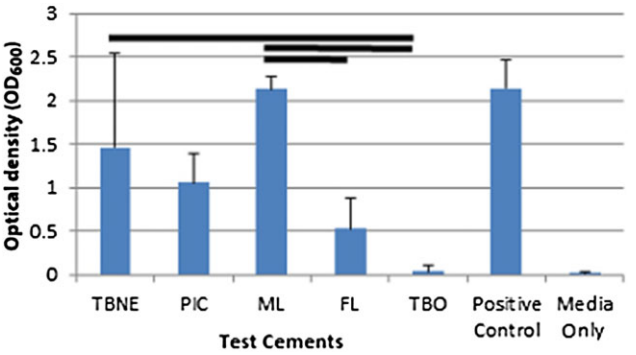


**Figure 4** Planktonic growth measurement by $OD_{600}$ test values for *Fusobacterium nucleatum* on different cements. Solid horizontal lines indicate differences between cements ($p < .05$). Means (SD) indicated. Note positive control not significantly different to ML ($p > .05$). FL = Fleck's Cement; ML = Multilink Implant; PIC = Premier Implant Cement; TBNE = Temp-Bond Non-Eugenol; TBO = Temp-Bond Original.

Although the general trend was for all cements except for ML to inhibit the growth of *A. actinomycetemcomitans* and *P. gingivalis*, in contrast, TBNE did not inhibit the growth of *F. nucleatum* compared with the positive control. This demonstrates a difference in the susceptibilities of *F. nucleatum*, *A. actinomycetemcomitans*, and *P. gingivalis*.

### Bacterial Biofilm Growth

Next, the ability of ML and TBO cements to support bacterial biofilm growth was determined. These cements were chosen for further study because they demonstrated the least and greatest ability to inhibit planktonic growth, respectively. Biofilm growth was determined by counting the number of bacteria able to stably adhere to the cement disks after incubation. A typical plate count assay is shown in Figure 5. Three separate experiments were performed for each cement with each bacterial species. *P. gingivalis* and *A. actinomycetemcomitans* adhered to the ML cement discs to such an extent that in all three experiments there were too many bacteria present to make an accurate assessment of extent of growth (>5,000, indicated as too numerous to count in Table 1). In contrast, TBO had significantly fewer *P. gingivalis* and *A. actinomycetemcomitans* adhering to the cement disks. This represents a difference in bacterial growth on the ML and TBO disks of at least a hundredfold. The ability of *F. nucleatum* to adhere to the ML and TBO disks revealed a similar but less extreme pattern. These data demonstrate that ML and TBO cement disks display a significant difference in their ability to promote bacterial adherence and biofilm formation. The data indicate that although TBO was superior to ML in preventing biofilm growth, it may have shown a selective advantage for *P. gingivalis* biofilm formation, although further studies will be necessary to determine if this trend is significant.

## DISCUSSION

The planktonic growth results indicated distinct differences, which is highly suggestive of large variations in the way cements interact with Gram-negative bacteria. The general trend for most of the test cements was to inhibit planktonic growth; the most consistent results were found with TBO. The inhibitory effect of this

**TABLE 1 Colony-Forming Unit Count For Cement Disks and Control**

| Cement | *Aggregatibacter actinomycetemcomitans* | *Porphyromonas gingivalis* | *Fusobacterium nucleatum* | Media Only (Negative Control) |
|---|---|---|---|---|
| ML | TNTC, TNTC, TNTC | TNTC, TNTC, TNTC | 760, 11, 264 | 0 |
| TBO | 0, 0, 73 | 132, 51, 12 | 0, 2, 10 | 0 |

TNTC = too numerous to count (>5,000).

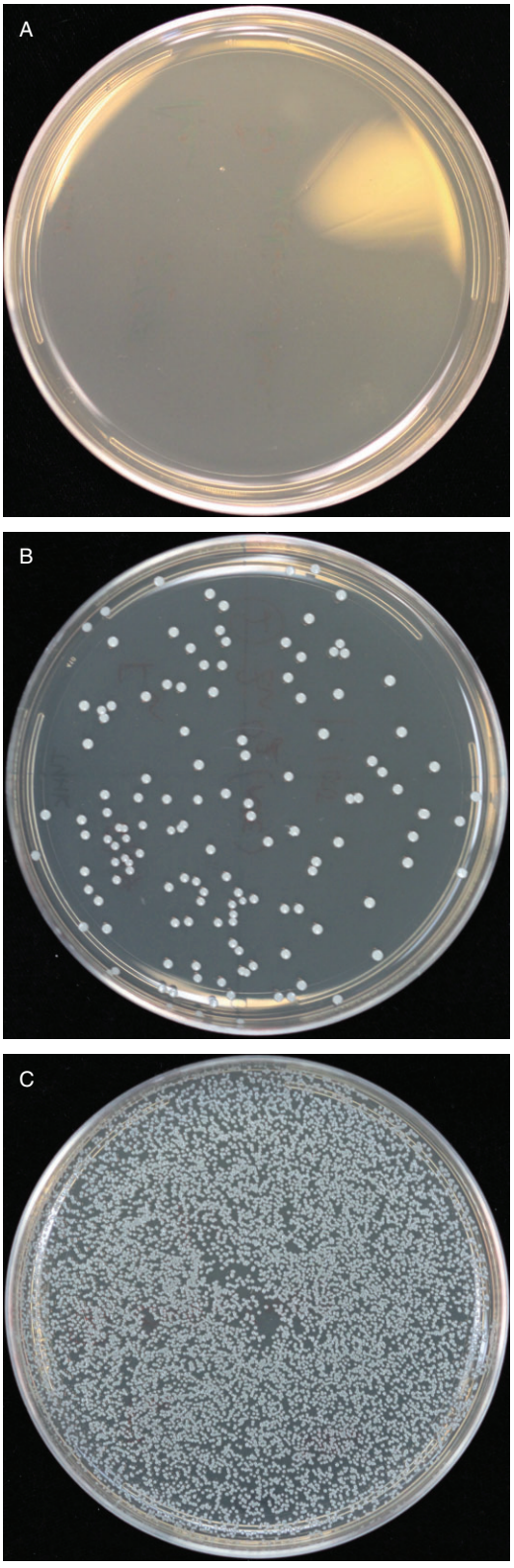


**Figure 5** Examples of agar plates with colony-forming units (CFUs) obtained from cement disks sampled for biofilm growth. *A*, no CFUs (TempBond Original with *Aggregatibacter actinomycetemcomitans*). *B*, 132 CFUs (TempBond Original with *Porphyromonas gingivalis*). *C*, CFUs too numerous to count (Multilink with *A. actinomycetemcomitans*).

cement was also noted when evaluating the biofilm growth (manifested as a low number of CFUs detected). The effect on microbial growth is likely to be a direct result of the formulation of this material, which contains both zinc oxide and eugenol.

The element zinc within TBO may have contributed to the antimicrobial effect, as similar inhibition (but to a lesser degree), with some selectivity with regard to the bacteria, was also noted with the other two zinc-containing cements, FL and TBNE. Zinc is known to have inherent antimicrobial properties, but this cannot account fully as to why TBO appeared to be so potent at inhibition; it is likely the combination with eugenol also played a role. Eugenol is a liquid extract from certain essential oils, especially from clove oil, nutmeg, cinnamon, and bay leaf. It is well known for its versatile pharmacological actions, with anti-inflammatory, anesthetic, antioxidative, and antibacterial properties.[23]

For the biofilm growth study, TBO performed well in preventing bacterial adhesion, especially with *A. actinomycetemcomitans* and *F. nucleatum*, where in some instances the CFU count was zero. This was in stark contrast to ML cement disks. Bacterial adhesion to surfaces is governed not only by chemical composition but other factors such as roughness and surface energy. To try and standardize the macro surface details, the cements were all fabricated in the same manner, that is, between flat, sterile glass surfaces. The adhesion of cement to the disks would clearly be confounded by material and physical factors; however, an attempt was made to minimize this. The chemical content of the cement would also contribute to the test bacteria adhering, and it it is also possible that the lack of adhesion to TBO was a direct result of the zinc and eugenol combination in TBO. This requires further evaluation.

The need for the restorative clinician to understand the impact of cement selection is critical. Wilson identified a positive relationship between excess cement and peri-implant disease. He found excess cement in 81% of cases that showed signs of peri-implantitis. The average time for signs of peri-implantitis to appear was 2.93 years, and although it was speculated that this was due to bacterial colonization of cement, no specific relationship with the cement type was established.[17]

This is particularly relevant for a patient who is periodontally susceptible, as periodontally susceptible patients present a greater risk of peri-implant disease.[1,4]

Consistent with this is the observation that in most peri-implantitis cases, the composition of the flora is similar to that of the subgingival flora of chronic periodontitis.[3] It has also been demonstrated that the bacteria in cases of partially edentulous implants may be more pathogenic (especially Gram-negative rods and spirochetes) than in the fully edentulous case, indicating a possible seeding mechanism from tooth pocket to implant crevice.[2]

Most dental cements have been produced for the natural tooth, where protection from caries-producing organisms is advantageous.[18] Cement selection for implant restorations is mostly arbitrary and frequently dictated by the retentive capability of the cement rather than its bacterial interations.[17] There has appeared no research to date on the interaction of cement used for restoring implants with the Gram-negative microbes that have the potential to cause peri-implant disease. The authors of this article believe this to be the first such research paper and believe further studies are merited.

Limitations of this comparative study include that the sample size was small and that the experimental data collection was intensive and so had to be limited in number of tests. Only three bacterial species were evaluated, and only five cement types. In this, a preliminary study, the bacteria were tested in isolation, whereas in vivo bacteria exist as a biofilm within a complex multimicrobial ecosystem; it is hoped further studies would include this. The methodology of bacterial removal from the cement disks and measurement may result in errors, as some precipitation on the glass well may occur; however, as a comparative study, it was assumed the amount of precipitation would be the same for all cements. Vortexing and sonic bathing may not remove all bacteria, depending upon the physical form of the surface; vortexing also has the potential to destroy bacteria, which may not then be viable for the agar media.

Different media may be employed that may result in differences in comparative growth; how the cements relate to one another with respect to inhibition may alter. It was not possible to control the surface texture of the cements, apart from fabrication between glass slabs; polishing would have potentially altered the surface chemistry, and some disks would break down. The cement disks were only incubated for a short period of time; it is possible the potency of the inhibitory effect may change over longer periods. This initial in vitro study was a relative or comparative study involving basic methodology but should provide a basis for further research. It is hoped future studies may involve other, more sophisticated methods, including confocal or polymerase chain reaction tests, for detecting microbial growth both on and around cements.

## CONCLUSION

Within the limitations of this study, the null hypothesis was rejected, with cements showing significant differences with respect to bacterial growth, both planktonic and biofilm. Zinc-based cements (TBO, TBNE, and FL) appear to have an advantage, especially compared to ML, in reducing the concentration of *A. actinomycetemcomitans*, *P. gingivalis*, and *F. nucleatum* in media broth. ML did not show any significant reduction in bacterial planktonic growth compared with the positive control for any of the bacterial species (*A. actinomycetemconitans*, *P. gingivalis*, *F. nucleatum*), whereas all other cement specimens significantly inhibited growth. TBO appeared to have the greatest resistance to bacterial biofilm development compared with ML.

## ACKNOWLEDGMENT

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