Antibacterial activity and tensile strength of provisional cements modified with fluoride-containing varnish

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Objective: To test three noneugenol provisional cements (TempBond NE, RelyX Temp NE, and Freegenol) for their antibacterial properties and to test the hypothesis that addition of fluoride varnish confers antibacterial properties on these provisional cements without compromising their tensile strength. **Method and Materials:** A total of 576 cylindrical samples were prepared (96 of each of six types) from three noneugenol provisional cements, both unmodified and modified by the addition of 5% w/w Duraphat fluoride varnish. The samples were aged in saline that was replaced twice a week for up to 90 days. Twenty-four samples of each type were removed from the saline at 1, 7, 30, or 90 days and assessed for antibacterial properties against *Streptococcus mutans* by using an agar diffusion test (n = 12) and tensile strength by using a diametral tensile strength test (n = 12). Data were subjected to one- and three-way ANOVA, the Tukey honestly significant difference test, and *t* test at a significance level of .002 ($P < .002$). **Results:** TempBond NE had no antibacterial activity in its unmodified form but showed antibacterial activity when modified by the addition of 5% w/w fluoride varnish. Freegenol had the highest antibacterial activity in its unmodified form, which was not altered by the addition of the varnish. RelyX Temp NE had mild antibacterial activity that was slightly enhanced by adding the varnish. Adding the varnish reduced the tensile strength of TempBond NE and Freegenol, but did not affect the tensile strength of RelyX Temp NE. **Conclusion:** Antibacterial activity was observed for the unmodified form of Freegenol and TempBond NE after the addition of the fluoride varnish. The addition of the fluoride varnish reduced the tensile strength of both TempBond NE and Freegenol. *(Quintessence Int 2013;44:107–112)*

Key words: antibacterial, cement, diametral tensile strength, fluoride varnish, provisional cement, *Streptococcus mutans*, zinc oxide–eugenol

Provisional cementation of provisional or permanent crowns and partial dentures is often applied during prosthodontic treatment. Such cementation is usually applied for short periods of time. Nevertheless, prolonged provisional cementation may be required in some cases, including follow-up periods to verify the endodontic prognosis of an abutment with periapical lesion or when adjunctive orthodontic or periodontal treatments are required prior to final prosthetic treatment. Waiting for osseointegration of implants placed under the pontics of provisional partial dentures is another instance requiring prolonged provisional cementation.

During such long periods, loss of the cement's seal may occur, allowing bacteria to penetrate and grow in the space between

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the crown and abutment tooth. This may occur especially with long acrylic provisional partial dentures that may undergo elastic deformation under functional masticatory forces.[1] Thus, antibacterial properties of provisional cements could be beneficial in the prevention of caries.

Previous attempts to create provisional cements with antibacterial and/or anticariogenic properties included the addition of stannous fluoride, chlorhexidine diacetate, or insoluble polyethyleneimine nanoparticles with no adverse mechanical effect to the cement.[2-4] On the other hand, some other additives do have an adverse effect on the mechanical properties of provisional cements.[5]

Fluoride-containing varnish has been already added to certain provisional cements and has shown to increase the retention of and reduce marginal leakage under crowns cemented with the modified cements.[2,6] Therefore, it was of interest to determine whether the addition of such fluoride varnish would also affect the antibacterial properties or tensile strength of provisional cements.

Most zinc oxide-based provisional cements currently in use avoid the traditional eugenol component.[7] Three such noneugenol, zinc oxide-based cements were chosen for the present study.

The tensile strength of a provisional cement should be sufficient to prevent accidental dislodgement of the crown but not be too high so as to allow safe dislodgement of the crown without causing damage to the abutment tooth or restoration.[8,9] The tensile strength of provisional cements should be lower than that of permanent cements such as zinc phosphate cement, which typically has tensile strength values ranging from 3.1 to 4.5 Megapascals (MPa).[10,11] Diametral tensile strength is usually used to evaluate the strength of such brittle materials.[12,13]

The present study was designed to test three noneugenol provisional cements for their antibacterial activity and to test the hypothesis that the addition of small amounts of fluoride varnish to these cements will confer antibacterial properties without compromising their mechanical properties.

METHOD AND MATERIALS

This study was designed to test the antibacterial activity and tensile strength of three commercially available provisional cements, both in their unmodified form and after the addition of a fluoride varnish.

Provisional cements

Three provisional cements were used: TempBond NE (Kerr), RelyX Temp NE (3M ESPE), and Freegenol (GC). The cements were used in either their unmodified form or modified form after the addition of Duraphat fluoride-containing varnish (2.26% sodium fluoride [NaF] w/w, Colgate Oral Pharmaceuticals). The cements were mixed according to their individual manufacturer's instructions. The fluoride varnish was added, when indicated, at 5% w/w, during mixing.

Sample preparation

A polytetrafluoroethylene (PTFE) mold was prepared in the form of a plate with 6-mm diameter cylindrical holes equipped with precision removable plugs. When the plugs were in place, the mold had 12 cylindrical wells that were 3 mm in height and 6 mm in diameter. A slight excess of each cement sample was introduced into the mold and a flat PTFE plate applied to its upper surface. The cement was initially allowed to set at 37°C and 100% humidity for 1 hour. After the initial setting, the flat upper plate and the lower plugs were removed, and each sample was extruded from the mold using a piston with a flat surface and a 6 mm diameter. Each sample was then checked for integrity of its surface at 10× magnification, and samples with nonperfect surfaces were discarded and replaced by similar samples with no surface defects. Ninety-six samples were prepared from each of the modified and unmodified cements, resulting in a total of 576 samples.

Bacteria

Streptococcus mutans strain 27351M, an anaerobic, bacitracin-resistant strain, was grown anaerobically from frozen cultures in a brain heart infusion (BHI) liquid medium (Difco, Becton & Dickinson) containing bacitracin (5 µg/mL, Sigma). The bacteria were passed twice in culture, checked microscopically for possible contamination (100×, phase contrast) and used to create a bacterial lawn on the surface of BHI agar plates containing bacitracin (5 µg/mL). A 100-µL drop of a 10^9 CFU/mL bacterial suspension from an overnight culture was first placed and spread evenly on the surface using a Drigalsky rotating table and applicator. The procedure was repeated three times to ensure the uniform spread of the bacteria to form a lawn, with a total of 300 µL of the suspension, after which the inoculum was allowed to dry for 3 minutes. All procedures were performed in an anaerobic chamber.

Aging of the samples

The samples were subjected to aging in 2 mL of phosphate-buffered saline containing bacitracin (5 µg/mL) at 37°C, which was replaced twice a week for up to 90 days. Samples were aged for 1, 7, 30, or 90 days. At each testing time point, 24 samples were retrieved from the aging liquid, dipped twice in sterile saline, dried with sterile absorbent paper, and used in the tests.

Agar diffusion test

For each time point, 12 samples of each cement type were placed on a freshly prepared bacterial lawn (four samples per agar plate).

The plates were then incubated anaerobically for 48 hours to allow the development of a uniform lawn of colonies. Inhibition of bacterial growth was easily detected as a clear circular area around a cement sample. The size of each inhibition zone was determined by measuring the largest diameter of the clear zone and adding it to the diameter perpendicular to it. This sum was divided by 2, and the diameter of the sample (6 mm) was then subtracted from it. The result (Δ inhibition zone) was expressed in mm and used as a parameter to compare the inhibitory potential of the cement samples. Large Δ inhibition zone indicated high antibacterial activity of the sample, while 0 Δ (no inhibition zone) indicated no antibacterial activity.

Diametral tensile strength test

Twelve samples were tested from every cement type using the diametral tensile strength test. Each sample was placed on a flat surface and tested using an Instron Universal Testing Machine (Instron 4502). A flat head was used to apply force to the upper surface of the sample, using a 10 KN load cell at a crosshead speed of 1 mm/minute, until failure was observed.

The force required to fracture the sample was registered and the diametral tensile strength (DTS) was calculated as follows:

$$DTS = \frac{2P}{\pi DT}$$

where P is the force required to break the sample, D is the diameter of the sample, and T is the height of the sample. D and T were constant in the present study at 6 and 3 mm, respectively. The results were expressed in MPa = N/mm².

Statistical analysis

The results were analyzed by one- and three-way analysis of variance (ANOVA) with the Tukey post hoc test with SPSS 10.0 (IBM) software; the significance level was set at 0.05. The effect of Duraphat incorporation for each aging period was analyzed by using the *t* test at a significance level of 0.02, in accordance to the Bonferroni test.

RESULTS

The unmodified cements varied in their antibacterial activity. Freegenol was inhibitory to bacterial growth, and this activity was enhanced by aging in saline (Fig 1). On the other hand, TempBond NE had negligible antibacterial activity, which was unaffected by aging. Unmodified RelyX Temp NE was less antibacterial than unmodified Freegenol ($P < .001$) (Fig 1).

Adding Duraphat varnish to TempBond NE resulted in antibacterial activity that was lower than that of modified Freegenol ($P < .001$) but similar to that of modified RelyX Temp NE (see Fig 1). Addition of varnish to Freegenol and RelyX Temp NE did not affect their antibacterial activity; however, the antibacterial activity of RelyX Temp NE was slightly elevated.

The unmodified cements varied in their tensile strength. TempBond NE and Freegenol had similar tensile strengths that

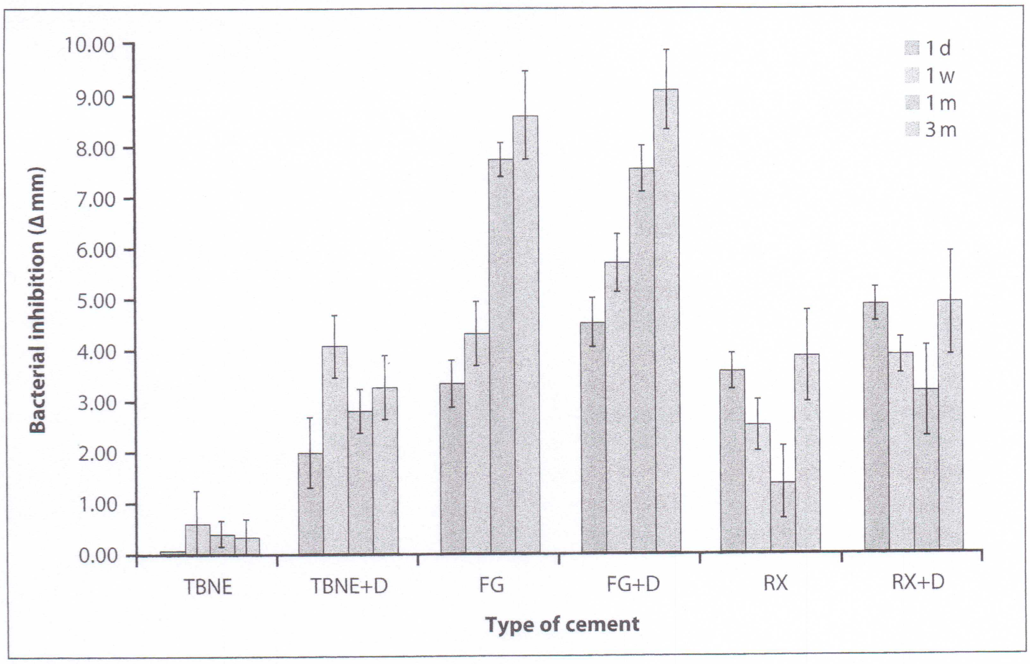

Fig 1 Effects of aging on the inhibition of bacterial growth by the unmodified and fluoride-varnish-modified provisional cements. TBNE, TempBond NE; FG, Freegenol; RX, RelyX Temp NE; +D, modified with Duraphat varnish. Each bar represents the mean (± SEM) of the Δ inhibition zone of 12 samples.

were higher than that of RelyX Temp NE ($P < .001$) (Fig 2). Aging in saline for up to 90 days did not affect the strength of unmodified Freegenol and TempBond NE cements (see Fig 2).

Addition of the varnish to the cements negatively affected the tensile strength of all three cements ($P < .001$). The initial strength was reduced after one day of aging by 52%, 57%, and 37% for TempBond NE, Freegenol and RelyX Temp NE, respectively (see Fig 2). Further aging had no significant effect on the strength of Freegenol or RelyX Temp NE. On the other hand, the strength of varnish-modified TempBond NE gradually increased with aging time ($P < .001$) (Fig 2).

DISCUSSION

Bacterial leakage and growth under loosened, provisionally cemented crowns and partial bridges can cause caries, especially during longer provsional cementation periods. A provisional cement with long-term antibacterial and anticariogenic activities may be beneficial in such conditions. Hence, a fluoride-containing varnish was tested as a potential additive to provisional cements. The 5% (w/w) sodium fluoride content of the varnish may confer antibacterial activity and reduce demineralization of the dentin of the abutment in case of caries development.

The loosening of a provisionally cemented provisional crown or partial denture results in the creation of a unique type of ecologic niche. Such a narrow, mechanically protected space may be filled with saliva, allowing bacterial biofilm growth that is not readily subjected to the pH-neutralizing effect of freely flowing fresh saliva. Hence, dentin caries may often develop in these conditions, especially in cases in which other abutments keep the loosened partial denture in place.

Provisional cements with antibacterial activity may be beneficial in such cases since they may serve as slow-release devices, maintaining effective antibacterial con-

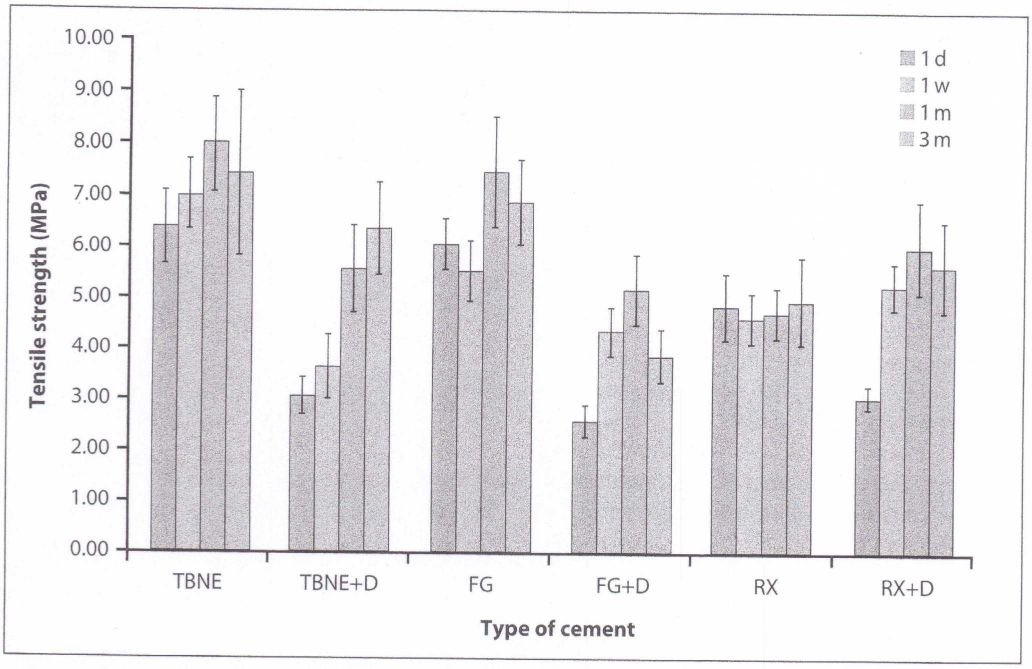

Fig 2 Effects of aging on diametral tensile strength (DTS) of the unmodified and fluoride-varnish modified provisional cements. TBNE, TempBond NE; FG, Freegenol; RX, RelyX Temp NE; +D, modified with Duraphat varnish. Each bar represents the mean (± SEM) diametral tensile strength of 12 samples.

centrations in the liquid in the gap, thereby preventing bacterial growth.

The agar diffusion test (ADT) was chosen for the present study since it is the best representation of the condition above: It is a bio-assay that measures the diffusion of the antibacterial agent into its surrounding environment. As such, it is more relevant for the issue at hand than the direct contact test (DCT), which is more suitable for testing the antibacterial activity of restorative composite resin surfaces.[14]

The three commercially available provisional cements tested in this study varied in their antibacterial activity and mechanical characteristics. Although the limited reported composition for these cements by the manufacturers is rather similar, substantial differences were seen in their antibacterial activity. TempBond NE had almost no antibacterial activity, whereas Freegenol had a substantial antibacterial effect that increased with aging. Such differences may suggest that Freegenol is likely to contain some antibacterial agent that is not reported in its publicly available formulation.

The results of the present study indicate that the antibacterial activity of provisional cements should be further investigated and that this information should be made available to users in the manufacturer's instructions. This would allow clinicians to make educated decisions regarding their preferred type of cement. Updates to the American Dental Association (ADA) specification no. 30 may also be required to ensure that manufacturers include such essential information in their instructions.

To the best of the authors' knowledge, this study represents the first report of antibacterial activity and tensile strength of provisional cements modified with fluoride-containing varnish.

The results demonstrate that the addition of fluoride varnish resulted in the appearance of antibacterial activity for TempBond NE cement, but did not alter the antibacterial activity of Freegenol cement. Hence, it may be of interest to determine whether the antibacterial activity of the modified TempBond NE was a result of the fluoride or some other antibacterial agent(s) in the varnish. It may also be worthwhile to determine whether the antibacterial activity observed for both the unmodified and modified cements has a broad spectrum or is limited to *Streptococcus mutans*. Nevertheless, such investigations were beyond the scope of the present study.

The diametral tensile strength of TempBond NE and Freegenol, both noneugenol cements, was drastically reduced by the addition of the varnish. This finding is consistent with previously published data, which show that the addition of Duraphat to Freegenol reduced the retention of the provisional crowns cemented with this agent.[2] However, the same study[2] showed that the addition of Duraphat increased the retention of provisional crowns cemented with TempBond, which is in agreement with the present finding that the strength of the modified TempBond cement increased with aging, reaching the strength of the unmodified cement after 90 days.[2] These results suggest that the relationship between crown retention and diametral tensile strength of provisional cements needs further investigation.

Taken together, the present results and those of former studies[2,3,6] indicate that the addition of Duraphat varnish to TempBond NE might be beneficial as related to antibacterial properties, retention, and marginal sealing of provisionally cemented crowns.

CONCLUSION

Within the limitations of the in vitro assays described in this report,

- Freegenol had high antibacterial activity against *Streptococcus mutans*, whereas TempBond NE lacked such activity and RelyX Temp NE had moderate activity against this bacterium.
- Adding a small amount of fluoride varnish conferred antibacterial activity on TempBond NE, whereas the effect was smaller or negligible for RelyX Temp NE and Freegenol, respectively.
- The addition of the varnish substantially reduced the tensile strength of TempBond NE and Freegenol, with a smaller effect on RelyX Temp NE.

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