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Research Article

Resistance of a Novel Alkaline Endodontic Cement to Microbial Penetration

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Abstract

The ability to resist microbial penetration is an important property of a root filling material. Alkaline cements can exert antimicrobial actions through the release of hydroxyl ions, and this characteristic supports their use in endodontics in situations where maintaining a seal against coronal leakage is essential. This study tested the resistance to microbial penetration of a novel calcium hydroxide cement (Supercal) and compared this to gutta percha with an epoxy resin sealer (AH Plus) and to a calcium hydroxide medicament (Pulpdent paste) over 60 days of continuous exposure to human saliva. The novel cement was superior to both the comparison materials, and more samples survived without showing microbial penetration through the root filling.

Keywords: Alkaline Cement; Calcium Hydroxide; Root Filling; Coronal Leakage; Gutta Percha; Obturation; Microbial Penetration

Abbreviations

BHI: Brain Heart Infusion; EDTA: Ethylenediaminetetraacetic Acid; MTA: Mineral Trioxide Aggregate.

Introduction

A key objective of endodontic treatment is to achieve complete obturation and sealing of the space of the root canal system, to prevent the ingress of fluids. In anterograde endodontics, the final phase of treatment, involving obturation of the root canal space, is typically undertaken using a combination of materials. A common technique involves the use of gutta percha points (made from natural rubber latex, with zinc oxide and a radiopacifier), which is condensed either cold or warmed. To fill voids, the gutta percha is used in combination with a sealer, such as AH26 or AH Plus® (Dentsply Maillefer, Tulsa, OK, USA). As well as reducing voids, the sealer can provide adhesion to the walls of the root canal, which improves the integrity of the root filling.

Despite the widespread use of gutta percha with epoxy resin sealers in endodontics, several issues with this approach have been identified, including the potential for long term staining (from the radiopacifying agents used in the sealer) [1], porosity in the sealers [2], and the inherent complexity of the obturation technique. Materials used for obturation in endodontics must have low solubility and display long-term dimensional stability [2]. Because of polymerization shrinkage, microscopic voids along the root canal wall will remain in the treated canal, and these can be a pathway for reinfection should there be coronal leakage, for example from loss of the coronal restoration or recurrent caries in the tooth.

To overcome such problems, an alternative approach would be the injection of a single material that would be applied in a viscous state (with user controllable setting properties) and then would set to a rigid form, be easily removed at a later date should preparation of a dowel space for a post be needed, be radiopaque but non-staining, and either be antimicrobial or otherwise able to resist the ingress of bacteria from events that allow coronal leakage to occur. By achieving a fluid proof seal, such a bulk filling material would inhibit communication between the oral cavity and the root canal system, and it would isolate any remaining microorganisms within the root canal system, preventing nourishment from the oral cavity or from periapical tissues reaching microorganisms within the root canal system.

Novel alkaline endodontic sealers based on calcium hydroxide or on mineral trioxide aggregate (MTA) have attracted recent interest. The alkaline pH that these materials can create results in useful antimicrobial activity [3,4]. A novel alkaline hydraulic cement (Supercal, Ozdent, Sydney, Australia) has been developed as a bulk filling material suitable for use in endodontics. It is able to resist microbial penetration [5] yet can be removed readily for later dowel space preparation [6]. The primary active ingredients

are calcium hydroxide and glycerol. These participate in dual setting reactions, which form rigid calcium glyceroxide complexes in a matrix of linear, cyclic and hyperbranched polymers of glycerol. Trapped within the matrix is also excess calcium hydroxide dissolved in traces of water that has been generated by the glycerol polymerization reaction. The set material can release hydroxyl ions when placed contact with water or saliva, providing therapeutic effects [7,8].

On the basis of its chemical properties, this novel alkaline cement should have passive antimicrobial properties and be more resistant to micorbial penetration from coronal leakage than a traditional root filling of gutta percha and epoxy resin sealer. The present study was designed to test this notion, using the well established two chamber model of Torabinejad., *et al.* [9] where treated roots are exposed on their cut coronal surface to continuous challenge from salivary bacteria. As a positive control for the antimicrobial actions of calcium hydroxide itself, a well known calcium hydroxide medicament paste (Pulpdent paste, Pulpdent Corporation, Waterton, MA, USA) was used for purposes of comparison. A recent study by Teoh *et al*. [5] used this same microbial penetration model, using comparing Supercal to MTA cement, a material that releases calcium hydroxide as part of its setting reaction.

Materials and Methods

A total of 100 extracted human maxillary molar teeth were collected from an oral surgery clinic, and used with human ethics approval (approval #1311). Each was assessed visually assessed with and without magnification in normal and transilluminated light, to identify and exclude teeth with cracks or defects. To remove traces of periodontal ligament, the teeth were first placed in a 5.25% sodium hypochlorite solution for 15 minutes, then the soft tissue remnants removed from the root surface by thorough debridement with a periodontal curette. The teeth were then stored in 0.15M phosphate buffer solution (pH 7.2) containing 0.1% thymol, until preparation of the root canals.

The teeth were sectioned below the level of the cemento-enamel junction to remove the palatal roots, giving a constant root length of 10 mm. The coronal cut surface of the palatal roots was flattened with a diamond bur. All sectioned roots were of comparable size, with single canals. The patency of all root canals was visually confirmed by placing an ISO 10 K file (Dentsply Maillefer, Switzerland) to a length of 1 mm beyond the apical constriction. The 100 roots were assigned randomly to one of the five groups (N = 20 roots per group), as follows.

- Group A: Positive control: roots with no obturation
- Group B: Negative control: roots sealed both coronally and apically with sticky wax
- Group C: Gutta percha with AH Plus, using the cold lateral condensation technique
- Group D: Pulpdent calcium hydroxide medicament paste
- Group E: Supercal alkaline cement.

The canal of each root was prepared to a working length 1 mm short of the apical foramen to a 0.04 taper ISO 40 master apical file. Rotary nickel titanium files (ProTaper Next™, Dentsply Maillefer, Switzerland) were used to prepare the canal in a crown down method. A standardized irrigation protocol was used, which included 1% sodium hypochlorite (Endosure Hypochlor 1% solution™, Dentalife, Ringwood, Melbourne, Australia) during preparation, followed by a 2 minute rinse with 15% w/v ethylenediaminetetraacetic acid (EDTA) with 0.85% w/v cetrimide (Endosure EDTA/C 15% solution™, Dentalife, Ringwood, Melbourne, Australia) at the end of preparation, to remove smear layer. The canals were then dried using paper points, and the apical patency of the canals was reconfirmed with an ISO 10 K file. At this stage, the treated roots were also examined once more to ensure that there were no cracks or other defects present. The designated material was then placed directly into the prepared canal. All treatments were standardized, and were undertaken in a single session by the one clinical operator, in order to remove effects of operator variability.

The obturated roots were then fixed into place within the challenge system, using sticky wax. The experimental model is shown in Figure 1. The details of the model have been described previously [5,9]. In brief, a total of one hundred 5 mL plastic specimen containers were used. A high speed handpiece was used to prepare holes approximately 6-8 mm in diameter in the specimen container screw-tops. A second screw-top was inverted, and a hole prepared in the bottom, and the lid placed above the specimen container, forming a reservoir. The two screw-tops were then joined together with sticky-wax. A root was then placed in the hole in each specimen container and secured with further sticky wax, on both the inner and outer surfaces of the specimen containers. Pink nail varnish was used to coat all the outer surface of the roots, except for the cut coronal surface and the apical third. This was done to seal off any undetected lateral canals or microscopic cracks in the root that would allow microbial penetration. The entire test assembly was then sterilized using gamma irradiation (25 kiloGrays over 24 hours) at a commercial gamma sterilisation facility (Steritech, Narangba, Qld, Australia).

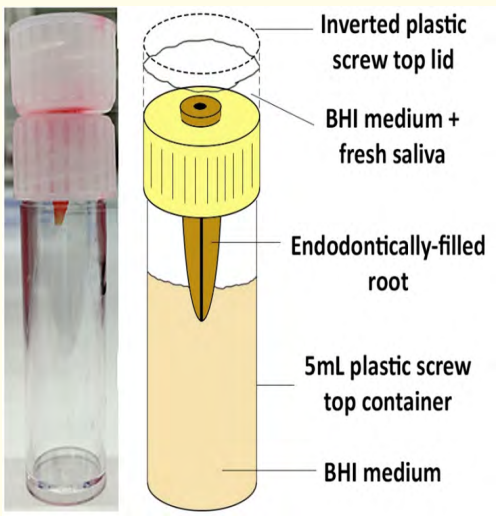

Figure 1: Photograph and schematic of the test system, based on Teoh., *et al.* [5].

The uppermost inverted lid served as a reservoir for the salivary inoculum, which was in contact with the coronal surface of the treated root. Once the whole assembly had been gamma sterilized, sterile growth medium was then placed into the lower chamber. A fresh salivary inoculum was placed in the uppermost reservoir every day.

The growth medium used to detect the penetration of viable bacteria through the root canals was Brain Heart Infusion (BHI) medium. This medium was prepared from stock powder (BBL Brain Heart Infusion™, Becton Dickinson, Franklin Lakes, NJ, USA) and autoclaved. Under sterile conditions in a laminar flow chamber, the sterile BHI medium was added into the lower part of the specimen containers so that the apical portion of each root was submerged by several millimetres. Prior to loading in the salivary inoculum, the whole assembly was incubated at 37°C for a 24 hr period, to check for microbial growth (which would indicate a procedural contamination of the test system).

To provide the challenge for the microbial penetration assay, human stimulated saliva was collected daily from a single healthy adult donor, stimulating their flow using pastilles of sterile paraffin wax (Saliva Test Buffer™ kit, GC Corporation, Tokyo, Japan). The collected whole saliva was mixed with sterile BHI medium to give a final dilution of 1:5 dilution. The resulting mixture was placed into the uppermost inverted lid, which served as a reservoir. Here the mixture was in direct contact with the coronal surface of the roots, exposing them to viable salivary bacteria.

The mixture of saliva and BHI was replaced with a fresh inoculum each day for a total of 60 days. The time of saliva collection and the donor were consistent for the duration of the study. Samples were kept in a dedicated humidified incubator at 37°C, and examined visually each day for turbidity of the BHI medium, the sign that indicated bacterial growth. Once this occurred, the date was recorded, and used to calculate the time at which microbial penetration had occurred. The time to contamination (as indicated by turbidity) was designated as the termination point for each sample in the study. Kaplan-Meier cumulative survival plots were calculated for each group. The significance of any difference in leakage resistance was determined using log-rank Chi square tests.

Results

All samples in Group A, the positive control group where the roots were unfilled, showed microbial growth within the first 48 hours (13 samples within 1 day, and the remaining 7 within 2 days). This indicates that the time for the assay system to indicate microbial penetration was less than 48 hours.

In the negative controls (Group B), some 19 of the 20 samples showed no growth, and the BHI medium in the lower chamber remained uncontaminated for the duration of the study. Examination of the one sample that showed growth revealed a small deficiency in the wax sealing of the sample, which allowed leakage to occur. These results indicate that the approach used for sealing the samples was adequate, and that the experimental model was working as expected.

The null hypothesis that there was no significant difference in microbial penetration between all the various experimental groups was rejected at all time points from day 3 to day 60 (Log-rank Chi-square $P<0.0001$). The novel alkaline cement was found to be the best material at resisting the challenge from continuous exposure to salivary microorganisms, having the most samples surviving for the full experimental period (Figure 2). At 60 days, 12 of the 20 roots that had been filled with Supercal had no microbial penetration, compared to only 5 for gutta percha, and 4 for Pulpdent. Differences between Supercal and gutta percha were significant ($P = 0.034$, hazard ration (95% CI): 0.42 (0.18 - 0.96)), and likewise between Supercal and Pulpdent ($P = 0.007$, hazard ration: 0.35 (0.16 - 0.79)). Differences between Gutta percha and Pulpdent paste were not significant ($P = 0.60$, hazard ration: 1.19 (0.59 - 2.41).

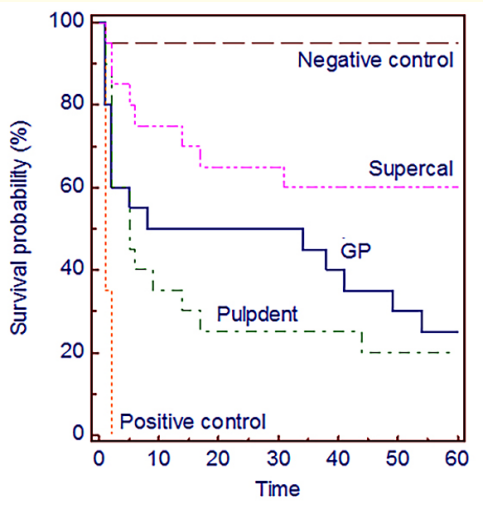

Figure 2: Kaplan-Meier survival curves for all experimental groups.

Discussion

This study explores the ability of a root filling to resist microbial penetration under a worst case scenario where the coronal root face is exposed fully to salivary microorganisms in a nutrient rich environment. Resistance to microbial penetration was greater for the novel alkaline cement Supercal, than for a conventional root filling of gutta percha and epoxy resin sealer, and for Pulpdent paste, a conventional water-based calcium hydroxide medicament paste that releases hydroxyl ions which impair microbial growth.

There are several reasons that likely explain these differences. Once the setting reactions are complete, Supercal is a dimensionally stable composite material with a stable polymer matrix and stable particles, both of very low solubility [7,8]. The first part of the setting reaction is calcium bridging to form crystals of calcium di-glyceroxide, and calcium glyceroxide in tetramer unit cells, as well as excess calcium hydroxide dissolved in free glycerol, and traces of water produced from the glycerol polymerization reaction. The released hydroxyl ions can exert antimicrobial actions. When coronal leakage occurs, this activates the release of these ions from the cement, however the cement itself does not dissolve. This explains why Supercal was superior to Pulpdent paste. The water vehicle of Pulpdent paste makes it susceptible to slowly dissolving or washing out over time when exposed to saliva. Supercal under the same conditions will not dissolve.

The second setting reaction of Supercal is polyglycerol formation, via etherification of one, two, or three of the glycerol hydroxyl group, to form ethers of glycerol with itself. The set cement is a mixture of both linear polyols and cyclic polyols. Hyperbranching polymerization gives a distribution of molecular weights. The polymers are thermally, dimensionally and oxidatively stable, and so the set cement does not change shape, and can maintain its seal over extended periods of time [7,8]. While the existence of polyglycerols was known from the 1930s, the technology used for their synthesis was never applied to dentistry because the processes used were complex, and involved heating glycerol in aqueous solution to temperatures above 200°C, under high pressure [10]. Despite this, in 2007, it was discovered that glycerol mixed with certain high levels of calcium hydroxide at room temperature would set hard within 12-24 hours with a clinically appropriate working time of 15 minutes. [7,8] In the present study, the superior resistance against microbial penetration of Supercal over gutta percha may reflect the fact that the set cement is dimensionally stable, and unlike epoxy resin does not shrink over time.

Resistance to microbial penetration and dimensional stability are not the only factors to be considered when choosing materials for obturation. The materials used must have sufficient Radiopacity. In the case of Supercal, radiopacity is gained from inclusion of non-staining radiopacifiers (barium sulphate and zirconium dioxide). In contrast, bismuth trioxide, which is used in AH 26 and in most brands of MTA, can cause staining [1,11].

This study provides further evidence that a novel alkaline cement could have potential for obturation of the root canal. It extends the results of prior studies [5] that showed superior performance of Supercal over MTA cements, which release calcium hydroxide when the set cement is exposed to water. Further studies of the performance of this novel calcium hydroxide cement are warranted.

Conclusion

This study shows improved resistance to microbial penetration for a novel calcium hydroxide cement versus to a conventional obturation approach using gutta percha with an epoxy resin sealer (AH Plus), and to a calcium hydroxide medicament (Pulpdent paste), in a scenario involving 60 days of continuous exposure to microorganisms from human saliva in a nutrient-rich growth medium. The differences reflect the physical and chemical properties of the materials being tested. The novel cement shows promise for further investigation.

Conflict of Interest

The author is a named co-inventor of Supercal calcium hydroxide cement.

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