Journal of Oral Science, Vol. 60, No. 1, 000-000, 2018

Original

# Release of antimicrobial compounds from a zinc oxide-chelate cement

Tamer Tüzüner[1)], Zeynep A. Güçlü[2)], Andrew Hurt[3)], Nichola J. Coleman[3)], and John W. Nicholson[4,5)]

[1)]Department of Paediatric Dentistry, Faculty of Dentistry, Karadiniez Technical University, Trabzon, Turkey
[2)]Department of Paediatric Dentistry, Faculty of Dentistry, Erciyes University, Kayseri, Turkey
[3)]Department of Pharmaceutical, Chemical and Environmental Sciences, University of Greenwich, Medway Campus, Kent, UK
[4)]Bluefield Centre for Biomaterials, London, UK
[5)]Dental Materials Science Unit, Institute of Dentistry, Queen Mary University of London, London, UK

(Received November 14, 2016; Accepted February 1, 2017)

**Abstract: This study examined the release of cetylpyridinium chloride and benzalkonium chloride from fatty acid chelate temporary dental cement and their antimicrobial effects. The cement was Cavex Temporary, and either cetylpyridinium chloride or benzalkonium chloride was added (1% or 5% by mass), incorporating into the base paste. Release of the additives was determined by reverse-phase high-performance liquid chromatography. Possible chemical interactions between the cement components and additives were examined by Fourier transform infrared (FTIR) spectroscopy. Antimicrobial effects were assessed by measuring the zone of inhibition around sample discs after 24 h in a *Streptococcus mutans* culture. FTIR spectroscopy showed no interaction with cement components. For both additives, release was by diffusion for approximately the first 6 hours, with equilibration after about 2 weeks. Diffusion coefficients were 1.76 $m^2 s^{½}$ to 8.05 × $10^{-12}$ $m^2 s^{½}$ and total release was 10.3 to 44.7% of additive loading. Zones of inhibition with additive were significantly larger than those for control discs. In conclusion, the antimicrobial properties of Cavex temporary cement are improved by the addition of the antimicrobial compounds cetylpyridium chloride and benzalkonium chloride, which are released by a diffusion process.**

Keywords: temporary cement; cetylpyridinium chloride; benzalkonium chloride; FTIR; HPLC; diffusion.

Correspondence to Dr. John W. Nicholson, Bluefield Centre for Biomaterials, 67-68 Hatton Garden, London EC1N 8JY, UK
E-mail: john.nicholson@bluefieldcentre.co.uk

doi.org/10.2334/josnusd.16-0791
DN/JST.JSTAGE/josnusd/16-0791

## Introduction

Several recent studies have reported the incorporation of antimicrobial compounds into tooth-colored dental restorative materials, namely composite resins (1-5) and glass-ionomer cements (6-9). Release of sufficient antimicrobial compound kills or at least inhibits offending microorganisms, thus protecting adjacent hard tissues from attack.

A number of substances have been used as antimicrobial additives in this way, including chlorhexidine (5) and silver (1) (in composite resins), and chlorhexidine (6), benxalkonium chloride (10), and cetylpyridinium chloride (11) (in the case of glass-ionomers). Even without additives, glass-ionomer cements have some antimicrobial characteristics that result from release of fluoride species (12). However, because fluoride release is low, the antimicrobial effect is limited, and glass-ionomers may not have clinically useful antimicrobial activity (13).

The addition of antimicrobial compounds to glass-ionomers substantially improved their antimicrobial

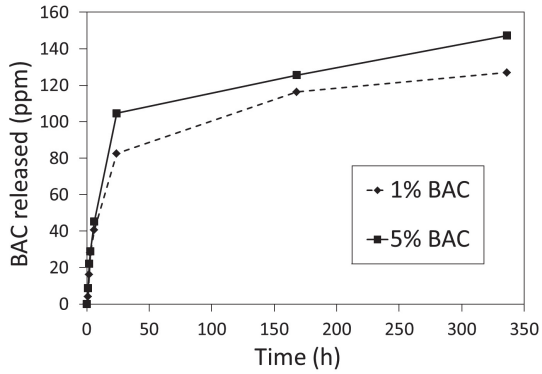


**Fig. 1** Release profiles for benzalkonium chloride (BAC)

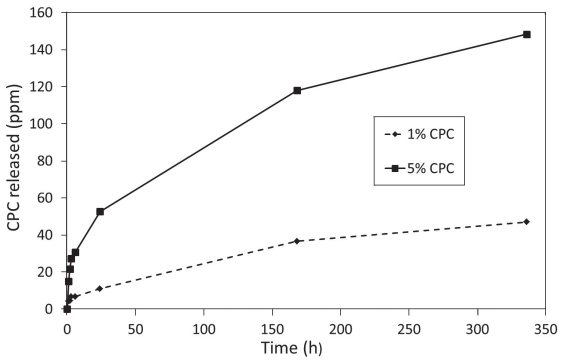


**Fig. 2** Release profiles for cetylpyridinium chloride (CPC)

properties *in vitro* (6,10). Unfortunately, antimicrobial compounds tend to impede the setting reaction, and this leads to weaker set cements (6,10) and lower surface hardness (7), as compared with specimens without additives. In addition, release of the antimicrobial compound is limited and cease within about 4 weeks. A high proportion of the additive therefore remains inside the cement indefinitely (6,10,11).

## Materials and Methods

Cavex Temporary Cement (Cavex BV, Haarlem, The Netherlands), was used in this study. It is a commercial eugenol-free temporary cement based on fatty acids chelating with zinc ions obtained from zinc oxide powder. The antimicrobial compounds used were cetylpyridinium chloride and benzalkonium chloride (both supplied by Sigma-Aldrich, Dorset, UK). The cement was mixed according to the manufacturer's instructions at a ratio of 1 g of 'base' paste to 0.5 g of 'catalyst' paste, and antimicrobial compound was incorporated at levels 1% and 5% by total mass by pre-mixing the necessary quantity of additive with the base paste. Manual mixing was done on a glass plate with the aid of a metal spatula.

The freshly mixed pastes were transferred to silicone rubber molds and placed between glass microscope slides to form the specimens (diameter 6 mm; depth 2 mm). They were allowed to cure fully in the molds at 37°C for 1 h. Each disc was then removed and placed in 5 $cm^3$ of deionized water held in polypropylene tubes. The samples were stored at 37°C, then at time periods of 1 h, 2 h, 3 h, 6 h, 24 h, 1 week, and 2 weeks, a volume of 0.1 $cm^3$ was removed from the storage solution and analyzed by reverse-phase high-performance liquid chromatography (HPLC). This analysis was carried out in triplicate for each sample-type.

HPLC analysis of either cetylpyridium chloride or benzalkonium chloride was done with an Agilent 1200 system fitted with a reverse-phase C-18 Kromasil column (length 150 × 4.60 mm). A 20 µL injection volume was used, and the flow rate was 1.0 $cm^3$/min. The mobile phase was an isocratic system consisting of 55:45 acetonitrile:water with an added drop of glacial acetic acid. A variable wavelength detector set to 254 nm was used for detection.

Once release had equilibrated, release profiles were used to calculate $M_t/M_\infty$ values (i.e., the ratio of release at time t and at equilibrium). These values were plotted against the square root of time, $t^{1/2}$ which yielded a straight line from which the diffusion coefficient was estimated by using the best-fit line was determined with least-squares regression. The Student *t*-test was used to evaluate the significance of any differences in release data and diffusion coefficients.

Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectra were obtained for the Cavex discs which had been cured for 24 h with and without the anti-microbial additives. The spectrometer used was a Perkin Elmer Spectrum One with a Universal Diamond ATR attachment. The range of data collection was 650 to 4,000 $cm^{-1}$, and 10 accumulated scans at a resolution of 2 $cm^{-1}$ were obtained.

The antimicrobial activities of Cavex cement alone and with varying amounts of antimicrobial additive were evaluated with the semi-quantitative Kirby-Bauer inhibition zone method. *Streptococcus mutans* NCIMB 13700 was used as the test microorganism. Experiments involved inoculation of 100 $cm^3$ of Nutrient Broth (Oxoid) with 0.1 $cm^3$ of an overnight culture of the bacterium. The resulting culture was then incubated for 24 h, with shaking, at 37°C. For each sample-type, a set of three 0.2 $cm^3$ volumes of the resulting culture were spread on nutrient agar plates and two cement discs were placed on each plate. These plates were then incubated at 37°C for 24 h, and examined for clear zones. The final population density of the spread plates was 1.5 (± 0.7) × $10^8$ colony-forming units *per* plate. The one-tailed

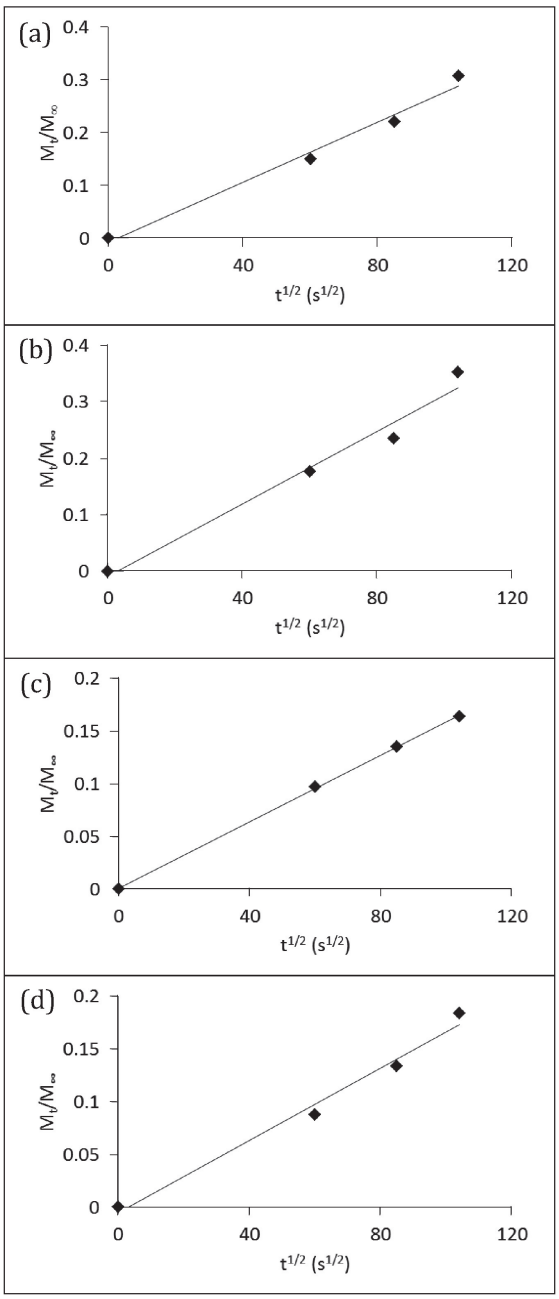


**Fig. 3** Diffusion plots for cement with (a) 1% BAC, (b) 5% BAC, (c) 1% CPC, and (d) 5% CPC

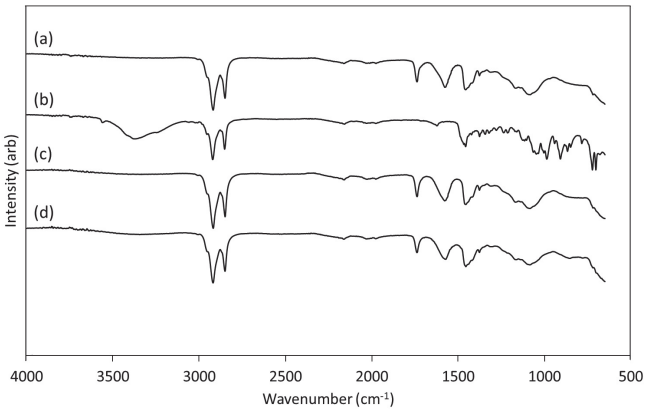


**Fig. 4** FTIR spectra of (a) cement, (b) BAC, (c) cement with 1% BAC, and (d) cement with 5% BAC

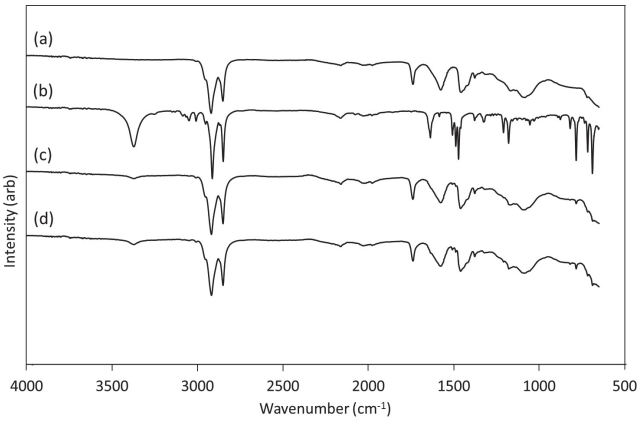


**Fig. 5** FTIR spectra of (a) cement, (b) CPC, (c) cement with 1% CPC, and (d) cement with 5% CPC

*t*-test was used to determine the statistical significance of differences between the antimicrobial activities of cement specimens with and without antimicrobial additives ($n = 6$, $P = 0.05$).

## Results

Samples released antimicrobial compound at all time points (Figs. 1, 2), and required about 2 weeks to reach equilibrium in all cases. Release profiles were used to determine $M_t/M_\infty$ values and the resulting diffusion plots of $M_t/M_\infty$ vs $t^{½}$ were linear for about the first 6 hours (Fig. 3). These plots were used to determine diffusion coefficients with the so-called Stefan approximation, ie by substitution into the equation:

$D = s^2\pi l^2/4$,

where s is the slope of the diffusion plot and 2l is the specimen thickness.

Release data for both additives at both loadings are shown in Table 1. In both cases the $R^2$ value was higher for release of the 1% loading than for the 5% loading, which indicates that the kinetics were closer to true diffusion at this loading. However, even at a 5% loading,

**Table 1** Diffusion and release data for benzalkonium chloride (BAC) and cetylpyridinium chloride (CPC)

| Property | 1% BAC | 5% BAC | 1% CPC | 5% CPC |
|---|---|---|---|---|
| Diffusion coefficient ($m^2s^{1/2}$) | $6.61 \times 10^{-12}$ | $8.05 \times 10^{-12}$ | $1.76 \times 10^{-12}$ | $2.27 \times 10^{-12}$ |
| $R^2$ | 0.985 | 0.974 | 0.999 | 0.985 |
| Maximum release (ppm) | 127 | 147 | 47 | 148 |
| % release | 44.7 | 10.3 | 16.5 | 10.4 |

**Table 2** FTIR assignments of major bands in components of Cavex temporary cement and the antimicrobial additives

| Assignment | Cavex ($cm^{-1}$) | BAC ($cm^{-1}$) | CPC ($cm^{-1}$) |
|---|---|---|---|
| C-H bend | | 703 | 689 |
| C-H bend | | 725 | 716 |
| C-H bend | | | 784 |
| C-O stretch | 1083 | | |
| C-O stretch | 1175 | | |
| C-N stretch | | 1050, 1134 | 1179 |
| C-H bend | 1377 | | |
| C-H bend | 1457 | 1459 | 1472 |
| C-C stretch | 1577 | 1574 | |
| C=C aromatic bend | | 1620 | |
| C=N stretch | | | 1637 |
| COOH stretching | 1738 | | |
| C-H stretching | 2851 | 2853 | 2849 |
| C-H stretching | 2920 | 2921 | 2913 |
| OH stretching | | | 3373 |

**Table 3** Inhibition zones for cement with and without additives (1% and 5% loadings)

| *S. mutans* | Cement | 1% BAC | 5% BAC | 1% CPC | 5% CPC |
|---|---|---|---|---|---|
| Zone of inhibition (mm) | $0.80 \pm 1.1^a$ | $2.8 \pm 0.80^b$ | $4.7 \pm 0.58^c$ | $2.6 \pm 0.55^b$ | $2.8 \pm 0.84^b$ |

Significant differences ($P < 0.05$) are indicated by different superscript letters.

the deviation from true diffusion was only slight. In all cases, substantial amounts of additive were retained in the cement at equilibrium, and higher loadings resulted in greater release of both additives.

FTIR spectra of cement specimens with and without additives are shown in Figs. 4 and 5. The bands in these spectra were identified, shown in Table 2. The presence of the additives did not result in new bands or shifts in existing bands. This finding is consistent with the absence of any chemical interaction between the additives and the cement components.

The antimicrobial effects of substances released from the Cavex cement are shown in Table 3. Cavex itself showed a small zone of inhibition, but the zone was much larger when either antimicrobial additive was present. The zone was significantly larger for 5% than for 1% benzalkonium chloride. However, the zone of inhibition did not differ significantly between the two loadings of cetylpyridinium chloride.

The zone of inhibition was larger when antimicrobial additives were present, and *t*-calc was greater than *t*-crit ($P = 0.05$). These differences were significant and antimicrobial release was sufficient to inhibit growth of the *S. mutans* test organism in all cases.

## Discussion

Both of the antimicrobial compounds studied were released in useful amounts from the fatty acid chelate cement Cavex. For about the first 6 h, release was diffusion-based in both substances, as shown by the linear dependency on $t^{1/2}$. Release equilibrated after approximately 2 weeks, at which time substantial amounts of antimicrobial compound remained in the cement. Similar behavior has been observed in other dental materials, including composite resins (4,5) and cements (6,8,14).

A previous study (11) of cetylpyridinium chloride release from a chelate cement reported that release was similar when the additive was mixed first with the base paste or with the catalyst paste. This is consistent with the absence of a chemical interaction between cetylpyridinium chloride and the cement components. However, the effect of varying the loading of the additive was not

previously studied. We found that both the diffusion coefficient and the total amount released increase when larger amounts of substance are incorporated. Nevertheless, the zone of inhibition for 5% cetylpyridinium chloride was not significantly larger than that for the1% loading. However, in both cases release yielded a zone of inhibition larger than that for the cement itself and there was a significant inhibitory effect on the *S. mutans* culture.

These findings are potentially useful clinically. Incorporation of broad-spectrum antimicrobial compounds into temporary cement might help maintain a substantially sterile region around a temporary crown or similar dental device. This could prove beneficial for patients, as it would limit caries development near the restoration. However, future studies should examine whether the amount released is sufficient to provide useful protection *in vivo*.

Both of the present antimicrobial compounds were inert within the chelate cement and, as indicated by FTIR, did not interact chemically with any cement component. Setting of this cement involves co-ordination of zinc ions from the zinc oxide filler with the carboxylic acid groups of the fatty acid. The characteristic absorption that shows lack of interaction is that due to the carbonyl group (C=O), which is present at at 1,738 $cm^{-1}$ in Cavex, and shows no significant shift in the presence of either cetylpyridinium chloride or benzalkonium chloride. Other bands in the 1,500 to 1,650 $cm^{-1}$ region also do not change, which confirms the absence of any chemical reaction. This inertness of the additives toward the components of the cement further suggests their usefulness in this chelate cement system.

**Conflict of interest**

None declared.

## References

1. Jedrychowski JR, Caputo AA, Kerper S (1983) Antibacterial and mechanical properties of restorative materials combined with chlorhexidines. J Oral Rehabil 10, 373-381.
2. Yamamoto K, Ohashi S, Aono M, Kokubo T, Yamada I, Yamauchi J (1996) Antibacterial activity of silver ions implanted in $SiO_2$ filler on oral streptococci. Dent Mater 12, 227-229.
3. Kawashita M, Tsuneyama S, Miyaji F, Kokubo T, Kozuka H, Yamamoto K (2000) Antibacterial silver-containing silica glass prepared by sol-gel method. Biomaterials 21, 393-398.
4. Imazato S, Edi N, Takahashi Y, Kaneko T, Ebisu S, Russell RRB (2003) Antibacterial activity of bactericide-immobilized filler for resin-based restoratives. Biomaterials 24, 3605-3609.
5. Leung D, Spratt DA, Pratten J, Gulabivala K, Mordan NJ, Young AM (2005) Chlorhexidine-releasing methacrylate dental composite materials. Biomaterials 26, 7145-7153.
6. Palmer G, Jones FH, Billington RW, Pearson GJ (2004) Chlorhexidine release from an experimental glasss-ionomer cement. Biomaterials 25, 5423-5431.
7. Takahashi Y, Imazato S, Kaneshiro AV, Ebisu S, Frencken JE, Tay FR (2006) Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach. Dent Mater 22, 647-652.
8. Türkün LS, Türkün M, Ertuğrul F, Ateş M, Brugger S (2008) Long-term antibacterial effects and physical properties of a chlorhexidine-containing glass ionomer cement. J Esthet Restor Dent 20, 29-44.
9. Tüzüner T, Ulusu T (2012) Effect of antibacterial agents on the surface hardness of a conventional glass-ionomer cement. J Appl Oral Sci 20, 45-49.
10. Botelho MG (2004) Compressive strength of glass ionomer cements with dental antibacterial agent. SADJ 59, 51-53.
11. Hurt A, Coleman NJ, Tüzüner T, Bagis B, Korkmaz FM, Nicholson JW (2016) Release of cetyl pyridinium chloride from fatty acid chelate temporary dental cement. Acta Biomater Odontol Scand 2, 1-6.
12. Forsten L (1977) Fluoride release from a glass ionomer cement. Scand J Dent Res 85, 503-504.
13. Forss H, Jokinen J, Spets-Happonen S, Seppa L, Luoma H (1991) Fluoride and mutans streptococci in plaque grown on glass ionomer and composite. Caries Res 25, 454-458.
14. Tüzüner T, Kusgoz A, Er K, Tasdemir T, Buruk K, Kemer B (2011) Antibacterial activity and physical properties of conventional glass-ionomer cements containing chlorhexidine diacetate/cetrimide mixtures. J Esthet Restor Dent 23, 46-55.