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Studies on the Incorporation of Benzalkonium Chloride and Cetylpyridinium Chloride Antimicrobial Agents nto Glass-Ionomer Dental Cements.

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ABSTRACT

The effect of the antimicrobial additives benzalkonium chloride and cetylpyridinium chloride on a restorative glass-ionomer dental cement (Fuji IX) has been studied. Both substances were added to the glass-ionomer at levels of 1%, 2% and 3% by mass at the mixing stage, and various effects were studied. Setting time was altered in most cases, but only slightly. Compressive strength and release were both determined using cylindrical specimens of size 4 mm diameter x 6 mm height. Additives were found to reduce the compressive strength, but differences were not significant. Release was studied into 5 cm^3 volumes of deionised water, and determined by UV/visible spectrophotometery at a wavelength of 259 nm for CPC and 214 nm for BC. Data showed that release occurred by a diffusion mechanism for the first 2-3 hours, with diffusion coefficients varying with concentration. Values were in the range 1.97×10^{-14} -1.78×10^{-12} m^2 s^{-1}. Release had ceased after six weeks, with total release representing between 2.15 and 4.84% of the initial additive loading.

Keywords: glass-ionomer; controlled release; benzalkonium chloride; cetyl pyridium chloride; diffusion.

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INTRODUCTION

Materials known as glass-ionomer cements are used extensively in dentistry [1]. They are used in a variety of ways, including as liners and bases, and also as direct restorative materials, mainly in children's dentistry [1, 2]. Among other applications, glass-ionomers can be used to cement orthodontic brackets [3], and they have also been studied as experimental bone cements [4, 5].

Glass-ionomer cements are prepared by reaction of powdered basic glass with aqueous solutions of polyacid, typically poly(acrylic acid) [6]. The glass is a complex substance, typically calcium or strontium fluoro-aluminosilicate. However, other experimental glasses have also been reported [7].

Glass-ionomer cements are considered anti-cariogenic [1]. One of the key features that promotes this is their ability to release fluoride [8], but it is also aided by their ability to buffer organic acids, such as lactic acid [9]. These acids are generated *in vivo* by oral micro-organisms, and their chemical effect is to erode the mineral phase of the tooth, the basis of dental caries [10].

Fluoride release is an important property of glass-ionomers. It is capable of being sustained for several years [11], and may be augmented by fluoride uptake in the presence of dissolved fluoride originating from toothpastes and/or fluoridated mouthwashes [12]. It also suggests that these materials can be used as controlled release devices, a topic of great interest in modern day pharmacy [13]. The most extensively studied substance for controlled release has been chlorhexidine [14], though the release of other substances, such as cetyl pyridinium chloride [15], benzalkonium chloride [15] and sodium fusidate [16].

Several aspects of the addition of these substances have been reported. For example, both cetyl pyridinium chloride and benzalkonium chloride have been shown to impart significant anti-microbial character to these materials, from which they can be released steadily, while at the same time weakening the set cement [15, 17]. Similar findings have been reported for chlorhexidine, which has also been shown to slow down the setting reaction slightly [14]. Sodium fusidate is another substance that has been studied. It has also been shown to be capable of being released from glass-ionomer cements, with release being a diffusion process. To date, there have been no reports of the effect of this particular substance on either the setting chemistry or eventual compressive strength.

The present study has been undertaken to extend our understanding of the effect of adding the cationic substances cetyl pyridinium chloride and benzalkonium chloride to glass-ionomer cements. Two different commercial restorative grade glass-ionomers have been used, and the effect of these additives on setting behaviour and compressive strength has been determined. In addition, release of them has been studied over periods of up to 6 weeks, using UV/visible spectrophotometry.

MATERIALS AND METHODS

Studies were carried out using the commercial restorative grade glass-ionomer cements ChemFlex (ex Dentsply, Germany) and Fuji IX (ex GC, Japan). Antimicrobial compounds used were cetyl pyridinium chloride (Sigma-Aldrich, Dorset, UK) and benzalkonium chloride (ex Fluka, Germany). These antimicrobial compounds were incorporated into these cements at the mixing stage at levels corresponding to 1%, 2% and 3% by mass. In addition, experiments were carried out on additive-free cements, as controls.

Setting times were determined for parent cements, and for cements containing the various levels of additive, using a Gillmore needle (28g mass), as specified in ISO9917 [18].

Compressive strength was determined using cylindrical specimens of dimensions 4 mm diameter x 6 mm height. Sets of five such specimens were prepared using stainless steel split moulds and loading them with freshly mixed cement pastes. Ends were made flat by clamping metal plates using a G-clamp. Specimens were cured in an oven at 37^{o}C for 1 hour, then removed from the moulds, and stored for a further 23 hours in water at 37^{o}C before testing. Testing was carried out on an Instron Universal Testing machine (Model 1193, Instron Corp., USA), and loads at failure were converted to strength. Means and standard deviations were determined for each cement/additive combination.

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Additive release was determined using specimens of the same dimensions using UV-visible spectrophotometry (with a Hewlett Packard 8453 spectrophotometer, and software provided by Agilent Technologies). Determinations were carried out at 259 nm for CPC and 214 nm for BC at every 15 min for an hour and then 1, 2, 3, 4, 5, 24 hours, and then weekly up to 6 weeks. Graphs of M_t/M_∞ were plotted, and the diffusion coefficient was determined from the linear portion of these graphs, taking the slope and substituting into the equation $D = s^2 \pi l^2/4$.

Data were examined for statistical significance using 1-way ANOVA, post hoc Tukey Honest Significant Difference (HSD) and Mann-Whitney U-tests.

RESULTS

The effect of additives at various levels on setting time is shown in Table 1. There were no clear trends, though some indication that setting time was extended slightly in some cases. This suggests that these additives have a slight inhibitory effect on the setting reaction.

Table 1: Setting time of Fuji IX with varying levels of addition

Additive	Setting time
None	4 min 35 s
1% BAC	4 min 40 s
2% BAC	4 min 30 s
3% BAC	4 min 30 s
1% CPC	4 min 38 s
2% CPC	4 min 45 s
3% CPC	4 min 25 s

Table 2: Compressive strength of Fuji IX with varying levels of addition (Standard deviations in parentheses)

Additive	Compressive strength/MPa
None	136.1 (33.2)
1% BAC	137.7 (6.2)
2% BAC	119.2 (12.4)
3% BAC	108.9 (8.5)
1% CPC	90.1 (10.8)
2% CPC	77.1 (16.2)
3% CPC	125.7 (4.7)

Compressive data are shown in Table 2. The initial result for cement without additive showed a wide scatter, which had the effect of making differences when additives were present not significant. Nonetheless, as for setting time, there was a general trend, with additives reducing compressive strength. Comparison of cements containing benzalkonium chloride with those containing cetyl pyridinium chloride showed the latter to be weaker for both 1% and 2% levels of addition.

Table 3: Release of benzalkonium chloride from Fuji IX doped samples. (Standard deviation in parentheses)

Time	1%	SD	2%	SD	3%	SD
15min	0.0057	(0.0002)	0.0081	(0.0006)	0.0131	(0.0013)
30min	0.0060	(0.0004)	0.0085	(0.0006)	0.0140	(0.0015)
45min	0.0065	(0.0008)	0.0087	(0.0006)	0.0149	(0.0018)
1 hr	0.0068	(0.0009)	0.0090	(0.0007)	0.0155	(0.0017)
2	0.0073	(0.0009)	0.0094	(0.0006)	0.0169	(0.0023)
3	0.0082	(0.0009)	0.0100	(0.0005)	0.0225	(0.0064)
4	0.0085	(0.0010)	0.0112	(0.0012)	0.0248	(0.0074)
5	0.0093	(0.0006)	0.0126	(0.0013)	0.0294	(0.0084)
24	0.0101	(0.0006)	0.0137	(0.0019)	0.0372	(0.0218)

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1 wk	0.0532	(0.0134)	0.0861	(0.0126)	0.1257	(0.0155)
2 wk	0.0805	(0.0157)	0.1215	(0.0293)	0.1342	(0.0136)
3 wk	0.1071	(0.0182)	0.1300	(0.0260)	0.1404	(0.0722)
4 wk	0.0964	(0.0150)	0.1539	(0.0308)	0.1852	(0.0208)
5 wk	0.1201	(0.0350)	0.1568	(0.0208)	0.1575	(0.0192)
6 wk	0.1249	(0.0158)	0.1210	(0.0315)	0.1672	(0.0234)

Table 4: Release of cetyl pyridinium chloride from Fuji IX doped samples (Standard deviation in parentheses)

Time	1%	SD	2%	SD	3%	SD
15 min	0.036	(0.004)	0.051	(0.002)	0.063	(0.003)
30 min	0.038	(0.004)	0.053	(0.002)	0.068	(0.003)
45 min	0.041	(0.003)	0.055	(0.002)	0.071	(0.003)
1 hr	0.043	(0.003)	0.057	(0.003)	0.074	(0.003)
2 hr	0.046	(0.003)	0.061	(0.004)	0.080	(0.004)
3 hr	0.047	(0.004)	0.062	(0.004)	0.084	(0.005)
4 hr	0.049	(0.002)	0.064	(0.008)	0.089	(0.001)
5 hr	0.048	(0.004)	0.066	(0.010)	0.090	(0.002)
24 hr	0.053	(0.005)	0.071	(0.009	0.111	(0.006)
1 wk	0.097	(0.007)	0.132	(0.042)	0.221	(0.010)
2 wk	0.100	(0.010)	0.191	(0.022)	0.263	(0.040)
3 wk	0.115	(0.005)	0.257	(0.040)	0.326	(0.049)
4 wk	0.149	(0.005)	0.235	(0.028)	0.297	(0.029)
5 wk	0.162	(0.004)	0.193	(0.037)	0.217	(0.027)
6 wk	0.152	(0.004)	0.204	(0.038)	0.322	(0.029)

Release data are shown in Tables 3 and 4 for benzalkonium chloride and cetyl pyridinium chloride respectively. Release generally occurred steadily over 6 weeks, by which time it was more or less equilibrated. Plotting the data in the form of M_t/M_∞ gave stright lines for the first 2-3 hours, as shown in Table 5.

Table 5: Linear regression equations and correlation coefficient for plots of Mt/M_∞ vs $\sqrt{t}/s$.

Additive (amount and type)	Equation	Correlation coefficient
1% Benzalkonium chloride	$y = 2.607 \times 10^{-4}x + 0.038$	0.988
2% Benzalkonium chloride	$y = 1.585 \times 10^{-4}x + 0.047$	0.996
3% Benzalkonium chloride	$y = 4.208 \times 10^{-4}x + 0.066$	0.992
1% Cetylpyridinium chloride	$y = 1.53 \times 10^{-3}x + 0.181$	0.991
2% Cetylpyridinium chloride	$y = 1.078 \times 10^{-3}x + 0.218$	0.925
3% Cetylpyridinium chloride	$y = 8.858 \times 10^{-4}x + 0.173$	0.995

Table 6: Diffusion coefficient of additives at various levels of addition

Additive (amount and type)	Diffusion coefficient $m^2 s^{-1}$
1% Benzalkonium chloride	5.34×10^{-14}
2% Benzalkonium chloride	1.97×10^{-14}
3% Benzalkonium chloride	1.39×10^{-13}
1% Cetylpyridinium chloride	1.78×10^{-12}
2% Cetylpyridinium chloride	9.13×10^{-13}
3% Cetylpyridinium chloride	6.16×10^{-13}

Diffusion coefficients, determined from the slope of the lines shown in Table 5, are listed in Table 6. For benzalkonium chloride, the highest value occurred for the 3% loading, but there was no order in the

results. By contrast, for cetyl pyridinium chloride, the highest value occurred with the 1% level of addition, and went down in order from 1% to 3%.

DISCUSSION

There have been a number of previous studies of the effect of antimicrobial additives on glass-ionomer cements [15-19]. Results have all been similar, in that the overall antibacterial effect of the cement has been improved, but at the expense of slightly prolonged setting times and reduced compressive strengths. A variety of types of compound has been studied, with several being cationic antimicrobials, as used in the present study. In addition, chlorhexidine, in the form of the diacetate [14] and sodium fusidate [16] have been studied.

The inhibition of setting that we have observed appears to occur with all types of additive. It has been suggested that the cationic compounds based on quaternary ammonium salts, such as benzalkonium chloride and cetyl pyridinium chloride, have a particular capacity to do this via interaction of with the poly(acrylic acid) component during setting [20, 21]. However, this effect has also been observed with neutral species, such as methanol [22], 2-hydroxethyl methacrylate [22] and sodium chloride [23]. Whatever the origin in terms of fundamental chemistry, it is widespread, and typically associated with a reduction in compressive strength.

The effect of benzalkonium chloride and cetyl pyridinium chloride on compressive strength of the conventional glass-ionomer cement Fuji IX has been reported previously [17]. In that paper, the compressive strength at 7 days was reported and, even for the 1% level of addition, reduction in strength was highly significant ($p<0.01$). In the case of the work in the current study, determining compressive strength at 24 hours may have been to soon to detect significant differences. Observing significant differences was also hampered by the large standard deviation in the compressive strength value for the cement sample without additive.

Release of benzalkonium chloride and cetyl pyridinium chloride has been shown previously from the enhancement in the antibacterial activity of the cements [17]. This was shown experimentally be measuring the zone of inhibition around cement discs placed in bacterial cultures using the agar diffusion method [17]. In the current work, release of benzalkonium chloride and cetyl pyridinium chloride has been measured directly using UV/visible spectrophotometry. This has enabled us to show that early release (2-3 hours) is diffusion based, with diffusion coefficients in the range.

Previously, release of chlorhexidine diacetate and of sodium fusidate have been shown to be diffusion processes in their early stages [14, 16]. For the latter, the diffusion coefficients were between 3.0 and 4.4 x 10^{-12} m^2 s^{-1}, *i.e.* slightly greater than those determined for benzalkonium chloride and cetyl pyridinium chloride in the present study. Total release of sodium fusidate was also much higher than that found for benzalkonium chloride and cetyl pyridinium chloride, at around 20-22% of total loading [16], compared with values of between 2 and 5% reported here.

Overall, our results confirm the usefulness of glass-ionomer cements as potential controlled release materials. This may have important clinical applications, for example if such modified materials could be used in the Atraumatic Restorative Treatment technique in underdeveloped countries [19], or for special needs patients with compromised levels of oral hygiene.

CONCLUSIONS

The effects of the antimicrobial additives benzalkonium chloride and cetyl pyridinium chloride on a restorative glass-ionomer dental cement (Fuji IX) previously reported [15, 17] have been confirmed. Both substances were found to have only minor effects on the setting time as determined with the Gilmore needle, and typically there was a slight increase in setting time, *i.e.* the setting reaction was inhibited to an extent. The compressive strength was found to be much lower than the manufacturer's claim, and to show considerable scatter. The additives generally reduced the compressive strength but not to a statistically significant extent. Cements containing cetyl pyridinium chloride at 1 and 2% were weaker to statistically significant extent than those containing benzalkonium chloride ($p<0.01$).

Additives were released into deionised water by a diffusion mechanism for the first 2-3 hours. Diffusion coefficients varied with concentration and were in the range 1.97 x 10^{-14} -1.78 x 10^{-12} m^2 s^{-1}. Total release varied with concentration, and was very low, i.e. between 2.15 and 4.84% of the initial additive loading, as previous reported [15].

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