

The antibacterial activity and release of quaternary ammonium compounds in an orthodontic primer

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ABSTRACT

The aim of this study was to evaluate the impact of 10 wt% benzalkonium chloride (TB-BAC) or 10 wt% cetylpyridinium chloride (TB-CPC) on the antimicrobial properties of the orthodontic adhesive primer, Transbond XT™ (TB). Antimicrobial activity was assessed using a zone of inhibition diffusion test and the release of the antimicrobial compounds was monitored by high performance liquid chromatography (HPLC). Shear bond strength (SBS) was tested using bovine enamel. Control, TB, specimens failed to demonstrate intrinsic antibacterial activity at 1, 7 and 14 days; whereas, TB-BAC and TB-CPC showed antibacterial effects at all times. HPLC analysis indicated no significant differences in

the release behaviour of TB-BAC and TB-CPC (*t*-test, $p > 0.05$), except for the 7-day release which was higher for TB-BAC ($p < 0.05$). By 14 days the extents of release were $27 \pm 2\%$ and $25 \pm 5\%$ of the total initial loading for TB-BAC and TB-CPC, respectively. The incorporation of 10 wt% BAC or CPC in Transbond XT™ adhesive primer also resulted in superior shear bond strength at 7 and 14 days (Fisher's LSD, $p < 0.05$) with no significant change in the mode of bracket failure under shear stress (Pearson's chi-squared, $p > 0.05$).

Key words: Antibacterial agents; Benzalkonium chloride; Cetylpyridinium chloride; Transbond self-etching primer.

La actividad antibacteriana y la liberación de compuestos de amonio cuaternario en un adhesivo imprimador ortodóncico

RESUMEN

El objetivo de este estudio fue evaluar el impacto del cloruro de benzalconio al 10% en peso del peso (TB-BAC) o de cloruro de cetilpiridinio al 10% del peso (TB-CPC) con propiedades antimicrobianas presentes en el adhesivo acondicionador ortodóncico, Transbond XT™ (TB). La actividad antimicrobiana se evaluó usando una zona de prueba de difusión de inhibición y la liberación de los compuestos antimicrobianos se controló mediante cromatografía líquida de alta resolución (HPLC). La resistencia de adhesión al corte (SBS) se probó usando esmalte bovino. Las muestras control, TB no lograron demostrar actividad antibacteriana intrínseca a 1, 7 y 14 días; mientras que TB-BAC y TB-CPC mostraron efectos antibacterianos en todo momento. El análisis por HPLC no indicó

diferencias significativas en el comportamiento de liberación de TB-BAC y TB-CPC (prueba *t*, $p > 0,05$), excepto en la liberación a los 7 días que fue más alta para TB-BAC ($p < 0,05$). A los 14 días, los grados de liberación fueron de $27 \pm 2\%$ y de $25 \pm 5\%$ de la carga inicial total para TB-BAC y TB-CPC, respectivamente. La incorporación de 10% en peso de BAC o CPC en el imprimador adhesivo Transbond XT™ también dio como resultado una resistencia superior corte a los 7 y 14 días (Fisher's LSD, $p < 0.05$) sin cambios significativos en el modo de falla del bracket bajo tensión de corte (Pearson's chi-cuadrado, $p > 0.05$).

Palabras clave: Agentes antibacterianos; Cloruro de banzalconio; Cloruro de Cetilpiridinio; Acondicionador autoadhesivo.

INTRODUCTION

It is well documented in the literature that orthodontic appliances may cause the demineralisation of enamel during fixed orthodontic treatment procedures¹⁻³. It is difficult, particularly for young patients, to remove the plaque around the brackets

which increases the risk of further cariogenic bacterial accumulation, white spot lesions and subsequent cavitated caries¹⁻³. To address these problems, various strategies have been employed to inhibit bacterial activity in the vicinity of orthodontic brackets. These include the application

of fluoride-releasing luting materials, topical fluoride therapy and the incorporation of antibacterial agents in toothpastes, gels and mouthwashes⁴⁻⁶.

In addition, the direct incorporation of antimicrobial compounds into orthodontic bonding materials, such as glass ionomers, zinc polycarboxylates and adhesive resins, is a subject of increasing interest⁷⁻⁸. A number of studies has indicated that a variety of antimicrobial compounds is effective in enhancing the antimicrobial character of these bonding materials, including chlorhexidine (CHX)⁹, cetylpyridinium chloride (CPC)¹⁰, cetrimide¹¹ and benzalkonium chloride (BAC)¹². Among the most popular antimicrobial agents in these applications are the quaternary ammonium salts (*i.e.* CPC and BAC).

The aim of this study was to investigate the impact of either 10 wt% benzalkonium chloride (BAC) or 10 wt% cetylpyridinium chloride (CPC) in the light-cured acrylate-based orthodontic adhesive primer, Transbond XT™ (3M, USA). The antimicrobial activities of the unmodified primer and of that admixed with BAC or CPC were compared using an inhibition zone assay with *Streptococcus mutans*. The *in vitro* release profiles of BAC and CPC from the admixed primer were monitored at 1, 7 and 14 days by high performance liquid chromatography (HPLC). The impact of lixiviation during component release on the integrity and texture of the primer was observed by scanning electron microscopy (SEM) and potential chemical interactions between the components of the primer and the quaternary ammonium compounds were investigated by Fourier transform infrared spectroscopy (FTIR). The shear bond strengths (SBS) of orthodontic brackets bonded to bovine enamel conditioned with the modified and unmodified primer, and the corresponding adhesive remnant indices (ARI), were compared at 1, 7 and 14 days.

MATERIALS AND METHODS

Sample preparation

This study employed a commercial light-cured orthodontic adhesive primer, (Transbond XT™ primer (code 712-034), 3M, CA, USA), which is principally composed of 45 – 55% triethylene glycol dimethacrylate and 45 – 55% bisphenol A diglycidyl ether dimethacrylate¹³. The antimicrobial compounds used were cetylpyridinium chloride (CPC) and benzalkonium chloride (BAC) powders (Sigma Aldrich, UK).

Sample discs (5 mm diameter x 1 mm depth) of the unmodified Transbond™ primer were produced according to the manufacturer's instructions by placing the liquid primer in polypropylene moulds and light-curing for 10 seconds (HiLux, LEDMAX, Europe). Sample discs incorporating antimicrobial compounds were prepared by manually pre-mixing either 10 wt% of BAC powder or 10 wt% of CPC powder with the liquid primer on a polypropylene surface with a metal spatula for approximately 1 minute until the powder had dissolved. The admixed liquids were then placed in polypropylene moulds and light-cured (as described above). All light-cured samples were stored in the dark for a maximum of 2 days in hermetically sealed polypropylene tubes until required. Unmodified Transbond™ primer samples were labelled 'TB' and those admixed with BAC and CPC were labelled TB-BAC and TB-CPC, respectively.

Zone of inhibition agar diffusion test

The antibacterial activities of the cured specimens (TB, TB-BAC and TB-CPC) against *Streptococcus mutans* were assessed by the Kirby-Bauer zone of inhibition agar diffusion test^{14,15}. The *S. mutans* was initially cultured on blood agar (Merck, Darmstadt, Germany) at 37 °C for 24 hours in 5% CO₂. Single colonies from the plates were transferred to BHI broth (Merck, Darmstadt, Germany) and incubated at 37 °C for 24 hours. McFarland 0.5 turbidity tubes were used to prepare a suspension of the strain in phosphate buffer solution, at approximately 1.5×10^8 cfu cm⁻³, which was then flood-inoculated onto the surface of BHI agar plates. The disc-shaped specimens for the TB control (n = 3), TB-BAC (n = 3) and TB-CPC (n = 3) were placed on the spread plates which were further incubated at 37 °C for 24 hours. The plates were then inspected for clear zones around the specimen discs which were measured at 3 different points. The discs were then transferred to freshly prepared test agar plates and stored at 2 – 4 °C. The inhibition zone agar diffusion procedures were then repeated at 7 and 14 days to appraise the ongoing antimicrobial activity of the specimens.

Release of antimicrobial compounds

One cured sample disc of either TB, TB-BAC or TB-CPC was placed in 5 cm³ of deionised water in a 15 cm³ screw-capped polypropylene centrifuge

tube and stored at 37 °C. Each sample type was prepared in triplicate. After 1, 7 and 14 days, 0.1 cm³ of the supernatant liquor was removed for HPLC analysis.

Concentrations of released cetylpyridinium ions were determined by HPLC using an Agilent 1200 series chromatograph fitted with a reverse-phase C-18 Primsep column of 150 mm in length and 4.60 mm in diameter. A total volume of 20 µl were injected into the instrument at a flow rate of 1.0 cm³ min⁻¹, with an isochratic mobile phase consisting of 55:45 acetonitrile:water with an added drop of concentrated sulphuric acid. Detection was by means of a variable wavelength detector set to 259 nm.

Concentrations of released benzalkonium ions were determined by HPLC using an Agilent 1200 series chromatograph fitted with a reverse-phase C18 Kromasil column of 150 mm in length and 4.60 mm in diameter. A total volume of 20 µl were injected into the instrument at a flow rate of 1.0 cm³ min⁻¹, with an isochratic mobile phase consisting of 55:45 acetonitrile:water with an added drop of glacial acetic acid. Detection was by means of a variable wavelength detector set to 254 nm.

Scanning electron microscopy

Secondary electron images of the surfaces of the TB, TB-BAC and TB-CPC discs prior to and following immersion in 5 cm³ of distilled water for 14 days at 37 °C were obtained from uncoated samples attached to carbon tabs on a JEOL (Welwyn Garden City, UK) JSM-5410 LV electron microscope with an Oxford Instruments (Oxford, UK) X-MaxN EDX detector in low vacuum mode. All images were obtained with an accelerating voltage of 1.0 kV at a working distance of 21 mm and a magnification of x100.

FTIR spectroscopy

FTIR spectra of the unset Transbond XTTM adhesive primer solution and of the cured TB, TB-BAC and TB-CPC discs were obtained in triplicate using a Perkin Elmer (MA, USA) Spectrum Two spectrometer with a Universal Diamond attenuated total reflectance attachment. Spectra were recorded with 16 accumulated scans between 4000 cm⁻¹ and 450 cm⁻¹ wavenumbers at a resolution of 4 cm⁻¹.

The degree of conversion (DC) was estimated by comparing the ratios of the intensities of the FTIR peaks for the reactive polymerising C=C bond (at

1639 cm⁻¹) and the unreactive C=O bond (at 1717 cm⁻¹) in the cured polymer and monomer using the following equation¹⁶:

$$DC = \left(1 - \frac{([C=C] \text{ in polymer} / [C=O] \text{ in polymer})}{([C=C] \text{ in monomer} / [C=O] \text{ in monomer})} \right) \times 100\%$$

Shear bond strength analysis

Ninety freshly extracted permanent mandibular bovine incisors with no visible imperfections were used for shear bond strength analysis. The root portion of each tooth was embedded in a 3 cm diameter cylinder of dental acrylic (Orthocryl, Dentarium, Ispringen, Germany) such that the buccal surfaces could be positioned parallel to the applied shear force. The buccal surfaces were polished with pumice, rinsed with water spray, air-dried, etched with 37% phosphoric acid (Super Etch, SDI, Australia) for 30 seconds, and again, rinsed and dried.

Unmodified Transbond XTTM adhesive primer (TB, n = 30) and the primer modified with either 10 wt% of BAC (TB-BAC, n = 30) or 10 wt% of CPC (TB-CPC, n = 30) were individually applied to the etched buccal surfaces of the teeth in accordance with the manufacturer's instructions. An Ormco mini 2000 metallic orthodontic premolar bracket (Ormco Corp., CA, USA) with 9.63 mm² base area was bonded to each tooth with TransbondTM adhesive (3M, CA, USA), *via* light-curing for 20 s (HiLux, LEDMAX, Europe). The brackets were positioned with orthodontic tweezers and excess adhesive was removed with a sharp scaler prior to curing. All samples were then stored in distilled water at room temperature until required for shear bond analysis. Ten of each sample-type (TB, TB-BAC and TB-CPC) were subjected to shear bond testing at 1, 7 and 14 days using a universal testing machine (Instron Corp., MA, USA) with a shear force blade at a cross-head speed of 1 mm min⁻¹ in the occluso-gingival direction. The results of each test were obtained as a force (N) which was then converted to a pressure (MPa).

The mode of bond failure was determined by observation using a stereomicroscope (Nikon, Tokyo, Japan) at x20 magnification. The failure sites on the tooth surface were classified according to their adhesive remnant index (ARI) score: 0 = no remaining adhesive; 1 = less than half of the adhesive remaining; 2 = more than half of the

adhesive remaining; and 3 = all of the adhesive remaining on the enamel with a distinct impression of the bracket mesh¹⁷.

Statistical analysis

Statistical analysis was performed with SPSS v17.0 for Windows (SPSS Inc., Chicago, USA) software. The comparison of the experimental groups for the agar diffusion and HPLC analyses at each time interval were carried out using the Mann Whitney U test and the Student's *t*-test, respectively, at a significance level of $p < 0.05$. The normal distribution of the shear bond strength data was confirmed using the Shapiro Wilk test, and these data for each group were compared using a two-way ANOVA and Fisher's LSD test at a significance level of $p < 0.05$. The Pearson's chi-squared test was used to compare the ARI scores among the groups at each time period.

RESULTS

During manual mixing with a metal spatula on a polypropylene surface, both 10 wt% BAC and 10 wt% CPC were visually observed to dissolve in the Transbond™ primer within one minute. The incorporation of these antimicrobial compounds did

not visibly appear to adversely affect the light-curing of the primer.

The zone of inhibition data for the control TB and experimental TB-BAC and TB-CPC specimens are listed in Table 1. The control TB specimens failed to demonstrate any antimicrobial activity against *S. mutans* throughout the duration of the study. Both BAC and CPC were observed to confer sustained antimicrobial character to the Transbond™ primer throughout the 14-day period. The incorporation of BAC into the adhesive primer resulted in significantly higher antibacterial activity ($p < 0.05$) compared with that of CPC at all times. In both cases, the extent of the antimicrobial activity was generally seen to diminish as a function of time.

The concentrations of the antimicrobial compounds released from the admixed Transbond™ primer samples into deionised water at 1, 7 and 14 days are listed in Table 2. Within the 14-day period, the total release of the antimicrobial compounds from TB-BAC and TB-CPC were, respectively, $27 \pm 2\%$ and $25 \pm 5\%$ of the total initial loading (as shown in Fig. 1). HPLC analysis indicated no statistically significant differences in the cumulative release behaviour of TB-BAC and TB-CPC ($p > 0.05$), with the exception of the release at 7 days ($p < 0.05$) which was higher for sample TB-BAC.

SEM analysis (not shown) of samples TB, TB-BAC and TB-CPC prior to and following exposure to distilled water indicates that the flat featureless surfaces of the Transbond™ primer remain unchanged after immersion in water for 14 days. The absence of voids or changes in texture confirms that the release of the added BAC and CPC from

Table 1: Median (and range) zone of inhibition data for TB, TB-BAC and TB-CPC

| Zone of Inhibition (mm) | | | |
|-------------------------|-------------------------|-------------------------|-------------------------|
| Sample | Day 1 | Day 7 | Day 14 |
| TB | 0 | 0 | 0 |
| TB-BAC | 17 (16-18) ^A | 15 (13-15) ^A | 13 (12-13) ^A |
| TB-CPC | 13 (12-13) ^B | 10 (9-11) ^B | 10 (9-10) ^B |

*In each column, different capital letters indicate significant differences between the groups ($p < 0.05$)

Table 2: Cumulative release of the antimicrobial quaternary ammonium compounds

| Concentration of antimicrobial quaternary ammonium compounds Mean \pm Standard deviation (ppm) | | | |
|---|-----------------------------|-------------------------------|-------------------------------|
| Sample | Day 1 | Day 7 | Day 14 |
| TB-BAC | 52.0 \pm 4.1 ^A | 196.0 \pm 14.0 ^A | 212.1 \pm 12.0 ^A |
| TB-CPC | 62.3 \pm 8.5 ^A | 111.0 \pm 12.8 ^B | 187.1 \pm 42.5 ^A |

*In each column, different capital letters indicate significant differences between the groups ($p < 0.05$) whereas the same letters indicate no significant differences ($p > 0.05$).

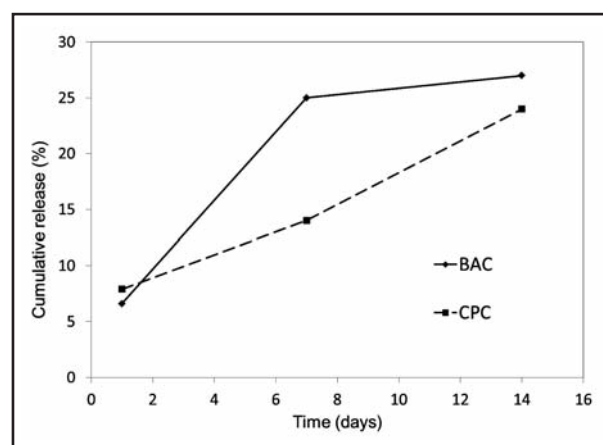


Fig. 1: Cumulative release of the antimicrobial quaternary ammonium compounds.

the primer does not impair the physical integrity of the resin.

The FTIR spectra of the monomeric Transbond™ primer solution, the antimicrobial agents (BAC and CPC), and the light-cured polymerised resins (TB, TB-BAC and TB-CPC) are shown in Fig. 2. The corresponding functional group assignments are given in Table 3¹⁸. According to the manufacturer's safety data sheet (13), Transbond™ primer comprises a mixture of triethylene glycol dimethacrylate, bisphenol A diglycidyl ether dimethacrylate, triphenylantimony, 4-(dimethylamino) benzeneethanol, DL-camphorquinone and hydroquinone. Present in these monomers are carboxylic acid, amine, carbonyl, alkane, alkene, aromatic and ether groups. All of these functional groups appear in the FTIR spectra of both the unset Transbond™ solution and light-cured polymerised discs (as indicated in Fig. 2 and Table 3).

The degree of conversion of light-cured acrylate-based resins is defined as the percentage of acrylate C=C groups from the various monomers which have undergone polymerisation¹⁶. Analysis of the FTIR spectra of the unset and light-cured Transbond™ primer indicated that this material achieved a degree of conversion of $52 \pm 2\%$ under the selected experimental conditions. It is not possible to estimate the extent of polymerisation of the primer in the presence of the antimicrobial agents, as their vibrational bands are concurrent with the carbonyl and acrylate bands of the primer. No new bands appear and none of the original bands is shifted by the incorporation of BAC or CPC in the primer, indicating that there is no direct chemical interaction between the antimicrobial agents and the components of the Transbond™ primer.

Shear bond strength (SBS) data for samples TB, TB-BAC and TB-CPC as functions of storage time in distilled water are given in Table 4. The initial 1-day SBS of the unmodified TB control sample was found to be significantly higher than its subsequent values at 7 and 14 days. The incorporation of either BAC or CPC in the primer did not have an impact on the 1-day SBS values relative to that of the unmodified control; although, after 7 and 14 days, both modified samples exhibited significantly higher bond strengths than those of the control. The initial 1-day SBS of TB-BAC was higher than that of TB-CPC; and there was found to be no difference between the two modified materials at the later time points.

Table 3: FTIR assignments of major bands present in TB, BAC and CPC¹⁸

| Assignment | TB (cm ⁻¹) | BAC (cm ⁻¹) | CPC (cm ⁻¹) |
|----------------|------------------------|-------------------------|-------------------------|
| C-H bend | 830 | 725, 703 | 690 |
| C-H bend | 884 | 786 | 715 |
| C-H bend | 937 | 985, 906 | 817, 785 |
| C-O stretch | 1251, 1160 | | |
| C-O stretch | 1316, 1294 | | |
| C-N stretch | | 1127, 1043 | 1181 |
| C-H bend | 1375 | | |
| C-H bend | 1408 | 1455 | 1473 |
| C=C aromatic | 1511 | 1620 | |
| C=C stretch | 1639, 1612 | | |
| C=N stretch | | | 1639 |
| C=O stretching | 1712 | | |
| C-H stretching | | 2856 | 2851 |
| C-H stretching | 2920 | 2925 | 2911 |
| OH stretching | 3480 | | 3381 |

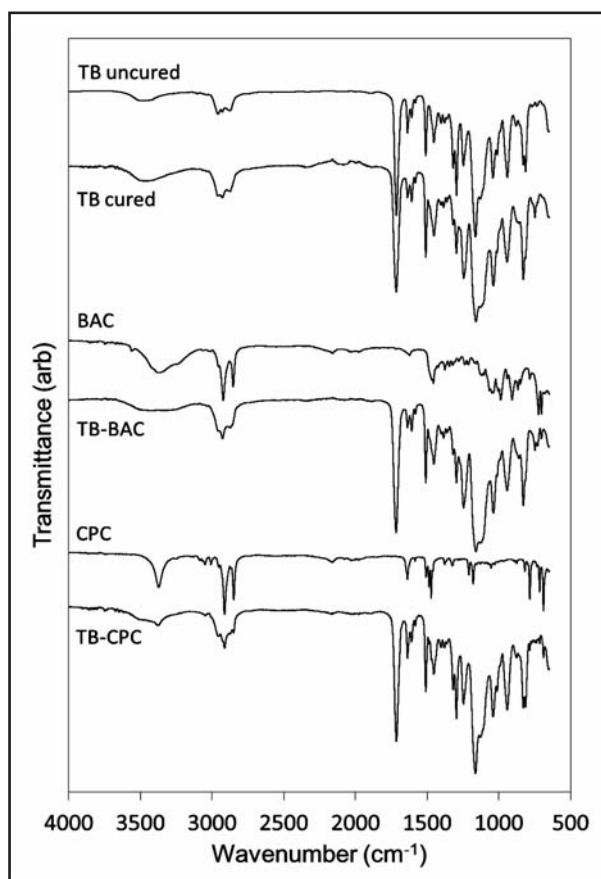


Fig. 2: FTIR spectra of cured and uncured TB, BAC, TB-BAC, CPC and TB-CPC.

Table 4: Shear bond strength data for TB, TB-BAC and TB-CPC

| Shear Bond Strength (MPa) | | | |
|---------------------------|-----------------------------|---------------------------|---------------------------|
| Sample | Day 1 | Day 7 | Day 14 |
| TB | 21.08 ± 4.51 ^{A,B} | 13.76 ± 8.67 ^A | 12.55 ± 4.87 ^A |
| TB-BAC | 23.51 ± 3.46 ^B | 19.63 ± 5.85 ^B | 24.51 ± 7.18 ^B |
| TB-CPC | 17.97 ± 4.21 ^A | 20.09 ± 3.99 ^B | 20.73 ± 6.45 ^B |

*In each column, different capital letters indicate significant differences between the groups (p<0.05) whereas the same letters indicate no significant differences (p>0.05).

Table 5: Distribution of adhesive remnant index scores for TB, TB-BAC and TB-CPC

| Sample (n=10) Frequency (and range) of ARI scores | | | | | | | | | | | | |
|---|---------|---|---|---|-----------|---|---|---|---------|---|---|---|
| Sample | Day 1 | | | | Day 7 | | | | Day 14 | | | |
| | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| TB | 2 | 4 | 2 | 2 | 4 | 1 | 1 | 4 | 2 | 2 | 4 | 2 |
| Median (Range) | 1 (0-3) | | | | 1.5 (0-3) | | | | 2 (0-3) | | | |
| TB-BAC | 0 | 6 | 3 | 1 | 1 | 3 | 3 | 3 | 2 | 4 | 2 | 2 |
| Median (Range) | 1 (1-3) | | | | 2 (0-3) | | | | 1 (0-3) | | | |
| TB-CPC | 1 | 2 | 7 | 0 | 1 | 6 | 2 | 1 | 1 | 6 | 2 | 1 |
| Median (Range) | 2 (0-2) | | | | 1 (0-3) | | | | 1 (0-3) | | | |
| p | 0.147 | | | | 0.145 | | | | 0.704 | | | |

The frequency distribution data for the ARI scores for samples TB, TB-BAC and TB-CPC as functions of storage time in distilled water are listed in Table 5. Differences in the median scores were observed among the groups, but, statistically, these differences were not found to be significant. These ARI data indicate that the incorporation of either BAC or CPC in Transbond™ primer has no impact on the mode of bracket failure under applied shear force.

DISCUSSION

Plaque accumulation around fixed orthodontic appliances is not easily removed by the patient and may cause enamel demineralisation, gingival inflammation and bracket debonding¹⁻³. Currently, the majority of commercial orthodontic primers, adhesives and luting agents exhibit limited or no intrinsic defence against bacterial accumulation^{8-10,14,15}. One strategy to address this problem is the direct incorporation of antimicrobial compounds into these bonding agents^{8-10,14,15}. In this respect, broad-spectrum antimicrobial cationic quaternary

ammonium compounds (QACs) are popular candidates. These compounds can be introduced either as a polymerisable monomer (e.g. methacryloyloxydodecylpyridinium bromide (MDPB)) which is permanently incorporated into the polymer matrix of the bonding agent¹⁹, or as an admixed component which is able to be released during service (e.g. benzalkonium chloride (BAC) and cetylpyridinium chloride (CPC))^{10,12}.

A number of studies has indicated that the addition of between 1 and 10 wt% BAC or CPC to orthodontic bonding agents such as glass ionomers, zinc polycarboxylates and methacrylate-based resins can confer enhanced broad-spectrum antimicrobial characteristics against cariogenic bacteria^{10,12}. However, there are also reports that these compounds can compromise the mechanical properties and bond strengths of these systems^{20,21}. In this current study, the antimicrobial compounds, BAC and CPC, were added to the adhesive primer in order to circumvent potential problems arising from the diminished mechanical integrity of the luting material. In addition, it is considered that the sustained release of antimicrobial compounds from the primer which is in direct contact with the enamel surface may be advantageous against bacterial accumulation in the immediate vicinity of the contacting surfaces.

During this investigation Transbond™ primer exhibited no intrinsic microbial resistance to *S. mutans* under the selected experimental conditions during the zone of inhibition agar diffusion test (Table 1). The literature contains few, yet conflicting, reports of the intrinsic antimicrobial properties of Transbond XT™ primer *in vitro*. Research by Çatalbaş *et al.*⁹ suggests that Transbond XT™ affords microbial resistance to *S. mutans*; although, the common consensus is that it possesses negligible inherent antimicrobial activity against this microorganism^{10,22}. The findings of the current study concur with this latter viewpoint. It should be noted that, the semi-quantitative zone of inhibition agar diffusion tests are not standardised, and that different experimental parameters (e.g. concentration of colony forming units, bacterial strain, incubation time, sample dimensions, type of growth medium and agar) impact upon the apparent antimicrobial activities of materials *in vitro*.

In a previous study, Al-Musallam *et al.*¹⁰ demonstrated that the addition of 10 wt% CPC to the Transbond

XT™ primer effected sustained release and antimicrobial activity against *S. mutans* throughout a 196-day observation period. The 14-day CPC release behaviour and antimicrobial activity of the TB-CPC system observed in the present study confirm these findings. The current study also indicates that BAC may be a more potent antimicrobial agent than CPC in the inhibition of *S. mutans* when incorporated into the Transbond XT™ primer, since the clear zones around TB-BAC were significantly greater than those around TB-CPC even though there was little difference between the release profiles of the two antimicrobial agents. The ongoing integrity and adhesive strength of modified bonding systems is of paramount clinical importance to the successful long-term functionality of fixed orthodontic appliances. *In vitro* shear bond strength analysis using bovine enamel is a convenient and commonly employed method to compare orthodontic adhesive systems²³. A number of studies has indicated significant differences between the SBS of human and bovine enamel, which appear to depend predominantly upon the method of enamel preparation, the nature of the adhesive system and the storage regime²³⁻²⁵. However, in many cases, for a given experimental procedure, a proportional relationship is reported between the SBS of human and bovine enamel which supports the use of bovine teeth as a relative proxy for human teeth in orthodontic adhesive bonding studies^{24,25}. It should be noted that there is some evidence to indicate a relationship between *in vitro* bond strength and the longevity of orthodontic restorations; although, at present, *in vitro* SBS tests cannot be used for direct predictions of clinical outcomes^{26,27}.

As previously mentioned, the incorporation of antimicrobial QACs can have a deleterious

impact on the mechanical properties and bonding strengths of orthodontic cements and adhesives^{20,21}. Furthermore, the incorporation of additives and modifications to orthodontic adhesives that increase the ARI score are generally regarded as unfavourable with respect to the ease and safety of removal of the residual resin remnants after debonding²⁸. The shear bond strength analysis carried out in this study indicated that after 7 and 14 days, both modified Transbond XT™ primer samples exhibited significantly higher SBS values than those of the control, despite the sustained release of the QACs from the resin matrix (Table 4). In this case, the improvement in SBS on addition of 10 wt% of either BAC or CPC was accompanied by no significant change in the mode of bracket failure under shear force (Table 5).

It is argued that polymerisable QAC monomers that are permanently immobilised within the adhesive resin matrix generally exhibit lower bactericidal efficacy than their 'free' QAC antimicrobial counterparts²⁹. In addition, permanently incorporated QACs may provide defence against biofilm formation on the resin surface, but they do not afford any sustained release of antimicrobial agents in the vicinity of the enamel. Hence, the direct incorporation of cheap 'over the counter' QAC compounds, such as BAC and CPC, offers clinicians a cost-effective, convenient method for enhancing the antimicrobial characteristics of orthodontic adhesives. In this respect, the incorporation of 10 wt% BAC or CPC in Transbond XT™ adhesive primer has been shown to confer significant antibacterial activity, sustained release and superior shear bond strength with no significant change in the mode of bracket failure under shear stress.

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