Chemical and Mechanical Removal of Staphylococcus Aureus from Orthodontic Retainers

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ABSTRACT

Aim. Recent studies have indicated that non-oral microorganisms are able to colonise intraoral devices such as orthodontic retainers. The aim of this study was to evaluate the effectiveness of chemical and mechanical elimination of Staphylococcus aureus biofilms (MRSA) from thermoplastic discs which were used to represent orthodontic retainers.

Methods and Results. Methicillin-resistant Staphylococcus aureus biofilms were grown on 5mm diameter thermoplastic copolyester discs in a Constant Depth Film Fermentor (CDFF). The efficacy of biofilm removal by Corsodyl, Steradent Active Plus, Endekay and Retainer Brite solutions were compared to a saline control against 1 and 2 day old biofilms. The discs containing the MRSA biofilms were exposed to each solution for 10, 30 and 60 minutes. All 4 antimicrobial agents were able to significantly reduce the numbers of viable organisms within the biofilm with Corsodyl showing the highest reduction in each case. Mechanical intervention was also investigated by using a commercially available sonicator. The combination of using both an antimicrobial agent and sonication showed no significant difference when compared to using the antimicrobial agent alone.

Conclusion. This study shows that chlorhexidine based mouthwash is the best chemical agent at reducing biofilm on the vacuum formed retainer, however residual microorganism retention could lead to re-growth and re-colonisation. Therefore, it is essential to look at other methods of cleaning such mechanical means to enhance the effect of chemical elimination of biofilm.

Key Words: Not available


INTRODUCTION

The human oral cavity provides an ideal environment for a wide variety of biofilms to form. A biofilm can form on any oral surface including those such as intraoral devices.¹ All the intraoral orthodontic appliances, including retainers, generate microbial biofilms which can infringe on the oral health of the patient.² Recent studies have indicated that non-oral microorganisms are able to colonise such intraoral devices.³ Indeed, Staphylococcus spp. was isolated from 50% of the orthodontic retainers and comprised over 8% of the viable microbiota.³ Staphylococcus species are the most common cause of mild infections such as impetigo and are also the cause of invasive diseases such as wound infections and osteomyelitis. Retainers could therefore be a reservoir for opportunistic pathogens and act as a source of cross-, self- and re-infection.

Although currently there is no evidence to link retention of opportunistic pathogens on orthodontic retainers and significant infectious diseases, biofilm formation near the gingival margin and subsequent prolong inflammation can result in breakdown of bone.⁴ Considering increased number of orthodontic patients that require long term retention to prevent relapse, colonisation of orthodontic retainers by opportunistic pathogens can affect oral health of the patients.³ Effective orthodontic retainer hygiene is therefore an important factor for maintaining oral health as well as the overall wellbeing of a patient. Removable orthodontic retainers such as vacuum formed
retainers and Hawley retainers can be cleaned mechanically, chemically, or by combination of the two. There are a variety of commercially available antimicrobial agents which can be used. However to date there is no evidence that shows the effectiveness of these antimicrobial agents in eliminating microorganisms on the surfaces of orthodontic retainer materials. This is especially important given the recent findings of opportunistic microorganisms being present in relatively high numbers on these devices. Furthermore, there has been significant interest in supplementing chemical agents with mechanical cleaning to achieve adequate plaque control given the resistant nature of biofilms.5

There has been recent interest in assessing the recalcitrance of MRSA biofilms using current therapies.6 However, there is no published literature to describe the effectiveness of the chemical elimination of Methicillin resistant Staphylococcus aureus on vacuum formed orthodontic retainer material. This laboratory-based study investigated the effectiveness of four types of antimicrobial agents and also evaluated the effect of ultrasonic vibration as a mechanical intervention in reducing the number of bacteria on the material.

MATERIALS AND METHODS

Production of biofilms
The Constant Depth Film Fermentor (CDFF; AC Service Group, Poole, UK) described by Pratten and Ready was used in this study to produce biofilms in a steady-state for antimicrobial testing.7 The glass vessel containing a stainless steel turntable holding 15 polytetrafluoroethylene (PTFE) sampling pans was used. Each sampling pan contained five thermoplastic copolyester discs which were prepared from an Essix ACE® plastic (Dentsply Raintree Essix Glenroe, FL 34243 USA). The discs were arranged around a central hole and recessed to a depth of 300µm. Methicillin resistant Staphylococcus aureus-15 (clinical isolate) inoculum and artificial saliva formulated by Russell and Coulter were allowed to drip onto the turntable holding the sampling pans (Figure 1) at the rate of 0.5ml per minute.8 The stainless steel turntable rotated under scraper bars maintaining a constant predetermined biofilm depth below the surface. Using a specially adapted tool, each of the pans was removed aseptically at specific time intervals via a sample port.

![Figure 1. Stainless steel tumtable containing sampling pans](image)

Effect of antimicrobial agents
Three series of experiments at 10, 30, and 60 minutes were carried out to analyse the effectiveness of exposure of the 4 antimicrobial agents (Corsodyl, Steradent Active Plus, Endekay and Retainer Brite) and a control (Phosphate buffered saline) to the MRSA biofilms. These chemical agents were prepared as described in manufacturer’s instruction.

The active ingredients and manufacturer’s details are listed in Table 1. The biofilms had been established for either 1 or 2 days on the surface of the thermoplastic copolyester discs.

Five sampling pans at a time were removed aseptically from the fermentor via the sampling port at prescribed time intervals. Each sampling pan containing five thermoplastic copolyester discs was immersed into the appropriate solutions for either 10, 30, or 60 minutes. The pans were then carefully removed from the solutions and the discs removed and vortexed in 1ml neutralising broth (DifcoBD, Oxford, UK) for 1 minute. Viable cells were enumerated via viable counting on Columbia blood agar (Oxoid, Basingstoke, UK).

Testing the effects of mechanical intervention
Two series of experiments were carried out to analyse the effectiveness of mechanical intervention (sonication) in eliminating MRSA biofilms. All the procedures in this experiment were similar to the previous experiments except the addition of mechanical intervention. In the first
experiment, five sampling pans were removed aseptically from the fermentor via the sampling port 1 and 2 days after the start of biofilm production. Each sampling pan was immersed into the cordless sonic cleaner (Ortho-Care Ltd, UK) containing the test solutions for 60 minutes. The sonic cleaners were switched on prior to the immersion.

The second experiment was carried out to analyse the effect of sonication alone. 2 day biofilms were immersed in Phosphate buffered saline (PBS) as a neutral solution. The first sampling pan (control) was immersed into the PBS for 120 minutes with the sonic cleaner turned off. The second, third and fourth sampling pans were immersed into the PBS for 30, 60 and 120 minutes with the sonic cleaner switched on.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Active ingredients</th>
<th>Manufacturers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corsodyl mouthwash</td>
<td>Chlorhexidine gluconate 0.2%, ethanol 7%, sorbitol, peppermint oil</td>
<td>GlaxoSmithKline, Brentford, Middlesex, UK</td>
</tr>
<tr>
<td>Endekay mouthwash</td>
<td>Sodium fluoride 0.05% ethanol, sorbitol, peppermint oil</td>
<td>Manx Pharma Limited, Warmick, UK</td>
</tr>
<tr>
<td>Retainer Brite tablet</td>
<td>Potassium persulfate, sodium perborate, sodium bicarbonate, sodium sulphate, sorbitol</td>
<td>Ortho-Care, West Yorkshire, UK</td>
</tr>
<tr>
<td>Steradent tablet</td>
<td>Sodium bicarbonate, sodium sulphate, sodium carbonate peroxide, potassium caroate</td>
<td>Reckitt Benckiser Healthcare, Slough, UK</td>
</tr>
</tbody>
</table>

**Statistical analysis**

Analysis of variance (ANOVA) followed by Bonferroni post hoc tests were performed to analyse the differences in mean volume of colony forming units per millilitre between the control group and the various chemical agent groups. Data were analysed using SPSS (Statistical Package for the Social Sciences, IBM, USA) software and the p-value was set at <0.05.

**RESULTS**

Figure 2 shows mean colony forming unit (cfu)/biofilm of 1 and 2 day old MRSA biofilms on the discs surfaces after 60 minutes exposure to the various antimicrobial agents. Corsodyl recorded the lowest cfu/biofilm for both 1 and 2 day old biofilms (1.90 x 10^6 and 1.78 x 10^7) while Endekay resulted in the highest cfu/biofilm (2.73 x 10^7 and 3.83 x 10^8) only second to the control.

The steradent Active Plus and Retainer Brite also showed some reduction in the mean cfu/biofilm on the discs surfaces. There were statistically significant differences between the mean log cfu/biofilm of the control (PBS) and each of the four antimicrobial agents (p<0.05 with 95% confidence).

Overall, the mean cfu/biofilm after 60 minutes exposure to each of different chemical agents was lower than the mean cfu/biofilm resulted from 10 minutes and 30 minutes exposures (data not shown). As expected all the four chemical agents recorded higher mean cfu/biofilm for the 2 day old biofilms than the 1 day old biofilms.

Figure 3 shows mean cfu/biofilm found on the thermoplastic copolyester discs after sonication using the cordless’ sonicator in PBS solution for 30, 60 and 120 minutes. The control (120 minutes exposure to PBS without sonication) produced 3.43x10^9 cfu/biofilm. The lowest mean cfu/biofilm was recorded by the disc which was sonicated in PBS for 120 minutes (1.63x10^8 cfu/biofilm) and followed by 60 minutes (2.68 x 10^8 cfu/biofilm) and 30 minutes (3.32 x 10^8 cfu/biofilm).

The mean cfu/biofilm decreased with an increase in sonication time. There was still large number of MRSA viable cells retained on the disc surfaces even after 120 minutes sonication although there were statistically significant differences between the mean log cfu/biofilm of the control (120 minutes in PBS without sonication) and three other groups (p<0.05 with 95% confidence).
Table 2. Colony forming units per biofilm of S. Aureus of 1 and 2 day old biofilms after 60 minutes exposure in various chemical agents. PBS = white bar, Endekay = diagonal lines, Coraodyl = dots, Steradent = horizontal stripe, Retainer Brite = grey bar, Error bars represent standard deviation

Figure 3. Colony forming unit per ml of 2 day old MRSA biofilms after sonication in PBS. Error bars represent standard deviation

Figure 4 shows mean cfu/biofilm of 24 and 48 hour old MRSA biofilms on discs surfaces after 60 minutes exposure to various antimicrobial agents in a ‘cordless’ sonicator. Corsodyl recorded the lowest mean cfu/biofilm for both 1 and 2 day (1.42 x 106 and 8.85 x 106cfu/biofilm) biofilms and followed by Steradent Active Plus (7.93 x 106 and 6.05 x 107cfu/biofilm). Among the four antimicrobial agents, Endekay resulted in highest mean cfu/biofilm (2.73 x 107 and 3.83 x 108) followed by
Retainer Brite (2.08 x 10^7 and 2.25 x 10^8 cfu/biofilm). None of the samples however showed clinically significant reduction in the mean cfu/biofilm compared to the samples which were exposed only to the antimicrobial agents for 60 minutes. However, there were statistically significant differences between the control (PBS) and the four antimicrobial agents (p<0.05 with 95% confidence) for both 1 and 2 day tests.

**Figure 4.** Colony forming unit per biofilm of MRSA biofilms after 60 minutes exposure to antimicrobial agents in combination with a ‘cordless’ sonicator. No agent or sonication = white bar, Endekay/sonication = diagonal lines, Cardosyl/sonication = dots, Steredent/sonication = horizontal stripe, Retainer Brite/sonication = grey bar. Error bars represent standard deviations

**DISCUSSION**

There were two aims to this study. Firstly, we investigated the effectiveness of antimicrobial agents alone in eliminating Methicillin resistant Staphylococcus aureus15 (MRSA-15) grown on the surface of discs made of thermoplastic copolyester sheets. The second part of the study investigated the effect of adding mechanical intervention using ultrasonic vibrations. MRSA-15 was chosen for these studies as a recent study has shown that opportunistic pathogens such as S. aureus, including methicillin-resistant strains of the organism colonise intraoral devices.1 The biofilms were grown on the surface of thermoplastic copolyester discs in a Constant Depth Film Fermentor (CDFF). The CDFF provides an environment similar to the human oral cavity. It also allows sampling of multiple biofilms at various intervals. Environmental factors such as nutrients, mechanical forces and temperature can be controlled to provide biofilms which are similar to those found in the mouth. The action of scraper blades simulates the masticatory and tongue movements which continuously remove the outmost layers of the oral biofilm. The maximum number of biofilm samples per cycle is limited to 75 and as such it is not possible to test more than 4 antimicrobial agents per cycle in order to have a reliable sample size and undertake at least 6 repeated measurements. The four antimicrobial agents were chosen for this study was based on their different chemical composition to each other. The results demonstrated that Corsodyl mouthwash was the most effective antimicrobial agent in eliminating MRSA biofilms on the disc surface. However further research is needed to study the staining effect of corsodyl mouthwash on the Essix retainer surfaces. To date there is no published data that describe the staining effect of corsodyl mouthwash on the Essix retainer.

Although increasing exposure time to the antimicrobial agents resulted in further killing of
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For all agents, there were still clinically significant amounts of viable MRSA cells present on the disc surfaces.

The outcome of this study demonstrates that even the most potent antimicrobial agent tested (Corsodyl mouthwash) was unable to completely eliminate the MRSA from the disc surfaces. Indeed, other studies have suggested that biofilm-specific resistant phenotypes may lead to the expression of protective mechanisms to combat the harmful effects of antimicrobial agents.5,10 It was clear from this study that an intact biofilm structure prevented total elimination of the MRSA cells from the discs after antimicrobial exposure and adding mechanical intervention by using a sonicator was not effective in disrupting the MRSA biofilms to increase their susceptibility to the antimicrobial agents. The likely explanation of this is that the strength of the mechanical vibration provided by the 'cordless' sonicator was not be adequate to disrupt the complex structure of the biofilm.

Corsodyl mouthwash is the most potent antimicrobial cleaning agents tested. Corsodyl mouthwash, Steradent Active Plus, Retainer Brite and Endeka mouthwash resulted in statistically significant reduction in the number of viable MRSA cells on thermoplastic copolyester disc surfaces. However, there were clinically significant numbers of viable MRSA cells still present on the discs surface after exposure indicating that this method alone is not effective in cleaning retainers. The residual microorganism retention could lead to re-growth and re-colonisation of the retainers and can affect oral health of the patients in the long term.3 Further research therefore still needs to be carried out to explore other methods of cleaning such as mechanical means to enhance the effect of chemical elimination of biofilm.

However these initial findings may be sufficient to suggest that patients should be informed to clean their retainers with an antimicrobial agent to reduce the numbers of harmful bacteria which may be present.

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