ANTIMICROBIAL EFFECTIVENESS OF SILVER-RELEASING ELASTOMERIC TIES

MATTHEW C. O’DELL

CLINICAL DENTISTRY

ABSTRACT

**Introduction:** Fixed appliances make satisfactory oral hygiene a greater challenge for the orthodontic patient. Improper oral hygiene during orthodontic treatment can cause enamel demineralization resulting in unesthetic white spot lesions. A new approach to providing protection against demineralization during treatment has been to incorporate antimicrobial agents into elastomeric ligature ties. A recent elastomeric ligature tie uses the antimicrobial characteristic of silver to help prevent formation of white spot lesions. The purpose of this in vitro study is to determine the antimicrobial effectiveness of a silver-releasing elastomeric ligature tie (OrthoShield Safe-T-Tie) against *Streptococcus mutans* and compare its results with a selenium-containing elastomeric (SeLECT Defense™) and a traditional non-antimicrobial elastomeric ligature tie (AlastiK™ Easy-To-Tie Ligatures).  

**Methods:** Two methods were used to evaluate the antimicrobial effectiveness of the elastomerics, agar diffusion and spectrophotometry. For the agar diffusion test, elastomerics were placed on *S. mutans* streaked agar plates containing Todd Hewitt (TH) Broth in .3% (3g/l). Plates were incubated (10% H₂, 10% CO₂, 80% N₂) at 37° C for a 24 hour period and then evaluated for zones of inhibition surrounding the elastomerics. For the spectrophotometer test, elastomerics were placed in test tubes containing 500 µl and 100 µl of *S. mutans* culture and incubated for six or eight hours. Optical densities of the *S. mutans* cultures were measured for each test tube by spectrophotometer (SmartSpec Plus Spectrophotometer) to determine bacterial cell
densities following incubation. Bacterial cell densities between groups were statistically analyzed using a non-parametric Wilcoxon Test. Generation times were calculated from cell density readings. **Results:** For the agar diffusion test, there were no measurable or visibly detectable zones of inhibition around any of the elastomeric samples. For the spectrophotometer test, silver-releasing elastomerics showed a statistical difference from the traditional elastomeric group in the 100 µl test but not the 500µl test. None of the elastomerics tested effectively reduced the generation times for a *S. mutans* cell population. **Conclusion:** Neither the silver-releasing nor silver-containing elastomeric ligature ties were effective in inhibiting growth of *Streptococcus mutans* in-vitro.
ACKNOWLEDGEMENTS

The author thanks Drs. John Ruby, Amjad Javed, Christos Vlachos, Andre Ferreira, and the faculty members and residents of the Department of Orthodontics, University of Alabama School of Dentistry, Birmingham, Alabama.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Oral Hygiene and Orthodontics</td>
<td>1</td>
</tr>
<tr>
<td>White Spot Lesions</td>
<td>2</td>
</tr>
<tr>
<td>Prevalence</td>
<td>2</td>
</tr>
<tr>
<td>Demineralization Process</td>
<td>3</td>
</tr>
<tr>
<td>Prevention of White Spot Lesions</td>
<td>4</td>
</tr>
<tr>
<td>Patient Oral Hygiene</td>
<td>4</td>
</tr>
<tr>
<td>Fluoride</td>
<td>5</td>
</tr>
<tr>
<td>Fluoride Elastomers</td>
<td>9</td>
</tr>
<tr>
<td>Antimicrobial Technology in Orthodontic Elastomers</td>
<td>11</td>
</tr>
<tr>
<td>Silver as an Antimicrobial Agent</td>
<td>12</td>
</tr>
<tr>
<td>Present Study</td>
<td>14</td>
</tr>
<tr>
<td>MATERIAL AND METHODS</td>
<td>15</td>
</tr>
<tr>
<td>Agar Diffusion Test</td>
<td>15</td>
</tr>
<tr>
<td>Spectrophotometer Test</td>
<td>16</td>
</tr>
<tr>
<td>Data Analysis</td>
<td>16</td>
</tr>
<tr>
<td>RESULTS</td>
<td>17</td>
</tr>
<tr>
<td>Agar Diffusion Test</td>
<td>17</td>
</tr>
<tr>
<td>Spectrophotometer Test</td>
<td>19</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>22</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>25</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>26</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean Cell Densities – 500 µl test</td>
</tr>
<tr>
<td>2</td>
<td>Mean Cell Densities – 100 µl test</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SRE plate after 24 hours incubation</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>SRE after 24 hours incubation</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>SCE plate after 24 hours incubation</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>SCE after 24 hours incubation</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>Traditional plate after 24 hours incubation</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>Traditional elastomeric after 24 hours incubation</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>Mean generation times after six hours incubation</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>Mean generation times after eight hours incubation</td>
<td>21</td>
</tr>
</tbody>
</table>
INTRODUCTION

Oral Hygiene and Orthodontics

The objectives of orthodontic treatment include not only achieving an optimum functional occlusion and esthetic smile, but also a healthy, disease free oral environment. Too often an ideal orthodontic occlusal result is tarnished by oral hygiene neglect. Improper oral hygiene during orthodontic treatment can result in white spot lesions, caries, and unhealthy periodontal tissues.

Fixed appliances make satisfactory oral hygiene a greater challenge for the orthodontic patient. Brackets, archwires, and ligatures complicate the use of conventional oral hygiene measures and promote areas of significant plaque accumulation. Tooth surfaces that were normally easy to clean become difficult to access for the patient once orthodontic appliances are in place. Specifically, orthodontic elastomeric ligature ties offer an area for increased dental biofilm accumulation that could be harmful to the teeth and gingival tissues. Souza et al. concluded in a study comparing two methods of archwire ligation that elastomeric ligatures promote significant retention of biofilm, cause significant clinical alternations of the plaque index and gingival bleeding index, and increase microorganisms associated with periodontal disease. Another study found that self-ligating appliances promoted less retention for Streptococci bacteria compared with elastomeric appliances.

Orthodontic appliances also provide a physical barrier that can block saliva flow to areas of plaque. This is important because saliva is responsible for the clearance of
plaque and encouraging a lower plaque pH in the presence of carbohydrates.\textsuperscript{4,5} These factors, along with length of treatment usually lasting two years, make oral hygiene of paramount importance to the patient and the orthodontist.

White Spot Lesions

Prevalence

Areas of enamel demineralization resulting in the formation of unesthetic white spot lesions are a common problem associated with orthodontic treatment. The reported overall prevalence of white spot lesions amongst orthodontic patients ranges from 2 to 96 per cent.\textsuperscript{4} Gorelick et al\textsuperscript{6} found that enamel demineralization after orthodontic treatment can involve up to 50\% of patients. Mizrahi et al\textsuperscript{7} completed a cross-sectional study that consisted of 527 patients examined prior to and 269 patients following orthodontic treatment. Results showed a significant increase in both the prevalence (72\%-84\%) and severity of enamel opacities after treatment.

Incipient lesions are often situated on the buccal aspects of teeth which normally show a low prevalence of caries.\textsuperscript{5,8} This can greatly decrease patient satisfaction concerning orthodontic treatment. One study showed that the frequency of white spot lesions on the facial surfaces of maxillary lateral incisors in orthodontically treated vs untreated patients was 25.5\% to 2.2\% respectively. Also, maxillary lateral incisors and canines had a higher frequency of white spot lesions than did maxillary first or second premolars.\textsuperscript{9} The high prevalence and esthetic location of orthodontic white spot lesions makes this a serious issue for the orthodontist and the orthodontic patient.
Demineralization Process

Dental caries develops overtime from a multitude of factors. Bacteria, host properties, and environmental factors such as diet combine to play a role in the development of dental caries. The earliest stage of a carious lesion is seen as an enamel opacity, or white spot lesion. An enamel opacity is due to subsurface mineralization with an increase in porosity and consequential changes in the optical properties of the enamel.  

The demineralization process is initiated by adherence of plague to the enamel surface. The two main groups of bacterial involved in the process are Streptococci mutans and lactobacillus. Rosenbloom and Tinanoff evaluated salivary Streptococcus mutans levels in patients before, during and after orthodontic treatment. S. mutans levels were significantly higher during active treatment for patients in fixed appliances compared to control groups. S. mutans is an acidogenic bacterium that possesses the ability to synthesize extracellular glucans from dietary sucrose. This process increases the cariogenicity of plaque by enhancing plaque mass. High levels of these acidogenic bacteria decrease the pH of the oral cavity and begin a series of chemical and physical reactions involved in the demineralization process. First, surface softening occurs where interprismatic substance is removed with mineral loss being most pronounced at the enamel surface. Next, organic acids diffuse into the subsurface enamel causing dissolution of calcium and phosphate. Porosities in the enamel rods alter the enamel refractive index in the affected area creating areas of enamel opacities.

The caries process is dynamic in nature. As the pH of the oral environment fluctuates, periods of demineralization and remineralization occur. Remineralization occurs when pH recovers and dissolved calcium and phosphate are allowed to precipitate.
on remaining mineral crystals.\textsuperscript{14} This process is responsible for formation of an apparently intact surface layer covering the body of the subsurface lesion.\textsuperscript{12} The presence of saliva is an important factor in remineralization. Saliva provides calcium, phosphate, proteins and lipids, and antibacterial substances that act as buffers to the acidic environment.\textsuperscript{12} Chang et al.\textsuperscript{4} reported that flow rate, pH, and cleansing capabilities of saliva strongly influence both caries risk and caries activity. Remineralization is a slower process than demineralization. It can reverse the damage done by demineralization if enough time allowed. It is when the demineralization process dominates, as is often the case with poor hygiene in orthodontics, that white spot lesions can occur. If there is an absence of the remineralization process for enough time, areas of demineralization can ultimately progress to carious lesions.\textsuperscript{14}

**Prevention of White Spot Lesions**

*Patient Oral Hygiene*

The most common cause of white spot lesions is poor oral hygiene. Chapman et al.\textsuperscript{15} found that patients with fair or poor pretreatment oral hygiene had 3 times the incidence of at least 1 white spot lesion compared with patients with good pretreatment oral hygiene. Methods of patient oral hygiene include brushing, flossing, oral irrigation devices, rinses, and other interdental aids. Toothbrushing is the best way to remove plaque on a daily basis.\textsuperscript{10} The option exists now for manual or electric toothbrushes. On the buccal and lingual surfaces, studies have shown that powered toothbrushes remove plaque more effectively than manual.\textsuperscript{16} Electric toothbrushes have the ability to increase patient motivation while offering an easier alternative to manual brushes.\textsuperscript{17}
In order for oral hygiene methods to be effective, good patient compliance is essential. Enamel demineralization can be a fast moving process. Ogaard et al. found that visible white spot lesions could form around ill-fitting orthodontic bands in as little as four weeks. Unfortunately, patient compliance is a frequent problem in orthodontics. Studies have shown that patient compliance in long term treatments such as orthodontics is only 50%. Boyd found 10-20% of adolescent patients had inadequate plaque removal during treatment. In a study by Geiger et al. testing the effects of a fluoride program on white spot formation, home care preventive protocol was judged to be poor in 52.5% of patients and another 20.8% of patients were only partially compliant. Geiger found in another study that only 13% of patients fully complied with a fluoride rinse protocol even after education efforts and a free supply of rinse. Orthodontic treatment is prematurely terminated on average in 5-10% of patients due to poor compliance. Even with appropriate patient education and motivation techniques by the orthodontist, patient cooperation with hygiene regimens remains problematic.

Fluoride

Fluoride has played an important role in reducing the incidence of dental caries for many years. The most notable effect has been through fluoridation of public water supplies. In orthodontics, the use of supplemental fluoride has also been used extensively in the prevention of enamel demineralization. The mechanisms of fluoride include: 1) inhibition of demineralization at the crystal surfaces inside the tooth 2) enhancement of remineralization at the crystal surfaces, and 3) inhibition of bacterial enzymes.
The mechanism by which fluoride inhibits demineralization is by reprecipitation of dissolved calcium and phosphate, thereby preventing these constituents from being leached out of the enamel into the plaque and saliva.\(^2\) When fluoride is present in the acid solution surrounding enamel mineral crystals, it is adsorbed strongly in the crystals and acts as a potent protection mechanism against acid dissolution of the crystal surface.\(^2\) This mechanism allows fluoride present in the plaque fluid at the time bacteria acid is generated to travel with the acid into the subsurface of a tooth and protect against being dissolved.\(^2\)

The principal action of fluoride is remineralization. When pH rises in the mouth, the demineralization process is slowed down and saliva saturated with calcium and phosphate allows minerals to go back into the tooth.\(^1\) When fluoride is present, it enhances this phenomenon by adsorbing to the surface and attracting calcium ions.\(^2\) As fluoride is introduced into this solution of calcium and phosphate, a new crystalline structure of fluorapatite \(\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2\) is formed instead of the natural occurring hydroxyapatite \(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\).\(^3\) The new surface of fluorapatite created is less soluble and more acid resistant.

Bacteria metabolism is inhibited when fluoride is taken up by the bacteria when they produce acid. Fluoride inhibits the enzyme enolase which is a bacterial enzyme responsible for breakdown of fermentable carbohydrates.\(^2\) This prevention of sugar breakdown and acid production is beneficial in arresting the caries process.

Topical fluoride therapies such as toothpastes, rinses, gels, and varnishes have been shown to reduce decalcification during orthodontic treatment.\(^4\) In the past, the most widely accepted method of topical fluoride toothpaste delivery was through conventional
fluoride toothpaste at 1000 ppm. More recent studies have shown that 5000 ppm fluoride toothpastes may be more effective in the reduction of demineralization. O’Reilly and Featherstone conducted a study to evaluate the ability of commercially available fluoride products to inhibit and/or reverse orthodontically related demineralization. They found demineralization occurred around orthodontic appliances after only 1 month even with the use of a proven fluoride dentifrice. Although, they further concluded that daily brushing with a fluoridated dentifrice, coupled with daily rinsing with a fluoride (0.05% sodium fluoride) mouthrinse, will provide complete protection by preventing demineralization or by promoting remineralization.

Multiple other studies have evaluated the use of fluoride rinses. One clinical study instituted a fluoride rinse program that involved using a daily fluoride rinse immediately before bedtime. The author concluded that a significant reduction in enamel white spot lesions can be achieved during orthodontic therapy through the use of a 10 ml neutral sodium fluoride rinse. The reduction achieved also depended on how much the patient complied with the prescribed use; the greater compliance seen the more likely they could expect a decrease in the occurrence of lesions. Alexander and Ripa conducted a study comparing the effectiveness of toothbrushing in combination with fluoride rinsing, fluoride gel brushing, and fluoride gel dentifrice brushing. The results indicated that a 1000-ppm dentifrice and an over-the-counter rinse have a very good effect in preventing demineralization. However, a greater degree of protection was provided by the daily use of a 5000-ppm fluoride gel along with toothbrushing with a fluoride paste or brushing twice daily with a 5000-ppm fluoride dentifrice alone. A
separate study found that a one-time topical application of acidulated phosphate fluoride
gel immediately after bonding had little benefit in reducing the incidence of white spots.\textsuperscript{19}

Another method of fluoride delivery is through professional applied fluoride
varnishes. This is beneficial because it allows patient compliance to be removed from the
fluoride delivery regimen. Todd et al\textsuperscript{29} performed a study to evaluate the ability of the
product Duraflor, a varnish containing 5\% sodium fluoride, to directly inhibit
demineralization of enamel surrounding orthodontic brackets. Results showed that the
teeth treated with Duraflor exhibited 50\% less demineralization than nontreated controls.
In a split mouth study conducted by Øgaard et al\textsuperscript{30}, orthodontic bands were attached to
premolars to be extracted for orthodontic reasons to induce enamel caries on the buccal
surfaces. Fluoride varnish was applied to 1 tooth of each pair with the contralateral
serving as the control. After 4 weeks the teeth were extracted and evaluated. Results
showed fluoride varnish application reduced enamel lesion depth by 48\% compared to
untreated controls. In a similar designed split mouth study, it was found that topical
application with a high concentration fluoride varnish can decrease enamel lesion depth
adjacent to bonded brackets by about 40\% for 3 months.\textsuperscript{31}

Fluoride-releasing orthodontic bonding agents are an additional option for
fluoride delivery that does not rely on patient compliance. The goal of these bonding
agents is to release steady low levels of fluoride over extended periods of time to aid in
the prevention of enamel demineralization. Gorton and Featherstone\textsuperscript{32} conducted a
double-blind randomized clinical trial to determine whether fluoride-releasing glass
ionomers used for bonding can significantly reduce the overall amount of
demineralization around orthodontic brackets in the mouth. Microhardness tests
concluded that the use of glass ionomer cement did significantly reduce enamel mineral loss due to dental caries around orthodontic brackets in patients’ mouths compared with non-fluoridated composite resin during a 4-week period. Schmitt et al\textsuperscript{33} found in an in-vitro study that teeth bonded with a resin-modified glass ionomer had 50\% smaller lesions depths than teeth bonded with composite resins. One potential drawback to these bonding agents is the inconsistent release of fluoride. Usually the fluoride is maximally released during the first few days and in some cases it can only be measured in minor quantities after 2 or 3 months.\textsuperscript{34}

\textit{Fluoride Elastomerics}

Most fluoride treatments require good patient compliance, increased dental visits, and/or increased chairside time. Fluoride releasing-elastomerics were developed as a fluoride delivery method during orthodontic treatment that does not rely on patient compliance. The goal of these elastomerics, which are impregnated with stannous fluoride (SnF\textsubscript{2}), is to provide a continuous source of fluoride around the orthodontic bracket. Mattick et al\textsuperscript{35} conducted a split mouth randomized clinical trial comparing the effects of fluoride-releasing elastomeric modules versus conventional modules on decalcification. He found that fluoride-releasing elastomerics showed significantly fewer serious decalcified lesions than conventional elastomerics. Similarly, a prospective controlled clinical trial by Banks et al\textsuperscript{36} found fluoride-releasing elastomeric modules and chains reduced post-fixed appliance treatment enamel decalcification scores per tooth by 49 per cent.

Wiltshire\textsuperscript{37} has completed studies that have addressed the claims of continuous
fluoride release from the elastomerics between orthodontic appointments. In an in vitro study of 200 fluoride-containing elastomeric ligature ties, he found fluoride release was characterized by an initial burst of fluoride during the first day and second day, followed by a logarithmic decrease. Of the total available fluoride, 63% had been leached out by the end of the first week and 88% by the second week. In a separate study, Wiltshire\textsuperscript{38} reported that in-vitro testing significantly underestimates the in-vivo residual fluoride release. He found elastomerics imbibed fluoride during treatment and fluoride-impregnated elastomeric ligature ties released significantly more fluoride than nonfluoride ties.

Studies have been conflicting in regards to effect of fluoride elastomerics on the bacterial environment around fixed appliances. Wilson et al\textsuperscript{39} conducted a clinical study of 24 patients to examine the effects of fluoride-releasing elastomerics on salivary \textit{Streptococcus mutans} numbers. The results showed that after the elastomers were placed, the percent of salivary \textit{S. mutans} decreased significantly. Although, no significant effects were seen after the elastomerics were in place for 2 or more weeks. Benson et al\textsuperscript{40} examined the effect on the microbiology of plaque by fluoridated elastomerics. Results showed the elastomerics were not effective at reducing local streptococcal or anaerobic bacterial growth after a clinically relevant time in the mouth.

While fluoride releasing elastomerics have shown to produce some benefit in the reduction of demineralization and bacterial growth around fixed orthodontic appliances, the advantages of these are still uncertain.\textsuperscript{35,36,41} It is questionable as to whether the fluoride release is sustained in the mouth long enough or in sufficient amounts to prevent the formation of white spot lesions. It is recommended that fluoride releasing
elastomerics be used as an adjunct with other oral hygiene methods in the prevention of white spot lesions. \(^{35}\)

**Antimicrobial Technology in Orthodontic Elastomerics**

Because fluoride elastomerics have shown promise but still have shortcomings, it has lead orthodontic companies to look for other solutions to improve elastomerics in the area of white spot prevention. New antimicrobial technologies have begun to be incorporated into orthodontic elastomeric ligature ties.

ClassOne Orthodontics incorporated their SeLECT\(^{TM}\) antimicrobial technology into orthodontic elastomeric ligature ties. This technology uses the properties of selenium for its antimicrobial effect to prevent plaque accumulation around the orthodontic elastomeric and bracket. Specifically, SeLECT\(^{TM}\) technology inhibits the growth of *Streptococcus mutans*, thus reducing enamel demineralization and white spot lesions.

Ortho Organizers also integrated their antimicrobial technology into orthodontic elastomeric ligature ties. Their OrthoShield antimicrobial technology utilizes the antimicrobial properties over silver to alter the microbial environment around fixed orthodontic appliances. Silver is embedded in the ligatures and released via a proprietary mechanism from zeolite to the surface of the elastomerics where it fights and prevents microbial growth. \(^{42}\) This silver release reduces the number of certain organisms related to enamel decalcification and periodontal infection by 99.9% within a few hours and remains active for up to 30 days. \(^{43}\)
Silver as an Antimicrobial Agent

Silver is a metal known for its broad-spectrum antimicrobial action against Gram-positive and Gram-negative bacteria, fungi, protozoa, and certain viruses.\textsuperscript{44,45,46,47} The antimicrobial action of silver is based on its ability to destabilize the bacterial cell wall, interrupt cell metabolism, and inhibit reproduction.\textsuperscript{43} Silver exhibits a strong affinity for zeolite, a porous crystalline material of hydrated sodium aluminosilicate, which can electrostatically bind silver ion up to approximately 40\%.\textsuperscript{48} These zeolite structures provide a good reservoir for the release of antimicrobial cations such as silver over extended periods of time.

Silver has long been used as an antimicrobial agent in medicine in many different aspects. The gold standard in topical burn treatment is silver sulfadiazine (Ag-SD), a useful agent in controlling microbial colonization and preventing burn wound infection.\textsuperscript{49,50} Silver coatings are also used to prevent bacterial colonization on medical devices. Silver coated catheters have been clinically tested with some success in helping prevent nosocomial infections such as urinary tract infections.\textsuperscript{51,52} Kollef et al\textsuperscript{53} found that patients receiving a silver-coated endotracheal tube had a statistically significant reduction in the incidence of ventilator-associated pneumonia (VAP) and delayed time to VAP occurrence compared with those receiving a similar, uncoated tube. Lee et al\textsuperscript{52} even found that silver-containing zeolite has an antimicrobial effect against methicillin-resistant \textit{Staphylococcus aureas} (MRSA).

More recently, silver as an antimicrobial agent has been found to have uses in dentistry. Combining silver into dental products can have a positive influence on bacterial control in the oral environment. Kawahara et al\textsuperscript{48} evaluated the antibacterial
effect of silver-zeolite against oral bacteria under anaerobic conditions. The study determined minimum inhibitory concentrations by using two-fold serial dilutions of silver zeolite in brain heat infusion broth. The findings suggested that silver zeolite may be a useful vehicle to provide antimicrobial activity to dental materials used even under anaerobic conditions such as deep in the periodontal pocket.

Because removable denture bases act as a reservoir for microorganisms that can contribute to infection in denture wearers, Casemiro et al.\textsuperscript{54} conducted a study to evaluate the antimicrobial activity of acrylic resins containing different percentages of silver and zinc zeolite. The antimicrobial activity against two strains of \textit{Candida albicans} and two strains of \textit{Streptococcus mutans} was assessed by agar diffusion methods. The addition of silver-zinc zeolite to acrylic resins showed antimicrobial activity against all bacterial strains. Casemiro concluded that adding silver-zinc zeolites to acrylic resins can be a valuable alternative for reducing microbial contamination of acrylic resin denture bases, acrylic baseplates of removable orthodontic appliances, and other devices.

Kreth et al.\textsuperscript{55} conducted a study on the antimicrobial efficacy of silver ion impregnation into endodontic sealer against \textit{S. mutans}. Growth inhibition studies and bacterial viability tests were performed. The results were obtained by measuring zones of inhibition and optical densities. Results showed that the silver ions enhanced the antimicrobial activity of the root canal sealer against \textit{S. mutans}. This technology has the possibility of reducing the presence of residual bacteria at the time of root canal completion and reducing the number of root canal treatment failures.
Present Study

Incorporation of silver into various medical and dental devices has been shown to be effective as an antimicrobial method. Although new technology has surfaced that incorporates silver into orthodontic elastomeric ligature ties, little research has been conducted to evaluate their efficacy against bacteria associated with orthodontic white spot lesions. The purpose of this in vitro study is to determine the antimicrobial effectiveness of a silver-releasing elastomeric ligature tie (OrthoShield Safe-T-Tie, Ortho Organizers, Carlsbad, CA) against *Streptococcus mutans* and compare its results with a selenium containing elastomeric (SeLECT Defense™, Element 34 Technology, Inc, Lubbock, TX) and a traditional non-antimicrobial elastomeric ligature tie (AlastiK™ Easy-To-Tie Ligatures, 3M Unitek, Monravia, Calif).
MATERIALS AND METHODS

Three elastomeric ligature ties were evaluated. A silver-releasing elastomeric (SRE) (OrthoShield Safe-T-Tie, Ortho Organizers, Carlsbad, CA), a selenium-containing elastomeric (SCE) (SeLECT Defense™, Element 34 Technology, Inc, Lubbock, TX), and a traditional non-antimicrobial elastomeric as a control (AlastiK™ Easy-To-Tie Ligatures, 3M Unitek, Monravia, Calif). All elastomers tested were sterilized using ethylene oxide. Two methods were used to evaluate the antimicrobial effectiveness of the elastomers; agar diffusion and spectrophotometry.

Agar Diffusion Test

*Streptococcus mutans* UA159 was grown anaerobically (Coy Laboratory Products, Inc, Grass Lakes, MI) under conditions 10% H₂, 10% CO₂, 80% N₂ at 37° C in Todd Hewitt (TH) broth for 12 hours. The *S. mutans* culture was streaked on three agar plates containing TH Broth in .3% (3g/l) agar. Elastomers were placed on the plates using sterile technique. Plate one contained three SREs. Plate two contained three SCEs. Plate three contained three traditional elastomerics. All plates were incubated (10% H₂, 10% CO₂, 80% N₂) at 37° C for a 24 hour period. Plates were evaluated for zones of inhibition surrounding the elastomers. Digital photos (Digital Rebel XTi with 100mm lens and ring flash, Canon U.S.A., Inc) were taken to record results.
Spectrophotometer Test

*S. mutans* UA159 was grown anaerobically (Coy Laboratory Products, Inc, Grass Lakes, MI) under conditions 10% H₂, 10% CO₂, 80% N₂ at 37° C in Todd Hewitt (TH) broth for 12 hours. *S. mutans* cell densities were adjusted to an Ab₆₀₀=1 and sent to Beckman Coulter Particle Characterization Laboratory (Miami, FL) for electronic enumeration using a Beckman Coulter MS3 Particle/Size Analyzer (Ab₆₀₀ of 1.0=1 x 10⁹ cells/ml). Twelve test tubes were prepared with 500 µl each of the *S. mutans* culture. One elastomeric was placed in each test tube using sterile technique. Three test tubes contained a SRE, three contained a SCE, three contained a traditional elastomeric, and three test tubes contained no elastomeric. All test tubes were incubated (10% H₂, 10% CO₂, 80% N₂) at 37° C for six hours. Optical densities of the *S. mutans* cultures were measured for each test tube by spectrophotometer (SmartSpec Plus Spectrophotometer, BioRAD, Hercules, CA) to determine bacterial cell densities following incubation.

This protocol was repeated with 100 µl of *S. mutans* culture and incubated for eight hours.

Data Analysis

Spectrophotometer cell density readings were compiled. Bacterial cell densities between groups were statistically analyzed using a non-parametric Wilcoxon Test.

Generation times were calculated from cell density readings by the following equations:

\[
\log_{10} N_r - \log_{10} N_i = \frac{t}{K} \quad \text{and} \quad \frac{t}{K} \times 60 = \text{Generation time (min)}.
\]
RESULTS

In detection of a potential antimicrobial effect of the SRE, two tests were applied: agar diffusion test and a spectrophotometer test.

Agar Diffusion Test

After a 24 hour incubation period, the three plates containing the elastomeric ligature ties were evaluated for zones of inhibition. There were no measurable or visibly detectable zones of inhibition around any of the elastomeric samples. These results suggest that neither the SRE nor SCE groups were successful in inhibiting bacterial growth adjacent the elastomeric ligature ties. Also, there were no differences seen between these groups and the control group of the traditional elastomeric ligature ties. Figures one through six show photographic results of the bacterial growth surrounding the elastomeric samples.

Fig. 1 SRE plate after 24 hours incubation.  
Fig. 2 SRE after 24 hours incubation.
Fig. 3 SCE plate after 24 hours incubation.

Fig. 4 SCE after 24 hours incubation.

Fig. 5 Traditional plate after 24 hours incubation.

Fig. 6 Traditional elastomeric after 24 hours incubation.
Spectrophotometer Test

Mean cell densities with respect to elastomeric type are recorded in Table 1 for the 500 µl S. mutans test. After six hours incubation, results showed there was no statistical difference in the cell densities between the traditional elastomeric group and the SRE group. A statistically significant difference was detected between the traditional elastomeric group and the SCE group (p=0.0495). However, the mean cell density for the SCE group was higher than the traditional elastomeric group. Mean generation times in minutes for the 500 µl test following the six hour incubation period were 55.0 for the no elastomeric group, 55.14 for the traditional elastomeric group, 55.20 for the SRE group, and 54.79 for the SCE group (Fig. 7).

Table 1

*Mean Cell Densities - 500 µl test*

<table>
<thead>
<tr>
<th>Elastomeric type</th>
<th>Initial 0 hours</th>
<th>Post 6 hours incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No elastomeric</td>
<td>1.6x10^7/ml</td>
<td>1.49x10^9/ml</td>
</tr>
<tr>
<td>Traditional</td>
<td>1.6x10^7/ml</td>
<td>1.48x10^9/ml</td>
</tr>
<tr>
<td>Silver-releasing</td>
<td>1.6x10^7/ml</td>
<td>1.47x10^9/ml</td>
</tr>
<tr>
<td>Selenium containing</td>
<td>1.6x10^7/ml</td>
<td>1.52x10^9/ml</td>
</tr>
</tbody>
</table>
Mean cell densities with respect to elastomeric type are recorded in Table 2 for the 100 µl *S. mutans* test. After eight hours incubation, results showed there was a statistically significant difference in the cell densities between the traditional elastomeric group and the SRE group (*p* = 0.0495). A statistically significant difference was also detected between the traditional elastomeric group and the SCE group (*p* = 0.0495). Mean generation times in minutes for the 100 µl test following the eight hour incubation period were 73.34 for the no elastomeric group, 72.71 for the traditional elastomeric group, 72.95 for the SRE group, and 73.12 for the SCE group (Fig. 8).
Table 2.

*Mean Cell Densities - 100 µl test*

<table>
<thead>
<tr>
<th>Elastomeric type</th>
<th>Initial 0 hours</th>
<th>Post 8 hours incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No elastomeric</td>
<td>1.6x10⁷/ml</td>
<td>1.49x10⁹/ml</td>
</tr>
<tr>
<td>Traditional</td>
<td>1.6x10⁷/ml</td>
<td>1.55x10⁹/ml</td>
</tr>
<tr>
<td>Silver-releasing</td>
<td>1.6x10⁷/ml</td>
<td>1.53x10⁹/ml</td>
</tr>
<tr>
<td>Selenium containing</td>
<td>1.6x10⁷/ml</td>
<td>1.51x10⁹/ml</td>
</tr>
</tbody>
</table>

Fig. 8 Mean generation times after 8 hours incubation
DISCUSSION

The aim of this study was to determine the antimicrobial effectiveness of a silver-releasing elastomeric ligature tie against Streptococcus mutans and compare its results with a selenium containing elastomeric and a traditional non-antimicrobial elastomeric ligature tie. Two separate techniques, agar diffusion and spectrophotometry, were conducted to examine the antimicrobial action of the elastomers.

The agar diffusion test is a means of measuring the effect of an antimicrobial agent grown in culture. It accomplishes this by measuring the zone of inhibition, area in which there is no growth of bacterial colonies, around the agent being evaluated. This is a method frequently used for antibiotic sensitivity testing. In this study, zones of inhibition were evaluated around the elastomeric ligature ties to determine if the SRE or SCE had a significant enough effect to prevent the colonization of S. mutans. Results showed that there were no zones of inhibition seen for any of the elastomeric samples tested. For the SRE, these results suggest that the concentration of silver released from the elastomeric ligature tie did not have an inhibitory effect on S. mutans. This is contrary to manufacturer claims that the silver released from the elastomerics prevents bacterial growth in the surrounding environment. Two different issues could be responsible for the inability for the SRE to inhibit S. mutans growth. One, the proprietary mechanism of silver release from the SRE may not have been efficient in releasing a large enough concentration of silver to inhibit bacterial growth. Second, the silver itself did not have an antimicrobial effect against S. mutans. The latter conclusion would not
agree with Casemiro’s study that silver-zinc zeolite yields antimicrobial activity against S. mutans. Results for the SCE suggest that the antimicrobial activity of the selenium contained within the elastomerics was also not sufficient enough to have an inhibitory effect on S. Mutans. This is contrary to manufacturer’s claims that decalcification causing bacteria can’t grow on SeLECT Defense™ coated surfaces.

The spectrophotometer test is another technique that can be performed for determining the inhibitory action of compounds on microorganisms. In this study, the elastomeric samples were tested in two different concentrations of S. mutans containing TH broth, 500 µl and 100 µl. A greater inhibitory effect was expected to be seen in the 100 µl test due to the smaller concentration of TH broth for the antimicrobial agents to diffuse into. This was seen for the SRE samples because they were statistically different from the traditional elastomeric group in the 100 µl test but not the 500µl test. The SCE samples were found to be significant in both the 500 µl and 100 µl test. Although a significant difference was seen in some instances, further examination reveals that not all the elastomerics had an inhibitory effect on S. Mutans. For example, the SCE group in the 500 µl test actually had a greater increase in bacterial density as compared to the traditional elastomeric group. Generation times also reveal that neither the SRE group nor the SCE group was very successful in inhibiting bacterial growth. Generation time is the time in which it takes for a cell population to double. Figure 7 and Figure 8 reveal the relationship of generations times among the SRE, SCE, and traditional elastomeric groups. In both the 100 µl and 500 µl tests, all generation times between groups were within approximately one minute of each other. These results show that none of the
elastomeric groups had an inhibitory effect capable of reducing the length of time it took for a *S. mutans* cell population to double in size.

There have been no published studies evaluating the antimicrobial effectiveness of silver-releasing or selenium-containing elastomeric ligature ties in which to compare results from this study. Although, results from this study do not support manufacturer’s claims that the SRE effectively kill 99.9% of certain microbes, including *S. mutans*, contained in plaque within a few hours. Results also do not agree with studies regarding the effectiveness of silver as an antimicrobial agent in other areas of dentistry. Because the SRE and SCE were not effective in inhibiting *S. mutans* in this in-vitro study, it could be expected that SRE or SCE would not be effective clinically in reducing white spot lesions during orthodontic treatment.

Limitations of this study include that this was an in-vitro study that did not fully simulate conditions of the oral environment. The oral environment is a constantly changing environment that is affected by multiple factors such as salivary flow. This study strictly provided a medium to test the growth of *S. mutans* around the elastomeric ligature ties in anaerobic conditions. For the spectrophotometer test, a limitation could be possible errors in the spectrophotometer readings due to machine or operator error. Further in-vivo studies would be beneficial in determining the effectiveness of the SRE and SCE clinically.
CONCLUSIONS

The following conclusions can be made under the conditions of this study:

- Neither the SRE nor SCE were effective in inhibiting growth of *Streptococcus mutans* adjacent to the ligature ties in-vitro.
- Neither the SRE nor SCE reduced generation times for *Streptococcus mutans* compared to traditional orthodontic elastomeric ties.
- The SRE and SCE would not likely be effective in preventing enamel demineralization during orthodontic treatment.
- Future in-vivo studies are necessary to confirm conclusion on clinical effectiveness of the SRE and SCE.
REFERENCES


47. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model from Gram-negative bacteria. Journal Colloid Interface Science 2004;275:177-182.


52. Lee JH. Laboratory study of the antimicrobial effect of silver zeolite in polybutylene terephthalate against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *enterococcus*. Exergen Corporation 2005.

