AN *IN VITRO* STUDY OF ANTIMICROBIAL PROPERTIES OF AN ORTHODONTIC SEALANT/ADHESIVE CONTAINING SELENIUM

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ABSTRACT

Michael T. Kelly: An *In Vitro* Study of Antimicrobial Properties of an Orthodontic Sealant/Adhesive Containing Selenium
(Under the direction of Lorne D. Koroluk)

Introduction: White spot lesions are a significant risk to patients undergoing orthodontic therapy. Antimicrobial agents are thought to reduce the incidence of white spot lesions due to their ability to kill cariogenic bacteria. The objective of this study was to characterize and quantify the antimicrobial properties of an orthodontic bonding system containing selenium (SeLECT Defense) compared to traditional orthodontic materials. **Methods:** SeLECT Defense sealant, adhesive, and band cement were compared to eight other materials. An Agar Diffusion Assay was used to demonstrate the inhibition of growth of *S. mutans* and *L. acidophilus.* A Direct Contact Inhibition Assay was used to quantify the bactericidal nature of the materials. **Results:** Several materials, including SeLECT Defense products, demonstrated antimicrobial properties in the Agar Diffusion Assay. Several materials, but not SeLECT Defense products, demonstrated bactericidal properties in the Direct Contact Inhibition Assay. **Conclusions:** Orthodontic bonding materials, including those containing Selenium, possess antimicrobial properties.

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WHITE SPOT LESIONS AND ORTHODONTICS

Introduction and Prevalence

A significant percentage of children have poor oral hygiene which can be attributed to a lack of motor skills, supervision, or motivation. Orthodontic appliances tend to increase plaque retention along their gingival margins and may increase periodontal inflammation if oral hygiene is inadequate.[1] Although rubber-cup prophylaxis has been shown to be effective in preventing gingival enlargement during orthodontic treatment,[2] most patients develop generalized moderate hyperplastic gingivitis within two months of the initiation of orthodontic treatment even in the presence of good oral hygiene.[1] Despite the periodontal insult caused by fixed orthodontic appliances, the loss of periodontal attachment has been shown to average 0.1mm or less during orthodontic treatment and is not different between treated and untreated patients.[3] Most of the observed changes during orthodontic treatment do not result in permanent damage of the periodontium.[1-3]

Of greater and more permanent consequence than the transient decrease in gingival health, orthodontic treatment can lead to the formation of white spot lesions on tooth enamel. White spot lesions result from cariogenic plaque accumulation due to inadequate oral hygiene and are usually located gingival to orthodontic brackets. White spot lesions can be defined as subsurface porosities or demineralizations with a chalky white appearance.[4] The lesions are characterized by a 50% loss of mineral content, underneath an intact layer of enamel which has

formed by remineralization.[5] White spot lesions 75 microns in depth can occur in as little as 4 weeks.[6] The decreased mineral content results in an alteration of the optical refractory index of enamel, which makes the white spot lesions appear lighter in color than the surrounding healthy enamel.[7] White spot lesions are permanent scars on the enamel, and if left untreated continue to be an esthetic problem for at least five years after treatment.[6] A survey has shown that 96% of patients, parents, orthodontists, and general dentists think white spot lesions decrease the esthetics of orthodontically-straightened teeth.[8] Although those surveyed agree that patients themselves are most responsible for the occurrence of white spot lesions, orthodontists are troubled by the high incidence of white spot lesion formation in their patients.[8]

Numerous studies have found that orthodontic patients have significantly more white spot lesions than untreated controls.[6,9,10] The incidence of white spot lesion formation during orthodontic treatment is reported to be as high as 72.9%, while the incidence of cavitated lesions is 2.3%.[11] A landmark study by Gorelick, et al. found that 24% of untreated patients had white spot lesions in locations on their teeth where white spot lesions are commonly observed in orthodontically-treated patients.[9] Fifty percent of patients who had undergone orthodontic treatment developed at least one white spot lesion, and no difference existed between patients treated with banded versus bonded appliances.[9] The authors noted that the labiogingival area of maxillary lateral incisors had the highest incidence of white spot lesions, while the maxillary posterior segment had the lowest.[9] No white spots were found on the lingual surface of mandibular incisors and canines after the use of a bonded canine-to-canine retainer.[9]

Hadler, et al. also concluded that orthodontic patients are at a higher risk for the development of white spot lesions compared to untreated controls.[10] Orthodontic patients and matched untreated controls were asked to adhere to an oral hygiene regimen consisting of

brushing twice daily with fluoride toothpaste, flossing, using a fluoride rinse, and using plaque disclosing tablets. Authors concluded that there is an inverse relationship between compliance with oral hygiene instruction and the incidence of white spot lesions, as those with good compliance had significantly fewer white spot lesions than those with moderate compliance, who in turn had significantly fewer white spot lesions than those with poor compliance.[10] Furthermore, treatment duration has been shown to be positively associated with new white spot lesion formation.[11]

Etiology

White spot lesions are the early manifestations of the caries process. Dental caries is a disease in which bacterial fermentation of sugars in a dental plaque or biofilm results in the production of lactic acid which demineralizes tooth structure.[12,13] White spot lesions form when lactic acid produced by the fermentation of carbohydrates diffuse through the porous subsurface enamel.[14] Lactic acid very rapidly dissociates to produce free hydrogen ions which dissolve hydroxyapatite, causing the release of calcium and phosphate from the lesion.[14] The resulting area of demineralized enamel is termed the incipient carious lesion and can progress to frank cavitation.[6] Caries progression is facilitated by aciduric bacteria such as mutans streptococci and lactobacilli, salivary dysfunction, and consumption of sugar.[12,13] Protective factors include salivary calcium, phosphate, fluoride, buffers and antibacterial proteins, as well as high salivary flow rate and the use of antibacterial agents.[12] There is an ongoing balance between demineralization of enamel caused by the caries process and remineralization of enamel caused by the protective factors.[12]

Central to the caries process is the presence of cariogenic aciduric bacteria, such as *Streptococcus mutans* and *Lactobacillus acidophilus*. While *L. acidophilus* is most commonly implicated in deep carious lesions, *S. mutans* is present in incipient caries, such as white spot lesions.[15] *S. mutans* has a variety of virulence factors that contribute to their ability to initiate the caries process to produce white spot lesions, while *L. acidophilus* utilizes its ability to thrive in the low pH environment of existing carious lesions.[15] Authors suggest that without the initiation of the white spot lesion by *S. mutans*, *L. acidophilus* will not be present.[15] Many studies have demonstrated that there are higher numbers and proportions of *S. mutans* in the saliva and dental plaque in patients with fixed orthodontic appliances.[16,17] Arneberg, et al. compared types of bacteria in plaque retained underneath orthodontic bands and plaque from unbanded surfaces.[17] During treatment, there was a progressive increase in the number of *S. mutans* and lactobacilli present in the plaque adjacent to orthodontic appliances, and all teeth with orthodontic appliances developed white spot lesions.[17] There were statistically significant higher proportions of *S. mutans* and lactobacilli present in the plaque adjacent to orthodontic appliances than in the plaque on untreated teeth.[17] The authors concluded that cariogenic environments such as those facilitated by orthodontic appliances result in the rapid selection of aciduric bacteria in plaque such as *S. mutans* and lactobacilli.[17] Similarly, a longitudinal study of ten patients undergoing orthodontic therapy with bonded brackets demonstrated that there is a significant progressive increase in the percentage of *S. mutans* in plaque adjacent to orthodontic brackets during treatment.[18] The authors concluded that the presence of orthodontic brackets appears to "favor selective increase of *S. mutans* numbers."[18]

Treatment

While white spot lesions tend to decrease in surface area and improve in appearance during the first two years after the completion of orthodontic therapy,[19] most can benefit from treatment. White spot lesions can be treated with several different modalities, including remineralization, bleaching, microabrasion, and dental restorations, depending on their severity. Despite the effectiveness of topical fluorides at preventing white spot lesions, the use of high-dose topical fluoride post-orthodontic therapy to treat white spot lesions is contraindicated because it can result in enamel staining due to rapid surface remineralization.[7] Remineralization of white spot lesions is best left to nature, and the physiologic levels of ions present in saliva.

Tooth whitening can be effective at masking the presence of white spot lesions. Ideally, healthy enamel will be whitened to a greater degree than white spots, with the effect being to lessen the contrast between the two. A study of 10 patients who underwent in-office bleaching followed by a 2-week regimen of in-home bleaching was conducted.[20] Using a colorimeter to assess color, it was determined that although both healthy enamel and white spot lesions were significantly lightened, the healthy enamel was affected more, and the result was to mask the white spots.[20] All ten patients reported being satisfied with the outcome.[20]

A newly-advocated treatment technique involves the microabrasion of the white spot lesions. Microabrasion is performed by using pumice or silicon carbide particles and hydrochloric acid to eliminate white spot lesions by physically removing demineralized areas of enamel.[21] Microabrasion has been shown to be effective in removing white spot lesions of up to 0.3mm in depth.[21] An *in vitro* study found that the appearance of white spot lesions can be

significantly improved by treatment with microabrasion, as well as microabrasion with MI Paste™ (GC America, Alsip, IL) treatment.[22]

A recently developed low-viscosity resin (Icon® by DMG America, Englewood, NJ) has been used to improve the appearance of white spot lesions following orthodontic treatment. The resin infiltrates porous white spot lesions and matches the refractory index of healthy enamel.[7] A study of teeth with post-orthodontic white spot lesions treated with Icon® found that 61% of lesions were completely masked, 33% partially masked, and only 6% remained unchanged.[23] This product offers a more conservative approach in the treatment of white spot lesions compared to microabrasion, although more data is needed concerning it durability and effectiveness over time.

Prevention

While remineralization, bleaching, microabrasion, and low-viscosity resins can be effective, severe lesions, cavitated lesions, or lesions that are unresponsive to other treatment modalities may benefit from resin composite restorations or porcelain veneers or crowns.[7] Due to their potentially high treatment costs, as well as their high prevalence and unaesthetic appearance, the best approach is to prevent the occurrence of white spot lesions prophylactically. All orthodontic patients are given very specific oral hygiene technique instruction and protocols which typically include the use of fluoride. During orthodontic treatment, the prevention of white spot lesions can be accomplished with topical fluoride. Fluoride inhibits the caries process by several different mechanisms. The fluoride ion is toxic to bacterial cells because it can diffuse through the bacterial cell wall and disrupt metabolism by interfering with the glycolytic enzyme, enolase.[12] Fluoride also alters the chemistry of enamel. In developing teeth, the

fluoride ion can substitute for a hydroxyl group in hydroxyapatite to form fluorapatite. Fluorapatite is less soluble than hydroxyapatite, and is therefore more resistant to acid-attack during the caries process.[12] Sound enamel typically contains 20 to 100ppm fluoride content due to fluoride ingestion during tooth development.[24] The previously described conversion of hydroxyapatite to fluorapatite by topical fluoride exposure results in enamel's "fluoride-rich, caries-resistant" outer layer, which contains 1,000-2,000 ppm fluoride.[24] Lastly, fluoride promotes remineralization of demineralized enamel by attracting calcium and phosphate ions from saliva to the enamel surface.[12] Fluoride levels as low as 0.04ppm can enhance enamel remineralization.[12]

Topical fluoride, in the forms of toothpaste, mouth rinses, gels, foams, and varnish, are commonly prescribed to the orthodontic patient. Fluoride ions present in drinking water and fluoride-containing products have been shown to reduce caries.[12] A systematic review was conducted to determine which topical fluoride formulation is most effective in preventing white spot lesions during orthodontic therapy. The authors concluded that the use of all topical fluorides in addition to fluoride toothpaste were effective in reducing the incidence of white spot lesions.[25] Different preparations of topical fluorides, including stannous fluoride gels, sodium fluoride rinses, and acidulated phosphate fluoride foams, all have the ability to reduce the incidence of white spot lesions and no single formulation is superior to the others.[25] There exists some evidence to suggest that higher concentrations of fluoride ion are more effective in preventing white spot lesions.[25] The highest available fluoride concentration exists in fluoride varnishes, which have been shown to result in up to 50% less demineralization of enamel than untreated surfaces.[26,27]

Some evidence shows that fluoride may not be sufficient to prevent the demineralization of enamel during severe cariogenic insults, and therefore the use of antimicrobial agents such as triclosan, xylitol, or chlorhexidine may be beneficial.[12,28] Chlorhexidine is a potent, substantive antimicrobial agent commonly used in the treatment of dental diseases. Daily use of chlorhexidine rinse for 2 weeks has been shown to kill *S. mutans* and prevent its recolonization on tooth surfaces for the following three to six months.[29] The application of chlorhexidine varnish has been shown to be effective in suppressing oral *S. mutans* levels for three to seven months after a single application when used within one month of the placement of fixed orthodontic appliances.[30] The use of chlorhexidine has its own risks. Prolonged use of chlorhexidine can result in the alteration of tooth color. Furthermore, there is question whether the presence of chlorhexidine decreases the bond strength of orthodontic brackets. Placement of chlorhexidine varnish immediately prior to bracket placement increases the rate of bond failure.[31,32] Similarly, the use of chlorhexidine rinse immediately prior to orthodontic bonding results in a statistically significant decrease in bond strength compared to the use of chlorhexidine rinse one week prior to bonding.[33] The use of chlorhexidine rinse one week prior to bonding orthodontic brackets did not decrease bond strength.[33]

MI Paste™ is a new topical agent thought to prevent and repair enamel demineralizations.[34] MI Paste™ contains casein phosphopeptide amorphous calcium phosphate, a protein that results in enamel remineralization.[34] MI Paste Plus™ is a second product that contains 900ppm fluoride in addition to casein phosphopeptide. A double-blind, prospective randomized controlled trial demonstrated that when MI Paste Plus™ is used every day by patients undergoing orthodontic treatment, there is a significant decrease in incidence and severity of white spot lesions compared to controls.[34] While this evidence suggests that MI

Paste™ is effective in preventing white spot lesions, it has not been shown to be able to remineralize existing white spot lesions. A study by Beerens, et al. compared the use of MI Paste™ to normal 1000ppm fluoride tooth paste to treat white spot lesions for 12 weeks following orthodontic treatment.[35] The double-blind randomized clinical trial measured the presence of white spot lesions using quantitative light-induced fluorescence. While both resulted in an increase in the mineral content of white spots, there was no statistically significant difference between MI Paste™ and fluoride toothpaste.[35] Furthermore, the size of the lesions did not change.[35] It has been concluded that there is no benefit to treating white spot lesions after orthodontic treatment with MI Paste™ alone,[22,35] although it has been used in combination with microabrasion to successfully improve the appearance of white spot lesions.[22]

The success of many of the aforementioned topical products at preventing white spot lesions depends on patient compliance. Unfortunately, patients cannot always be relied upon to adhere to oral hygiene regimens prescribed by their orthodontist. A study of 206 orthodontic patients found only 13% compliance with the daily use of a fluoride mouth rinse.[36] The study found that 42% of patients used the fluoride rinse every other day, and the remaining 45% used it less frequently.[36] As expected, significantly fewer white spot lesions developed in patients who used the fluoride rinse at least every other day compared to those who used it less frequently.[36]

Banding and Bonding Materials

While these agents can be effective in preventing white spot lesions, they are all applied at intervals and do not provide constant protection against cariogenic bacteria during the entire course of orthodontic treatment. Orthodontic sealants and primers used during bonding form a mechanical barrier to prevent acid attack on enamel. By etching and sealing the entire facial

surface of a tooth during orthodontic bonding, no enamel is exposed and therefore no enamel is susceptible to acid demineralization. This method of prevention requires the use of sealants containing filler particles for mechanical strength. An *in vitro* study examined the incidence of white spot lesions on teeth with orthodontic brackets bonded with a filled orthodontic sealant (Pro Seal™ by Reliance Orthodontic Products, Itasca, IL) compared to brackets bonded with an unfilled sealant and brackets bonded without a sealant. Each surface was exposed to 15,000 strokes of tooth brushing with non-fluoridated paste, and then cycled for 14 days through demineralization and remineralization processes.[27] Microhardness testing found that the filled sealant group had significantly less demineralization than the unfilled sealant group and the no sealant group.[27] The authors concluded that filled sealants can be used to prevent white spot lesions in orthodontic patients.[27] Despite their effectiveness, filled sealants require replacement as susceptible enamel surfaces become exposed due to toothbrush abrasion of the resin surface. Manufacturers of many commonly used filled orthodontic sealants, including SeLECT Defense™ (Element-34 Technologies, Lubbock, TX), Opal Seal™ (Opal Orthodontics, South Jordan, UT), and Ortho Solo™ (Ormco, Glendora, CA), recommend reapplying their products every three to six months to maintain a physical barrier on the tooth surface, although Pro Seal™ only needs to be reapplied after two years. Although it has been reported that sealants result in enamel loss, cracks and scratches,[37] the prevention of white spot lesions has a greater impact on the final esthetic outcome of the dentition. Therefore, the use of sealants should be strongly considered.

The adhesives used to cement fixed orthodontic appliances can also impact the development of white spot lesions. The use of glass ionomers to bond orthodontic brackets and bands is also common due to the high fluoride release of the material. During the acid-base

setting reaction of glass ionomers, fluoride is released from the aluminosilicate glass particles when they contact polyacrylic acid.[38] The greatest amount of fluoride is released on the first day with a significant decrease afterwards.[38] A split-mouth study compared the incidence of white spot lesion formation on teeth with brackets bonded with glass ionomer cement versus resin composite adhesive.[39] At completion of orthodontic treatment, 24% of teeth that had brackets bonded with glass ionomer cement had developed white spot lesions, compared to 40.5% of teeth bonded with resin composite.[39] It was also found that for patients who had longer treatment time, white spots were more frequent on teeth with resin composite adhesive.[39] The authors concluded that the use of glass ionomer cement for bonding results in fewer white spot lesions than resin composite adhesive in orthodontic patients.[39]

Despite the reported success of glass ionomer cements in the prevention of white spot lesions, their use in orthodontic bonding is not very common due to their poor mechanical characteristics. Resin-modified glass ionomer cements have improved mechanical properties and adhesive strength compared to traditional glass ionomers.[40-42] Similar to traditional glass ionomers, resin-modified glass ionomers have also been shown to be superior at preventing enamel demineralization compared to resin composites adhesives.[43] A randomized clinical trial investigated the incidence of demineralization around orthodontic brackets bonded with a resin-modified glass ionomer compared to a resin composite adhesive.[44] Microhardness testing concluded that after four weeks, there was significantly less demineralization around brackets bonded with the resin-modified glass ionomer compared to resin composite adhesive.[44] These results were supported by a second clinical trial which found that the use of resin-modified glass ionomer cements results in a decreased frequency of caries compared to resin composite adhesives in enamel at depths of up to 30 microns, and at distances of up to 200 microns cervical

to the orthodontic brackets.[45] The study found that there was 21% mineral loss next to brackets bonded with resin-modified glass ionomers compared to 33% next to brackets bonded with resin composite.[45] The authors also noted that the microhardness of the enamel under the bracket was the same for both treatment groups, so the observed differences in mineral loss could not be explained by the acid-etching in the resin composite bonding process.[45] An *in vitro* study also found that brackets bonded with resin-modified glass ionomer cement result in white spot lesions of more shallow depth and less mineral loss than resin composite bonding methods.[43]

It is believed that glass ionomer cements and resin-modified glass ionomer cements are anticariogenic due to their release of fluoride.[46,47] While some studies have shown that levels of fluoride in saliva of patient with brackets bonded with glass ionomer cements are not increased,[44] others have reported the opposite effect.[48] On the first day of orthodontic appliance cementation with glass ionomer, the amount of salivary fluoride is doubled and then rapidly returns to baseline levels.[48] A split-mouth study compared fluoride levels in 48-hour-old plaque next to brackets bonded with glass ionomer cement and resin composite adhesive at days 3, 8, 28, and 180 after appliance placement.[49] Results demonstrated that there were significantly higher fluoride amounts in plaque next to brackets bonded with glass ionomer cement at each time point.[49] Authors concluded that glass ionomer cements may act as long-term local fluoride-releasing adhesives.[49]

Glass ionomer cements also have the potential to alter the bacterial composition of dental plaque adjacent to orthodontic brackets. A study by Hallgren, et al. has shown that there is a higher tendency for *S. mutans* and lactobacilli to colonize around resin composite orthodontic adhesive compared to glass ionomer cement.[47] The authors concluded that glass ionomer

cements are anticariogenic due to antibacterial properties.[47] *In vitro* studies have confirmed this conclusion in agar diffusion and growth inhibition assays.[50]

In addition to their anticariogenic properties, resin-modified glass ionomer cements don't result in changes in tooth surface after fixed appliance removal like resin composite adhesives.[51] Despite these potential advantages, most practitioners use resin composites to bond orthodontic brackets due to their superior handling and mechanical properties. Glass ionomers have lower adhesive strength compared to resin composites and therefore are not favored.[52]

In attempt to combine the anticariogenic properties of glass ionomer cements with the handling and mechanical properties of resin composite adhesives, manufacturers have developed fluoride-releasing resin composites. Studies using ion-specific electrodes demonstrated that resin composite adhesives marketed for their fluoride-release did release significantly detectable levels of fluoride, though the amount released was significantly less that amounts released by glass ionomer cements and resin-modified glass ionomer cements.[38,53] A study by Ahn, et al. demonstrated the recharging potential of fluoride-releasing cements and adhesives.[54] Resin-modified glass ionomers and fluoride-releasing resin composites release high amounts of fluoride upon setting (initial release of resin-modified glass ionomers was 100ppm per gram, initial release for resin composites was 7ppm per gram) and then decrease to low baseline levels of fluoride release.[54] The authors demonstrated that all of the tested fluoride-releasing materials could be recharged to release increased amounts of fluoride for two days before returning to baseline levels.[54] Resin-modified glass ionomers released significantly more fluoride after being recharged compared to fluoride-releasing resin composites.[54] The study found that recharging the cements and adhesives with more highly concentrated fluoride topicals such as acidulated phosphate fluoride foam and sodium fluoride rinse resulted in higher fluoride release compared

to recharging with fluoride toothpaste.[54] Levels of fluoride released after recharging resin-modified glass ionomers and fluoride-releasing composites ranged from 2-35ppm per gram of material.[54] While such small amounts of fluoride are probably not effective at inhibiting bacteria *in vivo*, sub-ppm levels of fluoride are effective at preventing caries by shifting the balance from a state of demineralization to remineralization.[55] Similar to glass ionomer cements, *in vitro* studies have shown that fluoride-releasing resins have antibacterial properties against *S. mutans* and *L. acidophilus*.[56,57]

An *in vitro* study comparing white spot lesion formation adjacent to orthodontic brackets in an artificial caries solution found that brackets bonded with resin-modified glass ionomer cement and fluoride-releasing resin composite were equally effective at reducing the incidence of white spot lesions compared to a non-fluoride-releasing resin composite adhesive.[58] A split-mouth study found that white spot lesions that formed on teeth bonded with fluoride-releasing resin composite adhesive had 48% reduced lesion depth compared to teeth bonded with a non-fluoride-releasing adhesive after 4 weeks.[59]

The surface roughness of resin composite adhesives increases their susceptibility to bacterial colonization.[60] To combat the affinity of oral bacteria for bonding materials, resin composites adhesives have been designed to contain antimicrobial agents incorporated into their filler particles or immobilized in their polymer matrices.[61] The main components of resin composites, silica and zirconia filler particles, methyl methacrylate, Bis-GMA, TEGDMA, and UDMA, do not have antibacterial properties.[61] Chlorhexidine has been added to composites, but its release is highly dependent on its initial concentration within the material.[62] There is a large initial release of chlorhexidine that rapidly decreases to a steady concentration.[62] Increasing the amount of chlorhexidine in the resin composite adhesive results in decreased polymerization and

poor mechanical characteristics and is therefore not a feasible product.[62-66] *In vitro* studies have shown that a resin composite adhesive that releases the antimicrobial agent benzalkonium chloride has antibacterial activity against *S. mutans*.[67] Unfortunately, the antibacterial effect decreased significantly over time, and concentrations of benzalkonium chloride greater than 0.75% are toxic to human gingival cells.[67] The addition of zinc oxide to a resin-modified glass ionomer cement has been shown to increase its antibacterial properties against *S. mutans* as demonstrated in an agar diffusion assay, but the product is not commercially available.[68] Several studies have demonstrated the antibacterial properties of a resin composite containing the monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB) *in vitro* against *S. mutans* and lactobacilli.[69,70] Clinical trials of an orthodontic adhesive containing MDPB have demonstrated a significant decrease in demineralization around orthodontic brackets compared to control adhesives, but no significant beneficial effect on periodontal health has been reported.[71,72] Similarly, orthodontic adhesives containing silver nanoparticles have been shown to be bactericidal.[61,73] The slow release of silver ions from carrier materials such as zeolite or silica-gel is used to give many household items such as kitchenware, washing machines, clothes, and toiletries, antibacterial properties.[61] Unfortunately, as with those containing MDPB, such resin composite adhesives have poor color stability, handling characteristics, and mechanical properties.[61,69,73]

Selenium

A recently developed orthodontic system called "SeLECT Defense™" has been released to market. "SeLECT" stands for "**Se**lenium **L**abeled **E**xtra-**C**ellular **T**oxicity." SeLECT Defense™ consists of a filled resin sealant, resin composite bracket adhesive, orthodontic band cement, orthodontic brackets, and elastomeric ligatures. The adhesives contain selenium within

their resin matrices, while the brackets and elastomers have selenium attached to their surfaces. The manufacturer of SeLECT Defense™ claims that the selenium in their products catalyzes the formation of localized and short-lived superoxide radicals which results in less plaque formation around orthodontic brackets, improved gingival health and fewer white spot lesions.[74] Whereas previous materials containing antibacterial components have displayed poor mechanical characteristics, SeLECT Defense™ adhesive has been shown to have clinically acceptable shear bond strength.[75]

Selenium is element number 34 on the periodic table and is usually classified as a nonmetal, although it has some characteristics of a metal. Selenium is naturally found in the human body, specifically in proteins in the plasma, thyroid, gastrointestinal tract, skin, liver, kidneys and brain.[76] Selenium is an active part of the glutathione reductases, which act as antioxidants,[77] and many selenoproteins are involved in the immune system.[78] Naturally present in plants, the average dietary intake of selenium is 20-300mcg per day.[76] Signs of selenium toxicity begin at intake levels of 1,500mcg per day, but much higher doses are tolerable before severe side effects are observed.[76] The use of selenium is very common in medicine, and especially in the development of anticancer drugs.[76] Selenium has been linked to prevention of atherosclerosis, cancers, arthritis, central nervous system pathologies, and altered function of the immune system.[79]

Selenium can be covalently linked to solid surfaces and catalyze the formation of superoxide radicals which are toxic to bacteria and prevent their attachment to a given surface.[80] Medical devices, such as hemodialysis catheters or contact lenses, can be coated with organo-selenium to prevent the formation of bacterial biofilms or prevent bacterial growth as demonstrated *in vivo* and *in vitro*.[80,81] An organo-selenium coating has been shown to inhibit the

formation of bacterial biofilms on cellulose[82] through the reduction of oxygen by organo-selenium to form superoxide radicals which damage bacterial cell walls and DNA.[83] Organo-selenium is then reduced by glutathione, which results in the formation of a second superoxide radical.[83] The half-life of the radical produced by selenium is 60 nanoseconds and its path length is 35nm.[84] While the radical is only toxic to cells in close proximity, formation of a biofilm on a surface coated with selenium can be inhibited.[84] It has been demonstrated that concentrations of organo-selenium as low as 0.1% can inhibit bacteria.[83]

The organo-selenium present in SeLECT Defense™ is a diselenylmethacrylate with the IUPAC name 3-[3-((2-{1-methyl-2-[2-(2-methyl-acryloyloxy)-ethoxycarbonyl]-ethoxycarbonyl}-ethyldiselenyl))-propionyloxy]-butyric acid 2-(2-methyl-acryloyloxy)-ethyl ester.[83] An *in vitro* study has compared the SeLECT Defense™ dental sealant (Element-34 Technologies) containing organo-selenium to a sealant from the same manufacturer without organo-selenium.[83] Disks containing the sealant were placed in culture media containing mutans streptococci and were analyzed for growth of a bacterial biofilm on the surface of the disk after 24 hours incubation.[83] Confocal laser scanning microscopy after fluorescent staining and colony forming unit plating on Tryptic Soy Agar plates were used to test for the presence of biofilms and viable bacteria on the surfaces of the disks, respectively.[83] It was found that the presence of organo-selenium in the sealant resulted in a significant reduction of biofilm formation and viable colony forming units.[83] Furthermore, an agar diffusion assay demonstrated the ability of the sealant containing organo-selenium to inhibit bacterial growth, while the sealant lacking organo-selenium did not.[83] To demonstrate that the antibacterial properties of the organo-selenium sealant were persistent over time, disks of the sealant were aged in phosphate buffered solution for two months and the assays were repeated with identical significant results.[83] These results

demonstrate that bacteria are prevented from adhering to dental materials containing organo-selenium, but do not necessarily suggest that they are killed by contact with organo-selenium. However, this study does provide evidence that dental sealants containing organo-selenium have potential to be effective at preventing white spot lesions when used in the orthodontic patient.

An *in vivo* study compared SeLECT Defense™ pit and fissure sealant to UltraSeal XT Plus™ (Ultradent, South Jordan, UT), a commonly used pit and fissure sealant.[85] This randomized, double-blind, split-mouth study measured the clinical retention, caries formation, plaque formation, leakage, and safety of the sealants in 120 adolescents of moderate and severe caries risk.[85] The sealants were evaluated every three months for a total of 12 months. Plaque formation and leakage were detected using quantitative light-induced fluorescence.[85] The study found that SeLECT Defense™ sealant had higher retention compared to UltraSeal XT Plus™ (96.2% vs 80.9%) after 12 months and significantly less plaque growth on the sealant.[85] No SeLECT Defense™ sealants displayed any bacterial plaque growth on their surface, which the authors attributed to the antimicrobial nature of SeLECT Defense™.[85] The authors did note that of the 12% of UltraSeal XT Plus™ sealants that displayed surface plaque growth, all were in the high caries risk group and had poor oral hygiene.[85] Neither sealant showed signs of leakage, oral mucosa side effects, or caries.[85]

An *in vitro* study using an artificial mouth with simulated tooth brushing investigated the SeLECT Defense™ orthodontic system. Extracted human teeth bonded with SeLECT Defense™ brackets using the SeLECT Defense™ adhesive and sealant were compared to teeth bonded with traditional brackets using traditional adhesives (Transbond XT™ (3M Unitek, Monrovia, CA) adhesive and Transbond SEP™ (3M Unitek) primer). The measured outcomes were white spot lesion formation, ability to withstand toothbrush abrasion, color/shade of the

teeth, and plaque accumulation. The simulation lasted 28 days in a carbon dioxide incubator with continuous flow of fluid over the teeth to simulate saliva, and the addition of sucrose every 6 hours to simulate consumption of food.[86] The teeth were inoculated with *S. mutans* and *L. casei*.[86] The formation of white spot lesions was detected using quantitative light-induced fluorescence, transverse microradiography, and polarizing light microscopy, and plaque was measured using quantitative light-induced fluorescence.[86] The study found that the SeLECT Defense™ system significantly prevented the formation of white spot lesions compared to the conventional system with 86% reduction of demineralization based on polarizing light microscopy.[86] SeLECT Defense™ sealant was able to withstand 28 days of brushing, resulted in a significant decrease in plaque accumulation, and the authors noted that it resulted in a "bluish clouding" of the tooth surface.[86] While the data indicate that SeLECT Defense™ can prevent the formation of white spot lesions, this study does not distinguish between prevention of white spot lesions due to antibacterial properties of SeLECT Defense™ and prevention due to the presence of a mechanical barrier. The control teeth were bonded with an unfilled sealant, while SeLECT Defense™ sealant is filled and therefore more likely to resist toothbrush abrasion and provide a constant mechanical barrier against acid demineralization.

An *in vivo* study has examined the formation of white spot lesions on enamel coated with SeLECT Defense™ sealant.[84] Enamel blocks were cut from extracted human teeth and bonded to the first or second molars of 30 adult patients.[84] The enamel blocks were unsealed, coated with SeLECT Defense™ sealant, or coated with a chlorhexidine varnish. After being in the mouth for 28 days, the enamel blocks were removed and analyzed for demineralization using transverse microradiography and polarized light microscopy.[84] The results showed a significant decrease in the number of white spot lesions formed on enamel surfaces coated with the SeLECT

Defense™ sealant compared to uncoated enamel and chlorhexidine.[84] Similar to the artificial mouth study, this study did not distinguish between prevention of white spot lesions by mechanical barrier and prevention of white spot lesions due to the antimicrobial properties of SeLECT Defense™.

While these studies suggest that SeLECT Defense™ products have inhibitory effects on the attachment and growth of bacteria,[83-86] the bactericidal properties of the materials have not been investigated previously. However, the killing of cariogenic bacteria is not required to prevent the development of white spot lesions in orthodontic patients, while mere inhibition of attachment and growth on the tooth surface would be sufficient. A randomized clinical trial comparing the incidence of white spot lesions in orthodontic patients bonded with SeLECT Defense™ products versus traditional filled sealants and adhesives would be critical to investigate these claims. Furthermore, no data exists regarding the effect of SeLECT Defense™ products on periodontal health.

Conclusion

In a profession where the goal of treatment is to provide a highly esthetic outcome, orthodontists need to be prepared to deal with white spot lesions. Due to the prevalence, and the problems associated with effective treatment of white spot lesions, patients and orthodontists should focus on the prevention rather than the treatment of such lesions. While topical agents, such as fluoride, chlorhexidine, and casein phosphopeptide have been shown to help prevent white spot lesions, the materials are dependent on patient compliance and must be reapplied at intervals to be effective. Orthodontic sealants and bracket adhesives that contain antimicrobial agents have much promise in providing constant protection against the development of white

spot lesions throughout the duration of orthodontic treatment. While many of these materials have decreased mechanical properties as well as inconsistent and unsustained release of antimicrobial agents, some of these materials, such as SeLECT Defense™, have permanently-incorporated antibacterial agents that maintain clinically acceptable physical properties. Carefully designed clinical trials will be necessary to determine if such sealants and adhesives are effective at preventing white spot lesions in patients over the entire duration of orthodontic treatment.

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AN *IN VITRO* STUDY OF ANTIMICROBIAL PROPERTIES OF AN ORTHODONTIC SEALANT/ADHESIVE CONTAINING SELENIUM

Introduction

A significant percentage of children have poor oral hygiene which can be attributed to a lack of motor skills, supervision, or motivation. Orthodontic appliances tend to increase plaque retention along their gingival margins and may increase periodontal damage [1] and the development of white spot lesions, which are defined as subsurface porosities or demineralizations of the enamel surface with a chalky white appearance (Fig 1).[2] White spot lesions result from cariogenic plaque accumulation located gingival to orthodontic brackets.[3] The bacteria most often implicated in the development of caries are mutans streptococci and species of lactobacillus.[4] White spot lesions can occur within 4 weeks [5], and if left untreated, can progress to frank cavitation. The incidence of white spot lesion formation during orthodontic treatment is reported to be as high as 72.9%.[6] Furthermore, 96% of patients, parents, orthodontists, and general dentists think white spot lesions decrease the esthetics of orthodontically-straightened teeth.[5]

White spot lesions can be treated with several different modalities. Remineralization of white spot lesions can be accomplished with topical fluoride. A newly-advocated treatment technique involves the microabrasion of white spot lesions and application of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).[7] If left to progress, white spot

lesions that become cavitated require a dental restoration; moderate to severe white spot lesions in the esthetic zone may require composite restorations, porcelain veneers, or crowns.

Due to their high prevalence, unaesthetic appearance, and potentially high treatment costs, the best approach is to prevent the occurrence of white spot lesions prophylactically. All orthodontic patients are given very specific oral hygiene technique instruction and protocols. Topical fluoride, in the forms of toothpaste, mouth rinses, gels, foams, and varnish, are commonly prescribed to the orthodontic patient. While fluoride varnish has been shown to result in 50% less demineralization [8], no specific formulation of topical fluoride delivery has been found to be superior to others.[9] Chlorhexidine rinses are also known anti-caries agents used in orthodontic patients, and the prophylactic use of casein phosphopeptide-amorphous calcium phosphate is thought to be protective as well.[10] While these agents can be effective in preventing white spot lesions, they are all applied at intervals and do not provide constant protection against cariogenic bacteria during the entire course of orthodontic treatment.

Orthodontic sealants and primers form a mechanical barrier to prevent acid attack on enamel. Filled sealants have been shown to significantly reduce enamel demineralization compared to unfilled sealants, surfaces treated with fluoride varnish, and untreated controls.[11] Despite their effectiveness, filled sealants require replacement as susceptible enamel surfaces become exposed due to toothbrush abrasion. The adhesives used to cement orthodontic appliances to teeth contain antimicrobial agents which may decrease the incidence of white spot lesions. Composite resins are being developed to release fluoride or other antibacterial agents, such as benzalkonium chloride.[12] Other composites contain antibacterial agents immobilized in their polymer matrix or embedded in their filler particles, which result in varying degrees of antimicrobial activity.[13] However, no significant effect on periodontal health has been

reported.[14] The use of glass ionomers to bond orthodontic brackets and bands is also favored due to the high fluoride release of the material, which has been shown to be bactericidal.[15] *In vivo* studies have shown that orthodontic brackets bonded with glass ionomer cement had fewer white spot lesions when compared to brackets bonded with resin composites.[16] Resin-modified glass ionomers have improved mechanical properties compared to traditional glass ionomers and have also been shown to be superior at preventing enamel demineralization compared to resin composites.[17] Despite these potential advantages, most practitioners use resin composites to bond orthodontic brackets due to their superior handling and mechanical properties.

A recently developed orthodontic system called "SeLECT Defense" consists of a filled resin sealant, resin composite bracket adhesive, orthodontic band cement, orthodontic brackets, and elastomeric ligatures. The adhesives contain selenium within their resin matrices, while the brackets and elastomers have selenium attached to their surfaces. Selenium can be covalently linked to solid surfaces and catalyze the formation of superoxide radicals which prevent bacterial attachment to a given surface.[18] Medical devices, such as hemodialysis catheters or contact lenses, can be coated with organo-selenium to prevent formation of bacterial biofilms or prevent bacterial growth.[19]

There exist claims that SeLECT Defense products result in less plaque formation around orthodontic brackets, improved gingival health and fewer white spot lesions due to their antibacterial properties.[20] An *in vitro* study demonstrated the inhibition of bacterial attachment and growth on the surface of SeLECT Defense sealant, and that this inhibition persists for at least two months.[21] The SeLECT Defense system has been shown to result in fewer white spot lesions on teeth in an artificial mouth study with simulated tooth brushing as compared to teeth with brackets bonded with traditional adhesives.[22] Another *in vivo* study found that fewer white

spot lesions formed on enamel surfaces coated with SeLECT Defense sealant compared to uncoated enamel, as well as enamel coated with chlorhexidine.[23] However, these studies did not distinguish between prevention of white spot lesions by mechanical barrier and antimicrobial properties of SeLECT Defense. To date, no study has characterized the antimicrobial properties of SeLECT Defense compared to other commonly used orthodontic products. The aim of this study was to characterize and compare the antimicrobial properties of SeLECT Defense orthodontic sealant, adhesive, and band cement to other orthodontic bonding and banding materials.

Methods

Agar Diffusion Assay

An Agar Diffusion Assay was used to determine inhibition of growth of *S. mutans* strain ATCC 10449 (serotype c) and *L. acidophilus* strain ATCC 4356 by the orthodontic materials listed in Table 1. Each bacterial strain was cultured at 37°C under anaerobic conditions. Bacterial suspensions were prepared to 0.5 MacFarland Standard. Wilkins-Chalgren agar plates were evenly inoculated with either *S. mutans* or *L. acidophilus* with a cotton swab using aseptic technique.

Disks of each orthodontic sealant (Table 1) were prepared by applying approximately 10mg of sealant to a 6mm diameter sterile paper disk. Disks of orthodontic adhesives and band cements (Table 1) were formed using a plastic mold measuring 6mm in diameter and 1mm in depth (approximately 120mg of material) using a plastic mold. A new mold was used for each material to prevent cross-contamination between products. All disks were light-cured using a Valo LED Curing Light (Ultradent, South Jordan, UT) for 40 seconds.

The disks were placed immediately onto freshly inoculated plates using aseptic technique. A specimen of each of the four orthodontic sealants was placed on an agar plate in an arbitrary position along with a blank paper disk in the center to serve as a control. A specimen of each of the seven orthodontic adhesives and band cements was placed on an agar plate in an arbitrary position. The agar plates were incubated aerobically at 37°C in the presence of 5% carbon dioxide for 24 to 48 hours to allow sufficient growth of the bacteria.

Following incubation, the diameters of the zone of inhibition of bacterial growth around each disk were measured in millimeters using digital calipers. The Agar Diffusion Assay was conducted in triplicate per orthodontic material for each bacterial species, and the assay was repeated three times.

Direct Contact Inhibition Assay

A Direct Contact Inhibition assay was used to determine the bactericidal properties of each of the orthodontic materials listed in Table 1. Strains of *S. mutans* strain ATCC 10449 (serotype c) and *L. acidophilus* strain ATCC 4356 were cultured at 37°C under anaerobic conditions. Bacterial suspensions were prepared to 0.5 MacFarland Standard. The *S. mutans* suspension was diluted 1:2 with Wilkins-Chalgren broth. The *L. acidophilus* suspension was not diluted further.

Approximately 10mg of each of the sealants (Table 1), 120mg of each of the adhesives, band cements, and fluoride varnish (Table 1), and 10µL of each of the oral rinses (Table 1) were applied to sufficiently coat the bottom of separate wells of a 96-well microtiter plate (Greiner Bio-One Cellstar, Monroe, NC). Each well was light-cured for 40 seconds using a Valo LED Curing Light. An empty well served as a control. One 96-well microtiter plate was designated

for inoculation with *S. mutans*, and a second plate was designated for inoculation with *L. acidophilus*. Each plate contained triplicates of each orthodontic material.

A volume of 10µL of bacterial suspension was deposited on the surface of the orthodontic material in the bottom of each well. The plates were incubated at 37°C under aerobic conditions in the presence of 5% carbon dioxide for one hour to allow sufficient time for all bacteria in the 10µL volume to gravitate to the surface of the orthodontic material. Following incubation, 90µL of Wilkins-Chalgren broth was added to each well, and the plates were agitated for 15 seconds using an automated vortex.

A series consisting of five ten-fold dilutions was produced from the control wells of the *S. mutans* and *L. acidophilus* plates. Spots of 7µL of each dilution were inoculated on blood agar plates designated as "Reference Plate." Additional blood agar plates were inoculated with 7µL spots of bacterial suspension from each well of the *S. mutans* and *L. acidophilus* plates. All blood agar plates were incubated at 37°C under aerobic conditions in the presence of 5% carbon dioxide. After 24 hours, the reference plates were used to quantify the reduction in number of colony-forming units (CFU) from each well of the microtiter plate.

The Direct Contact Inhibition Assay was performed twice by inoculating plates immediately upon preparation of the orthodontic materials, and twice after allowing the orthodontic materials to age for 7 days in the microtiter plates at room temperature.

Statistical analysis

All statistical analyses were performed using statistical software (SAS version 9.3, Cary, NC). The Mantel Haenszel row mean score test was used for the Agar Diffusion Assay to assess whether, within each general type of material (sealant, adhesive, or band cement), differences

existed between the mean diameter of zones of inhibition. The Mantel Haenszel test was then used to determine if there were statistically significant differences between the diameters of the zones of inhibition of SeLECT Defense products compared to other products within the material type. The Mantel Haenszel row mean score test was used for the Direct Contact Inhibition Assay to assess whether, within each general type of material (sealant, adhesive, or band cement), differences exist between the observed reductions of colony-forming units. The Mantel Haenszel test was then used to determine if there were statistically significant differences between the reductions of colony-forming units of SeLECT Defense products compared to other products within the material type. The Mantel Haenszel test was used because of concerns about the dispersion of the data, even though means and medians were similar. $P < 0.05$ was considered significant.

Results

Following 24 to 48 hours of incubation, a bacterial "lawn" had grown on the Agar Diffusion Assay plates (Fig 2). Disks of specific orthodontic materials reproducibly resulted in circular zones marked by an absence of bacterial growth, termed "zones of inhibition" (Fig 2). Diameters of the zones of inhibition in the Agar Diffusion Assay are listed in Table 2.

All four sealants resulted in zones of inhibition against *S. mutans*, and statistically significant differences between the mean diameters of the zones created by each material exist ($P<0.0001$). The zones created by SeLECT Defense sealant against *S. mutans* were larger in diameter than those created by Ortho Solo ($P<0.0001$), but smaller than Transbond Plus SEP and Transbond XT Primer ($P<0.0001$). SeLECT Defense adhesive, Fuji Ortho LC, and Transbond Plus resulted in zones of inhibition against *S. mutans*, while Transbond XT did not ($P<0.0001$).

The differences in means of the zones of inhibition produced by SeLECT Defense adhesive compared to Fuji Ortho LC and Transbond Plus were not statistically significant. SeLECT Defense band cement resulted in a zone of inhibition against *S. mutans*, while Fuji I and Ultra Band-Lok did not ($P<0.0001$).

Transbond Plus SEP resulted in a zone of inhibition against *L. acidophilus*, while SeLECT Defense sealant and Ortho Solo did not ($P<0.0001$). One of the ten Transbond XT Primer samples resulted in an 8.5mm diameter zone of inhibition on an *L. acidophilus* plate. Fuji Ortho LC and Transbond Plus resulted in zones of inhibition against *L. acidophilus*, while SeLECT Defense adhesive and Transbond XT did not ($P<0.0001$). Fuji I band cement was the only band cement to result in a zone of inhibition against *L. acidophilus*, while SeLECT Defense band cement and Ultra Band-Lok did not ($P<0.0001$).

Spot-plating of the ten-fold dilutions of the control well in the Direct Contact Inhibition Assay resulted in a "Reference Plate" (Fig 3). Visual comparison of the orthodontic materials' spot plates (Fig 4) with the reference plate allowed the quantification of bacterial killing as a reduction of colony-forming units. When possible, colony-forming units in spots in reference plates and materials' spot plates were manually counted to aid visual comparison. Materials that resulted in greater than 99.9% reduction in CFU were termed "clinically significant" while materials that resulted in a reduction in CFU of less than 99.9% were termed "clinically insignificant." The reductions of CFU as a result of contact with orthodontic materials in the Direct Contact Inhibition Assay are listed in Tables 3 and 4 for newly-prepared and aged samples, respectively.

Of newly-prepared orthodontic materials samples, Transbond Plus SEP and Ortho Solo sealants resulted in clinically significant reduction of *S. mutans* CFU, while SeLECT Defense sealant and Transbond XT did not (Table 3) ($P<0.0001$). No adhesives or band cements resulted in a clinically significant reduction in *S. mutans* CFU, though the clinically insignificant reduction of *S. mutans* CFU produced by Fuji Ortho LC was significantly different than SeLECT Defense adhesive ($P = 0.001$). Fresh samples of ACT fluoride mouth rinse and Peridex chlorhexidine mouth rinse also resulted in clinically significant reduction of *S. mutans*. Fuji Ortho LC and Transbond Plus adhesives, Fuji I and Ultra Band-lok band cements, and Duraphat fluoride varnish each demonstrated clinically insignificant reduction of CFU when *S. mutans* was inoculated immediately following their preparation.

Of newly-prepared orthodontic materials samples, Transbond Plus SEP and Ortho Solo sealants resulted in clinically significant reduction of *L. acidophilus* CFU, while SeLECT Defense sealant and Transbond XT did not ($P<0.0001$). No adhesives or band cements resulted in a clinically significant reduction in *L. acidophilus* CFU, though the clinically insignificant reduction of *L. acidophilus* CFU produced by Fuji I was significantly different than SeLECT Defense adhesive ($P = 0.019$). Fresh samples of ACT fluoride mouth rinse and Peridex chlorhexidine mouth rinse also resulted in clinically significant reduction of *L. acidophilus*.

When the orthodontic materials were allowed to age for 7 days prior to inoculation, only Transbond Plus SEP resulted in clinically significant reduction of *S. mutans* and *L. acidophilus* ($P<0.0001$). After aging one week, ACT fluoride mouth rinse and Peridex chlorhexidine mouth rinse resulted in a clinically significant reduction of *S. mutans* and *L. acidophilus*. Fuji Ortho LC adhesive, and Fuji I and Ultra Band-lok band cements demonstrated clinically insignificant

reduction of *S. mutans* CFU, though only Fuji Ortho LC's reduction was statistically different than its SeLECT Defense counterpart (P=0.002).

Discussion

Previous studies by Amaechi found that SeLECT Defense products prevent the formation of white spot lesions.[22,23] However, these studies did not distinguish between prevention of white spot lesions due to antibacterial properties of SeLECT Defense and prevention due to the presence of a mechanical barrier, as filled sealants were not used on the control teeth. While the study by Tran et al. reported the antibacterial properties of SeLECT Defense sealant [21], there was no comparison made to other commonly used orthodontic sealants, and the inhibition was not characterized as bacteriostatic or bactericidal. The current study is the first study to characterize the antimicrobial properties of SeLECT Defense and compare them to other commonly used orthodontic products.

The Agar Diffusion Assay suggests that SeLECT Defense sealant, adhesive and band cement possess antimicrobial properties against *S. mutans*, as evidenced by a zone of inhibition. This finding supports the findings and conclusions of Tran, et al.[21] The Agar Diffusion Assay suggests that *L. acidophilus* is not sensitive to SeLECT Defense materials. The Agar Diffusion Assay suggests that many other commonly used orthodontic bonding and banding materials possess antibacterial properties as well. Previous studies have reported positive findings of antibacterial properties of Fuji I, Fuji Ortho LC, and Transbond Plus, and negative findings for Ultra Band-Lok and Transbond XT in agar diffusion and growth inhibition assays [15,24,25], which are supported by the findings of this study.

The zones of inhibition of the tested materials vary considerably in diameter (Table 2), yet their magnitudes may be of little clinical significance. A more appropriate interpretation of the results may be to consider the presence or absence of a zone of inhibition because white spot demineralization occurs at the enamel surface in direct contact with a cariogenic bacterial plaque. The formation of a zone of inhibition in this assay suggests that these materials have the potential to prevent bacterial growth on a tooth surface *in vivo*, while the magnitude of the diameter cannot be directly related to clinical events.

A zone of inhibition indicates that an antimicrobial agent diffuses from the sample disk into the surrounding agar to either kill or inhibit bacterial growth on the surface of the plate. The proposed antimicrobial mechanism of action of the SeLECT Defense products is selenium's ability to catalyze the formation of superoxide radicals which are bactericidal in nature.[18] During phases of bacterial growth, radical ions have the opportunity to irreversibly damage DNA as it is replicated. A material that produces radical ions which kill bacterial cells in this way would be considered bactericidal. There also exist radical-mediated pathways which inhibit metabolic enzyme function to prevent bacterial growth. Such a material that does not reduce the number of colony-forming units, but prevents any measurable bacterial growth would be considered bacteriostatic. To provide the proper environment to permit both mechanisms of antimicrobial action to occur, the assays were performed under aerobic growth conditions. The presence of oxygen was necessary to facilitate radical formation. Culture broth containing glucose, amino acids, and nucleic acids was necessary to promote mitosis, which would permit the opportunity for a radical-ion attack on replicating DNA. Furthermore, WC agar was used instead of agar containing heme in order to reduce oxygen radical scavenging molecules in the assay.

Superoxide radicals are, by definition, short-lived due to their highly reactive nature. Previous studies have reported that these radicals have a half-life of 60 nanoseconds.[23] They are limited to the selenium-coated surfaces of SeLECT Defense products, and do not leach out into the oral environment beyond 35 nanometers.[23] The Agar Diffusion Assay used in this study is not sensitive enough to detect zones of inhibition of such small magnitude, yet SeLECT Defense products resulted in measureable zones of inhibition. The data suggest that SeLECT Defense products have antimicrobial properties, but the assay cannot determine if the antibacterial agent is a selenium-catalyzed superoxide radical.

The proposed antimicrobial agents present in the other tested materials are listed in Table 1, but are also not confirmed by these assays. For example, Transbond Plus SEP consistently displayed antimicrobial properties across all assays. While it is reported to be fluoride-releasing, it also contains concentrated phosphoric acid which is meant to demineralize the enamel surface of a tooth to facilitate bonding an orthodontic bracket. In a clinical setting, the acid in the self-etching primer is naturally controlled and buffered by the ions released during demineralization of tooth structure. In the assays used in this study, no buffering agent was present, and likely the robust antimicrobial property of this product was due to the presence of the acid. Transbond XT Primer displayed antimicrobial properties in the Agar Diffusion Assay, despite its lack of fluoride release. The data from Transbond Plus SEP and Transbond XT Primer demonstrate the inability of the assays used in this study to determine the identity of a material's antimicrobial agent, but only the assays' ability to detect the presence of some unidentified antimicrobial agent. Many of the orthodontic products tested in the assay are reported to release fluoride (Table 1), and therefore should theoretically inhibit bacteria.[15] Fuji Ortho LC, Transbond Plus, and Fuji I are all known to release fluoride and resulted in zones of inhibition against *L*.

acidophilus, yet Ortho Solo sealant, which also releases fluoride, did not. Such findings may suggest that a threshold amount of fluoride release is necessary to result in a zone of inhibition for a given bacterial species.

The Agar Diffusion Assay demonstrated that all four sealants tested have antimicrobial properties. This is an encouraging finding because sealants can be used to protect the entire facial surface of the tooth when bonding orthodontic brackets. While a smaller percentage of the orthodontic adhesives and band cements demonstrated zones of inhibition, their antimicrobial properties may be of less clinical significance compared to sealants because white spot lesions form around orthodontic brackets more frequently than underneath them (Fig 1).

The Direct Contact Inhibition Assay did not provide evidence that selenium-containing orthodontic materials have bactericidal properties. The assay provided one hour to allow bacteria in the inoculum to settle via gravity to the surface of the orthodontic material, come into contact with an antimicrobial agent, and be killed. As opposed to the Agar Diffusion Assay, which cannot distinguish bacterial killing from mere growth inhibition, the Direct Contact Inhibition Assay provides evidence of bactericidal properties. If bacteria were killed or irreversibly inhibited upon contact with the orthodontic material, no viable bacteria would remain in the inoculum when spot-plated on agar (Fig 4, material #2). Bacteria that were unaffected or merely inhibited while in contact with the orthodontic material would be able to form colonies when removed from the presence of orthodontic materials in the microtiter plate well, and spotted on the agar (Fig 4, materials #1, 3, and 4).

The Food and Drug Administration defines an antibacterial product as being capable of killing 99.9% of bacteria. This guideline was used to define a "clinically significant" reduction

in CFU in the Direct Contact Inhibition Assay. This assay only demonstrated clinically significant reduction of CFU by Transbond Plus SEP, Ortho Solo sealant, ACT fluoride rinse, and Peridex chlorhexidine rinse. Any reduction of CFU less than 99.9% was deemed insignificant. It is possible that other materials tested in this assay are capable of clinically significant reductions in bacterial CFU, but require longer than one hour of contact for sufficient killing to occur. However, previous studies have shown that bacterial exposure to orthodontic cements for one hour was sufficient to result in a significant inhibition of bacterial growth.[24]

Of the nine orthodontic materials that demonstrated positive zones of inhibition against *S. mutans* or *L. acidophilus*, in the Agar Diffusion Assay, only two (Ortho Solo and Transbond Plus SEP) can be characterized as bactericidal by the Direct Contact Inhibition Assay. The other seven materials are at a minimum bacteriostatic because their presence prevents bacterial growth (Fig 2). Clinically, bacteriostatic and bactericidal materials would equally prevent white spot lesions, because white spot formation is dependent on the growth of bacterial plaque and subsequent production of lactic acid, not simply the presence of cariogenic bacteria.

When the Direct Contact Inhibition Assay was repeated with orthodontic materials samples that were aged 7 days prior to inoculation, Ortho Solo sealant did not retain its ability to kill *S. mutans* or *L. acidophilus*. This may suggest that the antibacterial agent present in this product is volatile in nature, and was not sufficiently present in the sample after one week of aging.

The data show that *S. mutans* and *L. acidophilus* are not equally sensitive to the same orthodontic materials, indicating that these species of bacteria may have different defense mechanisms. SeLECT Defense materials only produced zones of inhibition on agar plates

inoculated with *S. mutans*. It is important to consider that *L. acidophilus* is most commonly implicated in deep carious lesions, while *S. mutans* may be present in incipient caries, such as white spot lesions.[26] *S. mutans* has a variety of virulence factors that contribute to their ability to initiate the caries process to produce white spot lesions, while *L. acidophilus* utilizes its ability to thrive in the low pH environment of existing carious lesions. Many authors suggest that without the initiation of the white spot lesion by *S. mutans*, *L. acidophilus* will not be present.[26] Therefore, it is of primary importance that antimicrobial orthodontic materials, such as SeLECT Defense, are effective against *S. mutans*, and secondarily effective against *L. acidophilus*.

While this study demonstrates that many orthodontic bonding materials, including those containing Selenium, possess antimicrobial properties, it is important to emphasize that this is a short-term, *in vitro* study testing bacterial species in isolation. Caries is a chronic, multifactorial disease that occurs in a complex environment in which many bacterial species coexist and interact. Furthermore, it is unclear whether or not these antimicrobial properties are maintained throughout the course of orthodontic treatment. The data does not provide evidence that any of the tested bonding materials reduce the incidence of white spot lesions during orthodontic treatment, for which a randomized, controlled clinical trial would be needed. The data does suggest, however, that many orthodontic bonding materials, including those containing Selenium, have the potential to prevent white spot lesions due to their antimicrobial properties as demonstrated in this study.

Conclusions

The Agar Diffusion Assay demonstrated that orthodontic sealants, adhesives, and band cements, including those containing Selenium, possess antimicrobial properties. The Direct

Contact Inhibition Assay demonstrated that orthodontic bonding materials, though not SeLECT Defense products, possess bactericidal properties. At this time, the antibacterial properties of SeLECT Defense products against *S. mutans* can be characterized as bacteriostatic and not bactericidal. A randomized clinical trial is needed to determine if the incidence of white spot lesion formation during orthodontic therapy can be decreased by using antimicrobial orthodontic sealants, adhesives, and band cements containing selenium, such as SeLECT Defense.

Tables

Table 1 - Orthodontic materials tested in Agar Diffusion Assay and Direct Contact Inhibition Assay.

Material	Type	Manufacturer*	Hypothesized Antibacterial Agent
C. Blank Paper Disk [a]	Control	BD	None
1. SeLECT Defense	Sealant/Bonding Agent	Element 34	Selenium
2. Transbond Plus SEP	Sealant/Bonding Agent	3M	Fluoride
3. Ortho Solo	Sealant/Bonding Agent	Ormco	Fluoride
4. Transbond XT Primer	Sealant/Bonding Agent	3M	None
5. SeLECT Defense	Adhesive	Element 34	Selenium
6. Fuji Ortho LC	Adhesive	GC America	Fluoride
7. Transbond Plus	Adhesive	3M	Fluoride
8. Transbond XT	Adhesive	3M	None
9. SeLECT Defense	Band Cement	Element 34	Selenium
10. Fuji I	Band Cement	GC America	Fluoride
11. Ultra Band-Lok	Band Cement	Reliance Ortho	Fluoride
12. Peridex[b]	Oral Rinse	3M	0.12% chlorhexidine
13. Duraphat[b]	Fluoride Varnish	Colgate	5% Sodium Fluoride
14. ACT Anticavity Rinse[b]	Fluoride Rinse	ACT	0.05% Sodium Fluoride (alcohol free)

[a] Blank paper disk served as a control in the Agar Diffusion Assay only. An empty microtiter plate well served as a control in the Direct Contact Inhibition Assay.
[b] Material tested in Direct Contact Inhibition Assay only.
* BD (Franklin Lakes, NJ), Element 34 (Lubbock, TX), 3M (Monrovia, CA), Ormco (Glendora, CA), GC America (Alsip, IL), Reliance Ortho (Itasca, IL), Colgate (New York, NY), ACT (Chattanooga, TN).

Table 2 - Zones of surface growth inhibition (diameter in mm, mean ± standard deviation) of orthodontic materials against *S. mutans* and *L. acidophilus* using Agar Diffusion Assay. n = 10.

Material	Type	Zone of Inhibition (mm, mean ± SD) against *S. mutans*	P value	Zone of Inhibition (mm, mean ± SD) against *L. acidophilus*	P value
C. Blank Paper Disk	Control	No Inhibition [a]		No Inhibition [a]	
1. SeLECT Defense	Sealant/Bonding Agent	8.1 ± 0.3		No Inhibition [a]	
2. Transbond Plus SEP	Sealant/Bonding Agent	22.3 ± 3.1	<0.0001	11.1 ± 2.0 [a]	<0.0001
3. Ortho Solo	Sealant/Bonding Agent	7.2 ± 0.2		No Inhibition [a]	
4. Transbond XT Primer	Sealant/Bonding Agent	12.9 ± 2.3		6.3 ± 0.8	
5. SeLECT Defense	Adhesive	8.0 ± 0.9		No Inhibition [a]	
6. Fuji Ortho LC	Adhesive	7.4 ± 0.5	<0.0001	8.0 ± 0.8	<0.0001
7. Transbond Plus	Adhesive	7.5 ± 0.5		11.2 ± 1.0	
8. Transbond XT	Adhesive	No Inhibition [a]		No Inhibition [a]	
9. SeLECT Defense	Band Cement	7.3 ± 0.9		No Inhibition [a]	
10. Fuji I	Band Cement	No Inhibition [a]	<0.0001	7.0 ± 0.0	<0.0001
11. Ultra Band-Lok	Band Cement	No Inhibition [a]		No Inhibition [a]	

[a] No diffusible zone of inhibition present surrounding the 6mm disk.

Table 3 - Reduction of colony-forming units of *S. mutans* and *L. acidophilus* inoculated on newly-prepared orthodontic materials using Direct Contact Inhibition Assay. n = 6.

Material	Type	Reduction of CFU of *S. mutans*	P value	Reduction of CFU of *L. acidophilus*	P value
C. Empty Well	Control	None		None	
1. SeLECT Defense	Sealant/Bonding Agent	None		None	
2. Transbond Plus SEP	Sealant/Bonding Agent	Clinically Significant [a]	<0.0001	Clinically Significant [a]	<0.0001
3. Ortho Solo	Sealant/Bonding Agent	Clinically Significant [a]		Clinically Significant [a]	
4. Transbond XT Primer	Sealant/Bonding Agent	None		None	
5. SeLECT Defense	Adhesive	None		None	
6. Fuji Ortho LC	Adhesive	Clinically Insignificant	<0.0001	None	1.00
7. Transbond Plus	Adhesive	Clinically Insignificant		None	
8. Transbond XT	Adhesive	None		None	
9. SeLECT Defense	Band Cement	None		None	
10. Fuji I	Band Cement	Clinically Insignificant	0.1194	Clinically Insignificant	0.0078
11. Ultra Band-Lok	Band Cement	Clinically Insignificant		None	
12. Peridex[a]	Oral Rinse	Clinically Significant [a]		Clinically Significant [a]	
13. Duraphat[a]	Fluoride Varnish	Clinically Insignificant		None	
14. ACT Rinse[a]	Fluoride Rinse	Clinically Significant [a]		Clinically Significant [a]	

[a] Material meets FDA definition of "antibacterial" by killing 99.9% of bacteria.

Table 4 - Reduction of colony-forming units of *S. mutans* and *L. acidophilus* inoculated on orthodontic materials aged 7 days using Direct Contact Inhibition Assay. n = 6.

Material	Type	Reduction of CFU of *S. mutans*	P value	Reduction of CFU of *L. acidophilus*	P value
C. Empty Well	Control	None		None	
1. SeLECT Defense	Sealant/Bonding Agent	None		None	
2. Transbond Plus SEP	Sealant/Bonding Agent	Clinically Significant [a]	<0.0001	Clinically Significant [a]	<0.0001
3. Ortho Solo	Sealant/Bonding Agent	None		None	
4. Transbond XT Primer	Sealant/Bonding Agent	None		None	
5. SeLECT Defense	Adhesive	None		None	
6. Fuji Ortho LC	Adhesive	Clinically Insignificant	<0.0001	None	1.00
7. Transbond Plus	Adhesive	None		None	
8. Transbond XT	Adhesive	None		None	
9. SeLECT Defense	Band Cement	None		None	
10. Fuji I	Band Cement	Clinically Insignificant	0.2425	None	1.00
11. Ultra Band-Lok	Band Cement	Clinically Insignificant		None	
12. Peridex[a]	Oral Rinse	Clinically Significant [a]		Clinically Significant [a]	
13. Duraphat[a]	Fluoride Varnish	None		None	
14. ACT Rinse[a]	Fluoride Rinse	Clinically Significant [a]		Clinically Significant [a]	

[a] Material meets FDA definition of "antibacterial" by killing 99.9% of bacteria.

Figures

Figure 1 - White spot lesions following removal of orthodontic appliances. Poor oral hygiene during orthodontic treatment resulted in white spot lesions located along the gingival margins of teeth. Note signs of gingival inflammation, as well as a carious lesion on the facial surface of the maxillary right canine.

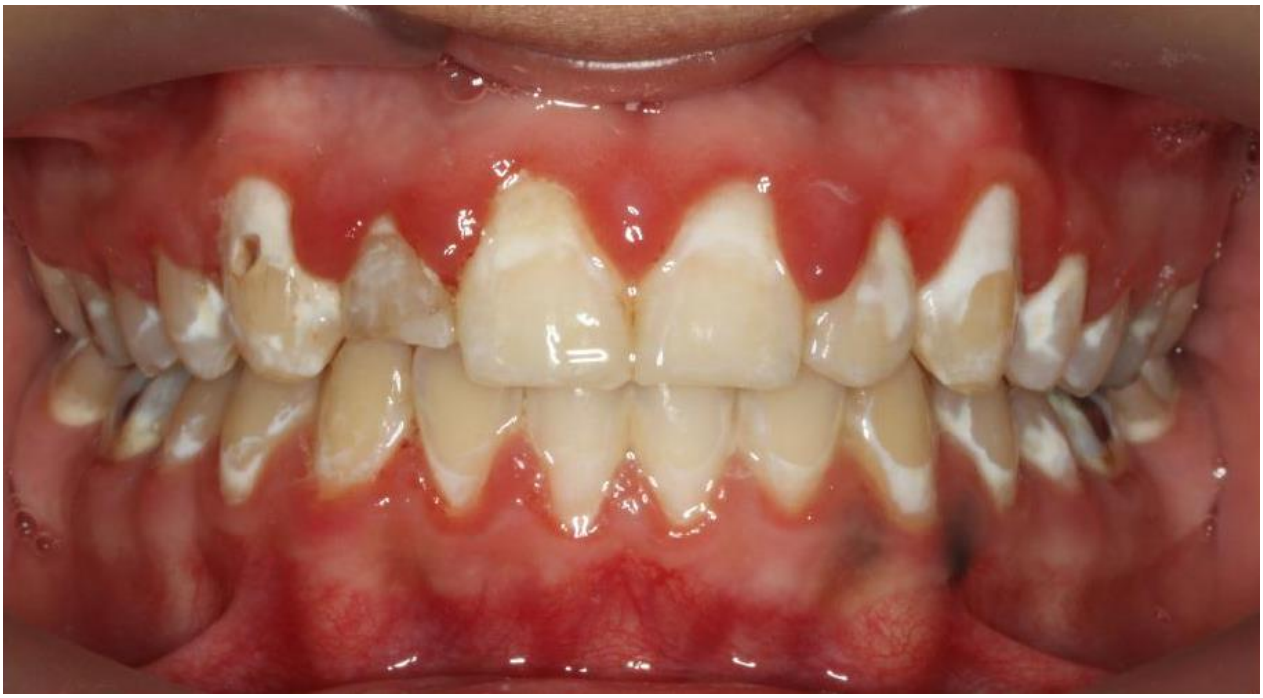


Figure 2 - Orthodontic material disks on WC Agar plates inoculated with bacteria following 48 hours of aerobic incubation at 37°C in the presence of 5% carbon dioxide. a. Orthodontic sealants on *S. mutans* plate. b. Orthodontic adhesives and band cements on *S. mutans* plate. c. Orthodontic sealants on *L. acidophilus* plate. d. Orthodontic adhesives and band cements on *L. acidophilus* plate. Numbers correspond to the orthodontic material as listed in Table 1.

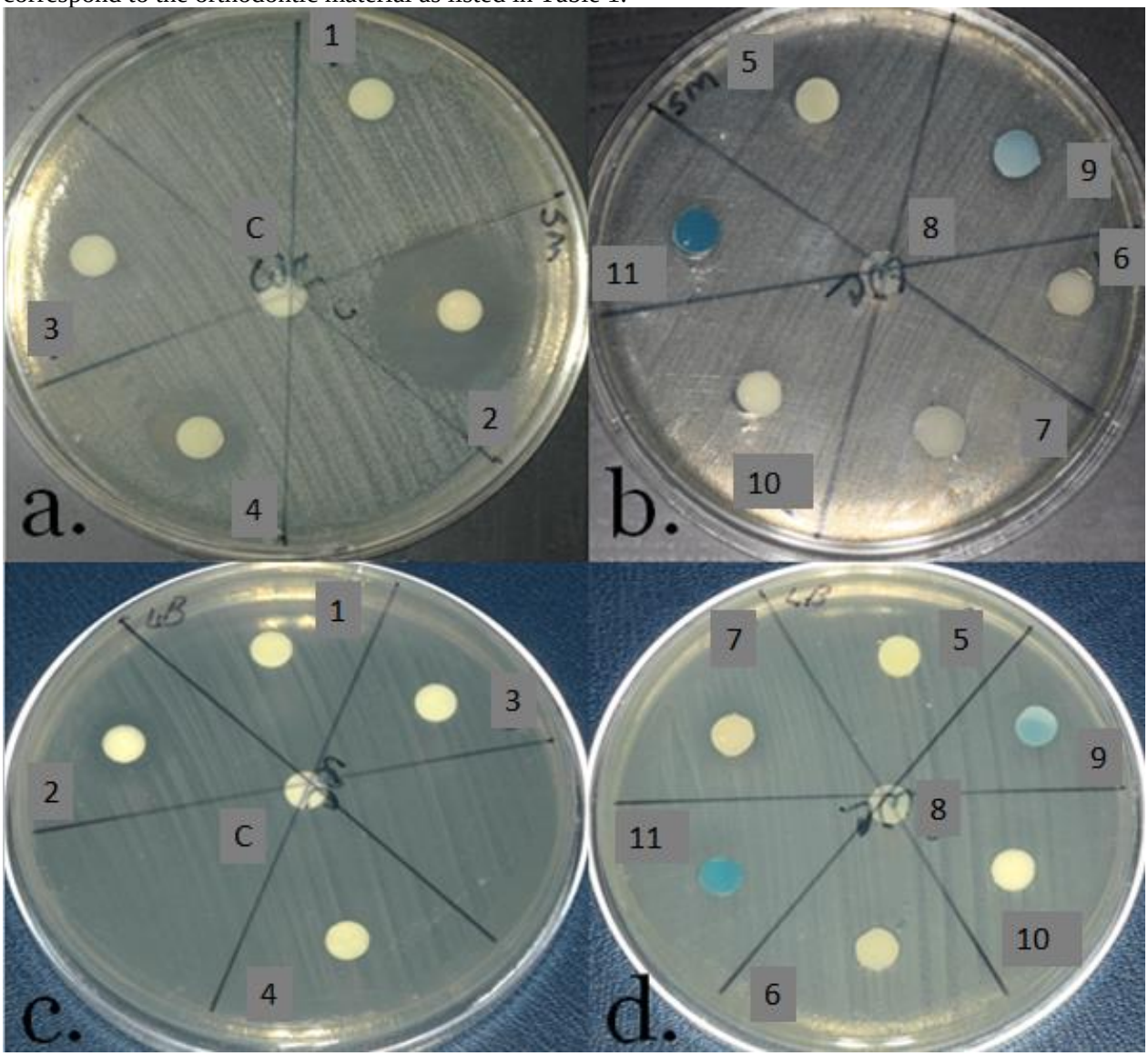


Figure 3 - *L. acidophilus* "Reference Plate" in triplicate. Percentages correspond to reduction of colony-forming units resulting from ten-fold dilution of inoculation of control (empty) microtiter plate wells.

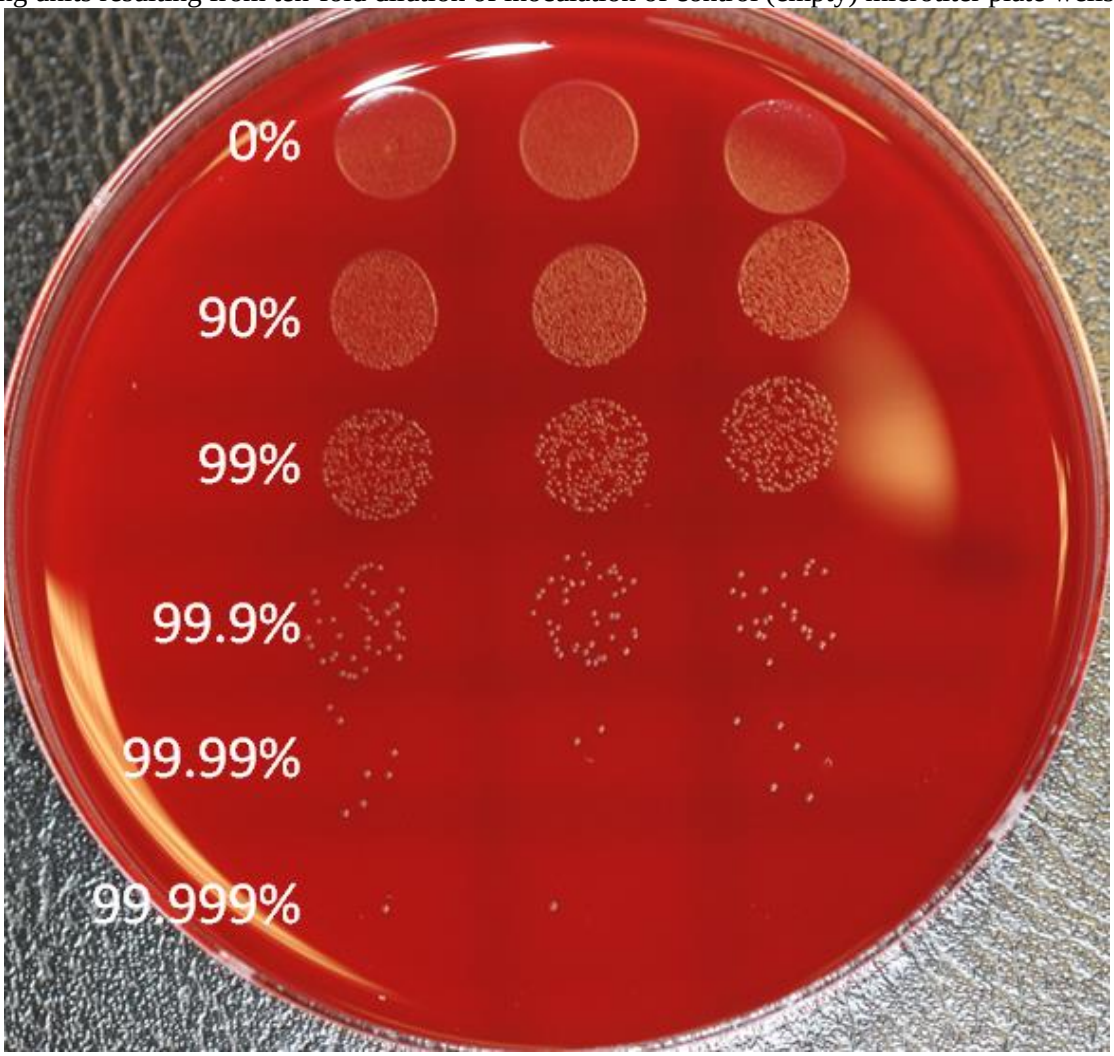
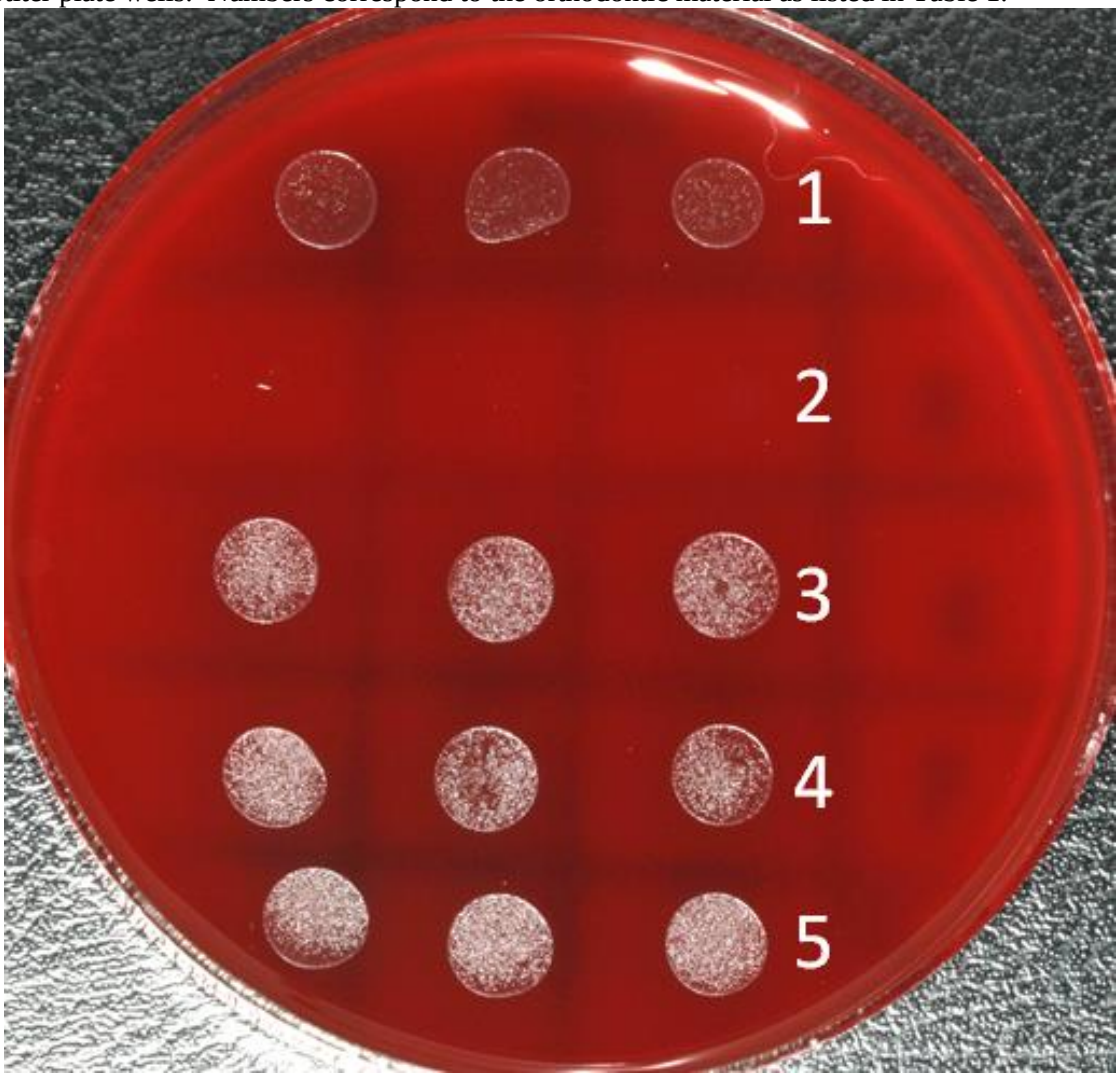


Figure 4 - Spot plate in triplicate of *L. acidophilus* inoculated onto 7-day aged orthodontic materials in microtiter plate wells. Numbers correspond to the orthodontic material as listed in Table 1.



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