Surface antibacterial properties of fissure sealants

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Abstract

Purpose: Sealants form a physical barrier between the oral environment and deep fissures that contribute to caries prevention. It is postulated that sealants possessing antibacterial properties are advantageous. The purpose of this study was to evaluate the antibacterial properties of four pit and fissure sealants using direct contact test (DCT) and agar diffusion test (ADT).

Methods: For the DCT, 8 samples of Helioseal F (Vivadent), Ultraseal XT (Ultadent-Weldent), Conseal F (SDI), and Dyract Seal (Dentsply) were placed on the sidewalls of wells of a 96-microtiter plate. After polymerization, freshly grown *Streptococcus mutans* cells (circa $1X10^6$) were placed on the surface of each sample for 1 hour at 37°C. Fresh media was then added to each well and bacterial growth was followed for 16 hours by temperature-controlled spectrophotometer. Similarly prepared samples were aged in phosphate buffered saline for 14 and 30 days and the DCT was repeated. The ADT was performed by placing samples in uniform wells punched in agar plates.

Results: Freshly polymerized samples in the DCT, Dyract Seal and Ultraseal XT possessed prominent antibacterial properties. Dyract Seal also demonstrated the most potent antibacterial properties, which lasted 14 days but faded within 30 days. In ADT, the halo in the bacterial lawn was measured after 48 hours, and only Dyract Seal demonstrated an inhibition zone.

Conclusions: The compomer-based sealant Dyract Seal possessed the most potent and longest lasting antibacterial activity.(*Pediatr Dent*. 2003;25:43-48)

KEYWORDS: PIT AND FISSURE SEALANT, BACTERIA, ANTIBACTERIAL, *S MUTANS*

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Caries develops when bacterial plaque cannot be removed from the deeper regions of the fissure. Over 80% of caries that occurs in children and teenagers is in the occlusal surface.[1] Pit and fissure sealants are effective in preventing occlusal caries in vitro.[2-4] A physical barrier formed between the tooth surface and the oral environment reduces carious lesions caused by *Streptococcus mutans.*[5,6]

Numerous studies concentrate on the tooth-restoration interface, which is considered to be the weak link in any restoration. In vitro studies have examined microleakage between the tooth surface and sealants placed on acid-etched enamel[7] with and without bonding agents.[8] Other in vitro studies have compared microleakage of sealants using different methods of mechanical preparation. No significant difference in microleakage has been found between sealants applied after conventional pumicing and bur preparation[9] and after using a bur technique or air abrasion.[10] However, microleakage is reduced when fissures are prepared using a high-speed diamond bur compared to those prepared with a low-speed round bur.[11] Since microleakage cannot be avoided, antibacterial properties of fissure sealant materials may contribute to the prevention of caries.

It is widely recognized that the interaction of restorative materials with microorganisms is important for the longevity and effectiveness of restorations.[12,13] Studies have been conducted to evaluate the antimicrobial activity of restorative materials in vitro.[13-16] In most of these studies, the agar diffusion test (ADT) was used as the standard assay despite its known limitations. Difficulties associated with this test include its semiquantitative nature, its limitation to measure the activity of soluble components and the difficulties in controlling a number of variables (ie, inoculum density,

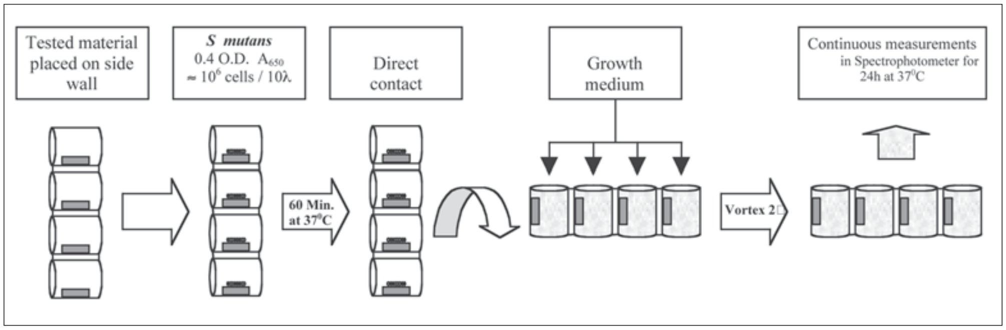

Figure 1. Schematic representation of the DCT experimental set-up. The test is performed in a 96-well, flat-bottom microtiter plate. The plate is held vertically and the sidewall of wells is evenly coated with the tested material. A 10-µL bacterial suspension is placed on the test material. Evaporation of the suspension's liquid (1 hour at 37°C) ensured direct contact between bacteria and the tested materials. The plate is then held horizontally and growth medium is added to each well followed by gentle mixing for 2 minutes. The plate is incubated at 37°C in the THERMOmax microplate photospectrometer where bacterial outgrowth is monitored.

medium content, agar viscosity, storage conditions of agar plates, size and number of specimens per plate, adequate contact between specimens and adjacent agar, and incubation time and temperature).[13]

ADT is essentially based on measuring the activity of those components, which are soluble and capable of diffusing into the surrounding aqueous media. Therefore, using ADT for any dental restorative material for which low solubility is a major requirement seems to be inadequate.

The direct contact test (DCT) was developed[17] to measure effect of direct and close contact between the test microorganism and the tested materials, regardless of the solubility and diffusability of the antimicrobial components.

The purpose of the present study was to evaluate the antibacterial properties of 4 different pit and fissure sealant materials by the agar diffusion test (ADT) and the direct contact test (DCT).

Methods

Two experimental methodologies were used in this study: the widely used ADT, which is qualitative; and the relatively new method DCT, which is quantitative and provides additional information.

Tested materials

Most commercially available pit and fissure sealant materials contain fluoride. Fluoride is incorporated into modern sealants because of its effect on bacterial metabolism, plaque pH and enamel remineralization.[18] Four fluoride-containing fissure sealants were tested: 3 composite resin-based materials—Helioseal F (Vivadent, Schaan, Liechtenstein), Ultraseal XT (Ultadent-Weldent South Jordan, Utah), and Conseal F (SDI Victoria, Australia); and a compomer-based material—Dyract Seal (Dentsply, Konstanz, Germany).

Test microorganism and growth conditions

S mutans, the primary etiological agent of caries and a frequent caries lesion isolate, has been widely used to test antimicrobial activity of restorative materials[19,20] *S mutans* 27351M was grown aerobically from frozen stock cultures in brain heart infusion (BHI) broth containing 0.5% bacitracin at 37°C. *S mutans* is naturally resistant to bacitracin, therefore, this antibiotic was added to growth media and buffers to prevent microbial contamination during the experiments.

The ADT was performed using both mitis salivarius agar plates and triptic soy blood agar plates. Each plate was inoculated with 200 µL of freshly grown *S mutans* (OD 0.6 at 650 nm). Eight holes, 4 mm in diameter, were punched in the agar of each plate, and the respective sealant material, in duplicate, was introduced and immediately polymerized. Plates were incubated for 48 hours allowing an unequivocal visual inspection for the presence of inhibition zone in the bacterial lawn. Where appropriate, the bacterial inhibition zone halo was measured in 2 perpendicular locations and expressed in millimeters. The ADT for each material and in each growth media was repeated at least 3 times.

The direct contact test[17] is based on turbidometric determination of bacterial growth in 96-well microtiter plates. The kinetics of the outgrowth in each well is recorded at 650 nm for 16 hours every 30 minutes using a temperature-controlled spectrophotometer set at 37°C (THERMOmax, Molecular Device Corp, Menlo Oaks Corp Center, Menlo Park, Calif). Auto mixing prior to each reading ensured a homogeneous bacterial cell suspension.

The experimental set-up is shown in Figure 1. A 96-well flat-bottom microtiter plate (Nunclon, Nunc, Copenhagen, Denmark) was held vertically (ie, the surface of the plate is perpendicular to the floor and the sidewall of 8 wells is coated evenly with a measured amount of the tested material). A thin coat is achieved by using a small-sized flat-ended dental spatula. The material was then polymerized in strict compliance with the manufacturer's recommendation. Special care was taken to avoid the flow of the material to the bottom of the well, which would interfere with the light path through the microplate well.

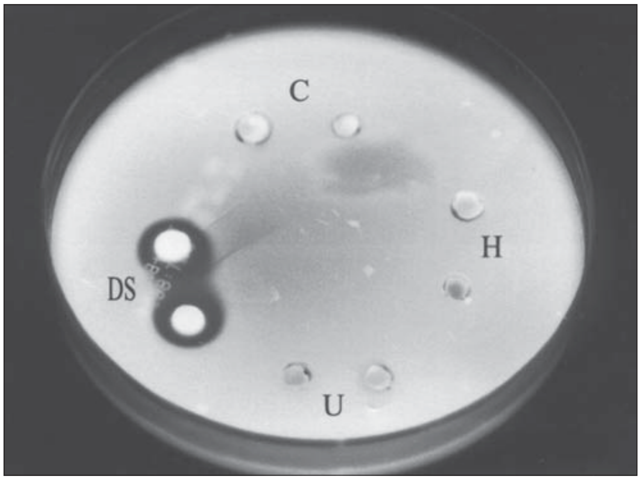

Figure 2. Agar diffusion test of 4 sealants. An inhibitory halo in the bacterial lawn around Dyract Seal (DS) is observed, whereas no inhibitory halo around other sealant materials—Helioseal F (H), Ultraseal XT (U) and Conseal F (C)—was observed.

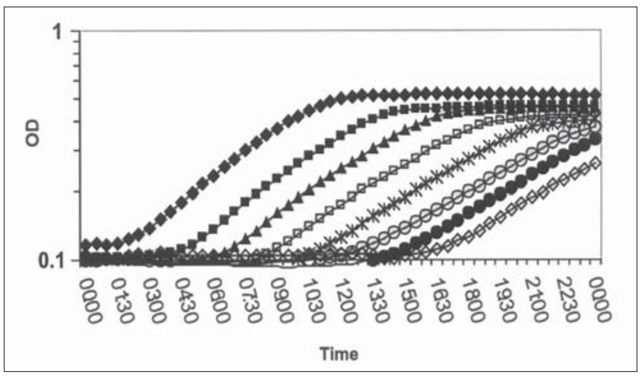

Figure 3. Parallel to the experimental set-up shown in Figure 1, calibration experiments were performed in each plate to establish bacterial growth under the experimental conditions. Each point on the growth curve is the average of the optical densities (OD) measured in duplicate wells at the same time. Starting inoculums for each curve were serially diluted 5-fold from the original inoculum. The gradual decrease in bacteria due to the serial dilution has no effect on the bacterial growth rate or the final density of bacteria at the stationary phase.

A 10-μL bacterial suspension (0.9 to 1.1X10^6 colony forming units, calculated from viable counts performed separately for each experiment) was placed on the test material, while the plate remained in a vertical position. Evaporation of the suspension's liquid ensured direct contact between bacteria and the tested materials, which usually occurred within 1 hour at 37°C. BHI broth with 50 μg/mL bacitracin (220 μL) was then added to each well and gently mixed for 2 minutes. Eight uncoated wells in the same microtiter plate served as positive control. That is, identical bacterial inoculum was placed on the sidewall of the uncoated wells and processed as in the experiment wells. The negative control consisted of a set of wells coated with the tested materials, as in experimental wells, containing equal volumes of uninoculated medium. The plate was then incubated at 37°C in the THERMOmax microplate reader and the optical density in each well at 650 nm was followed for 16 hours.

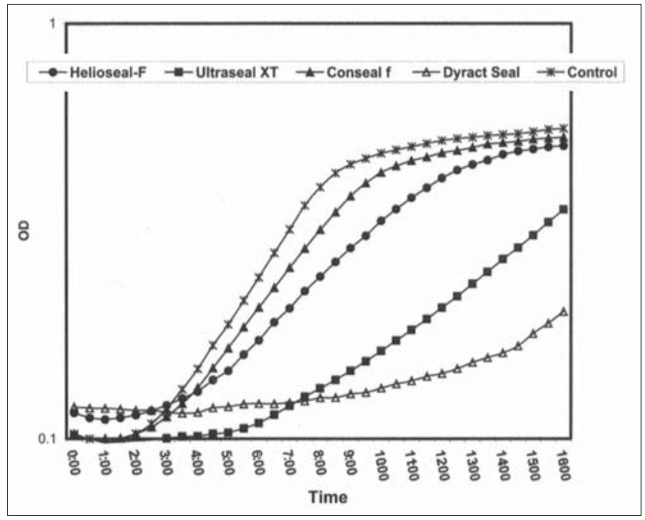

Figure 4. Bacterial growth after direct contact with fresh material. Each point on the curve is the average optical densities (OD) measured in 8 separate wells at the same time.

The recorded data were plotted as semilogarithmic growth curves. The linear portion of the curve, which correlates with bacterial growth rate, was transferred and expressed as a linear mathematical formula. ANOVA and Tukey multiple comparison procedures were applied on the slope of these linear formulas.

Similar experiments were carried out where the tested materials were allowed to age for 14 and 30 days, respectively. Aging was performed in the presence of phosphate-buffered saline (PBS) containing 50 μg/mL bacitracin, which was replaced every 48 hours.

Parallel to the experimental set-up, calibration experiments were carried out in each plate to establish bacterial outgrowth under experimental conditions. For this purpose, 10 μL of bacterial suspension was placed on each sidewall of 2 wells in a 96-well microtiter plate as in the experimental set-up. Then, 275 μL of fresh medium was added and the plate gently mixed for 2 minutes. From each well, 55 μL was transferred to an adjacent set of wells, respectively, which contained 220 μL fresh medium. This was repeated 7 consecutive times.

Results

ADT was performed on samples of the 4 pit and fissure sealants. Results demonstrated an inhibitory halo in the bacterial lawn with a diameter of 6.62±0.51 mm around Dyract Seal, whereas no inhibitory halo around the other sealant materials was observed (Figure 2).

For the DCT, calibration growth curves, in which bacteria were diluted by a factor of 5, were performed in each experiment; an example is shown in Figure 3. Each point on the curve was the average of 2 wells measured at the same time. DCT was performed on 8 samples of each of the 4 materials tested. The growth curve for each well was analyzed and a regression line was performed on the linear segment of the curve. The R^2 ranged between values of 0.99 to 0.96. Two-way ANOVA indicated a significant difference

Table 1. Rate of Bacterial Growth as Demonstrated by the Slope of the Linear Portion of the Growth Curve*			
Sealant material	Fresh material	Aged two weeks	Aged one month
Control	2.872±0.4981	3.165±0.3695	2.888±0.2604
Conseal F	2.659±0.401	2.915±0.06325	3.149±0.307
Helioseal F	1.859±0.2288	3.140±0.1963	3.835±0.1181
Ultraseal XT	0.9250±0.9547	2.327±0.197	2.914±0.1369
Dyract Seal	0.07714±0.1459	0.1025±0.00276	2.880±0.2658
One-way ANOVA	$P<.0001$	$P<.0001$	Not significant

*Each number in the table is the average ([x10^{-2}]±standard deviation [x10^{-2}]) of the slope of bacterial growth in 8 separate wells in the same microtiter plate. Vertical lines connect values, which do not differ significantly (Tukey's comparison).

in bacterial growth rate (slope) between the sealants in a combination of time and material ($P<.001$).

Dyract Seal had the most potent antibacterial properties immediately after polymerization. Ultraseal XT also demonstrated distinct antibacterial properties, whereas Helioseal F had very little antibacterial effect and Conseal F had none (Figure 4 and Table 1).

In the 14-day-old samples, Dyract Seal maintained its antibacterial activity (Figure 5), followed by Ultraseal XT with less potent antibacterial properties. Helioseal F and Conceal F showed no antibacterial activity and were similar to the control (Table 1).

None of the tested materials maintained their antibacterial property after 30 days (Figure 6 and Table 1).

Discussion

The use of pit and fissure sealants is recognized as a state-of-the-art method in prevention of initial occlusal caries. It is also recognized that antibacterial properties of these materials may contribute to their clinical performance.

Two methods were used to evaluate the antibacterial properties of 4 common sealants in the present study. The ADT indicated that only the compomer Dyract Seal possessed antibacterial activity, while the resin-based sealants showed no inhibitory effect. However, when the DCT was used, all materials possessed antibacterial properties when tested immediately after polymerization. The most potent antibacterial property was exhibited by Dyract Seal, which lasted for at least 14 days, followed by Ultraseal XT.

In the present study, 2 experimental methodologies were used and compared. The ADT is qualitative in its nature and much of the currently available information is derived from publications using this method. The DCT, a relatively new method, is quantitative and provides information on bacterial growth rate and viability.[17] It was designed to measure the effect of direct and close contact between a monolayer of the test microorganism and the tested materials, regardless of the solubility and diffusability of the antimicrobial components under controlled in vitro conditions.

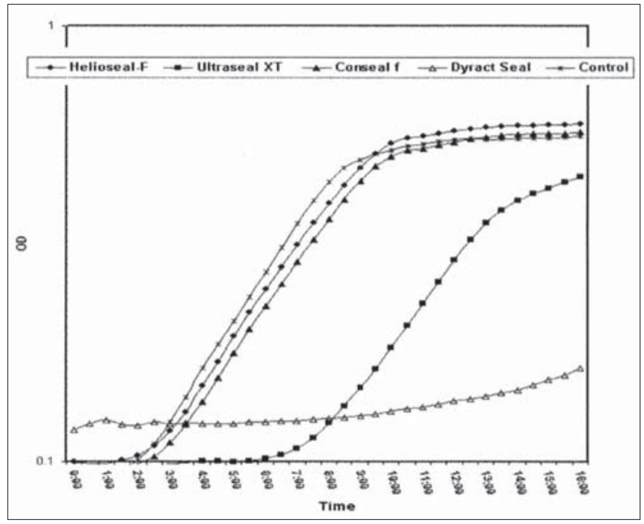

Figure 5. Bacterial growth after direct contact with 14-day-old material. Each point on the curve is the average optical densities (OD) measured in 8 separate wells at the same time.

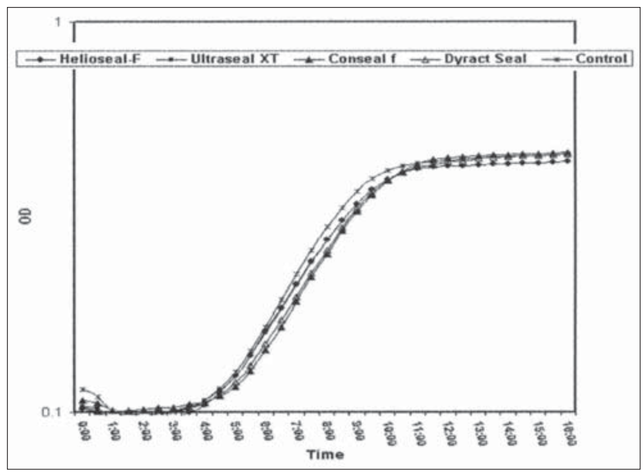

Figure 6. Bacterial growth after direct contact with 30-day-old material. Each point on the curve is the average optical densities (OD) measured in 8 separate wells at the same time.

Using the temperature-controlled spectrophotometer and the appropriate software, the DCT achieves accurate and highly reproducible data.

It is possible to estimate the number of viable bacteria at the end of the direct contact incubation period using the calibration growth curves. Starting with wells containing 10^6 viable bacteria, for example, the consequent curves represent outgrowth of 200,000, 40,000, 8,000, 1,600, 320, 64 and 12.8 bacteria per well, respectively (Figure 2). It follows that experimental wells showing no growth had virtually no viable bacteria at time 0. The curves also demonstrated that the gradual decrease in viable bacteria due to serial dilution at time 0 had no effect on the growth rate and the final density of bacteria at the stationary phase in this system. The 5-fold decrease in the viable bacteria at time 0 resulted only in a delay lag time in the exponential growth. Therefore, changes in the slope in the experimental curves can be contributed solely to bacteria-material interaction.

The fact that the ADT demonstrated no inhibition zone around Conseal F, Ultraseal XT and Helioseal F may be attributed to very low solubility of the polymerized resin. The DCT demonstrated potent antibacterial activity in the 2 latter materials, which could not be detected by the ADT. On the contrary, the significant inhibitory zone around the Dyract Seal samples in the ADT could be contributed to its solubility in aqueous medium. This was also observed in the DCT set-up where Dyract Seal was the only material to show complete elimination of bacteria. The minimal elevation observed in the growth curve for this material was identical to the negative control wells, where no bacteria were present (not shown). This observation could be interpreted in part by the material's solubility, as indicated by the manufacturer test values (2.71±0.42 $\mu g/mm^3$, Dentsply Dyract Seal portfolio).

It has been suggested[21] that fluoride release by sealants is able to produce inhibition of *S mutans*. Comparative in vitro studies report that a fluoride-containing sealant significantly improves the caries resistance of enamel in close proximity to the sealant.[22,23] Previous studies show that the fluoride level in plaque growing on glass ionomer is much higher than that on composite resins which seems to affect the level of *S mutans* in dental plaque.[24] All products examined in the present study contained fluoride: 3 were composite resin-based and 1 was a compomer, which is a composition of resin and glass ionomer.

Notwithstanding the claim made by the manufacturers for a long-term fluoride release, in this study, the antibacterial characteristic of the materials did not withstand a period of 14 days for Conseal-F and Helioseal-F and 30 days for Ultraseal-XT and Dyract Seal.

Pit and fissure sealants enable a prolonged reduction of *S mutans* presence in situ.[3] Different methods for improving the techniques of placing the sealant and reducing microleakage have been previously described.[8-11] Even laser irradiation combined with an adhesive system does not eliminate microleakage.[25]

It is accepted that the antibacterial property of any restorative material will amplify its potential to prevent caries. It may be advantageous immediately after preparing the fissure site when clinically complete bacterial elimination cannot be ascertained. Antibacterial properties are also advantageous in the long-term, when it might prevent secondary caries. Unfortunately, this study's in vitro results showed that aging sealants in PBS for 30 days resulted in no antibacterial activities. Further development and improvement in material technology is needed to address these issues.

Conclusions

With the limitations of the in vitro methodology used in the present study, the following conclusions can be drawn.

1. All resin-based sealant materials demonstrated various degrees of antibacterial activity.
2. Dyract Seal, the compomer-based sealant, possessed the most potent and long-lasting antibacterial activity. Dyract Seal was also the only sealant demonstrating inhibitory halo in the ADT, indicating that its antibacterial components can diffuse into the surrounding milieu.
3. The antibacterial properties of all tested materials fade within a month.
4. The correlation between in vitro antibacterial activity and clinical performance of the materials has to be established.

Acknowledgments

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References

1. NIH. Consensus development conference statement on dental sealants in the prevention of tooth decay. *JADA*. 1984;108:233-236.
2. Ripa LW. An update of the effectiveness of pit-and-fissure sealants. *Caries Res*. 1993;1:77-82.
3. Mass E, Eli I, Lev-Dor-Samovici B, Weiss EI. Continuous effect of pit and fissure sealing on S mutans presence in situ. *Pediatr Dent*. 1999;21:164-168.
4. Boksman L, Carson B. Two-year retention and caries rates of UltraSeal XT and FluoroShield light-cured pit and fissure sealants. *Gen Dent*. 1998;46:184-187.
5. Hamada S, Slade HD. Biology, immunology and cariogenicity of S mutans. *Micobiol Rev*. 1980;44: 331-384.
6. Loesche WJ. Role of the Streptococcus mutans in human dental decay. *Microbiol Rev*. 1986;50:353-380.
7. Baratieri LN, Monteiro S, Jr. Influence of acid type (phosphoric or maleic) on the retention of pit and fissure sealant: an in vivo study. *Quintessence Int.* 1994;25:749-755.
8. Tulunglu O, Bodur H, Uctasli M, Alacam A. The effect of bonding agent on the microleakage and bond strength of sealant in primary teeth. *J Oral Rehab*. 1999;26:436-441.
9. Boj JR, Xalabrade A, Gracía-Godoy F. Microleakage of fissure sealants after enameloplasty. *Pediatr Dent*. 1995;17:143(abstract).
10. Hatibovic-Kofman S. Microleakage of sealants after conventional, bur, and air-abrasion preparation of pits and fissures. *Pediatr Dent*. 1998;20:173-176.
11. Geiger SB, Gulayev S, Weiss EI. Improving fissure sealant quality: Mechanical preparation and filling level. *J Dent*. 2000;28:407-412.
12. Brannstrom M, Nordenvall KJ. Bacterial penetration, pulpal reaction and the inner surface of concise enamel bond composite filling in etched and unetched cavities. *J Dent Res.* 1978;57:3-10.
13. Tobias RS. Antibacterial properties of dental restorative materials: A review. *Int Endod*. 1998;21:155-160.
14. Leinfelder LF. New development in resin restorative systems. *JADA*. 1993;128:573-581.

15. Meiers JC, Miller GA. Antibacterial activity of dentin bonding systems, resin-modified glass ionomers and polyacid-modified composite resins. *Oper Dent*. 1996;21:257-264.
16. Palenik CJ, Setcost JC. Antimicrobial abilities of various dentine bonding agents and restorative materials. *J Dent.* 1996;24:289-295.
17. Weiss EI, Shalhav M, Fuss Z. Assessment of antibacterial activity of endodontic sealers by a direct contact test. *Endod Dent Traumatol*. 1996;12:179-184.
18. Van Loveren C. Antimicrobial activity of fluoride and its in vivo importance: identification of research questions. *Caries Res*. 2001;35(suppl)1:65-70.
19. Herra M, Castillo A, Bravo M, et al. Antibacterial activity of resin adhesives, glass-ionomer and resin-modified glass-ionomer cements and compomer in contact with dentin caries samples. *Operat Dent.* 2000;4:265-269.
20. Willershausen B, Callaway A, Ernst CP, Stender E. The influence of oral bacteria on the surface of resin-based dental restorative materials—an in vitro study. *Int Dent J*. 1999;49:231-239.
21. Loyola-Rodriguez JP, García-Godoy F. Antibacterial activity of fluoride-releasing sealants on mutans streptococci. *J Clin Pediatr Dent*. 1996;20:109-111.
22. Jensen ME, Wefel JS, Triolo PT, Hammesfahr PD. Effects of a fluoride-releasing fissure sealant on artificial enamel caries. *Am J Dent*. 1990;3:75-78.
23. Hicks MJ, Flaitz CM. Caries-like lesion formation around fluoride-releasing sealant and glass ionomer. *Am J Dent*. 1992;5:329-334.
24. Forss H, Jokinen J, Spets-Happonen S, Seppa L, Luoma H. Fluoride and mutans streptococci in plaque grown on glass ionomer and composite. *Caries Res*. 1991;25:454-458.
25. do Rego MA, de Araujo MA. Microleakage evaluation of pit and fissure sealants done with different procedures, materials and laser after invasive technique. *J Clin Pediatr Dent*. 1999;24:63-68.

Abstract of the Scientific Literature

Performance-enhancing Substances and Their Use Among Adolescent Athletes

The purpose of this study was to review the history and classification, the epidemiology and prevalence, and the clinical aspects of performance-enhancing substances (PES). This article lists the names and actions of PES, grouping them into those that are considered supplements, prescription drugs and illicit or banned substances and describes the signs and symptoms of anabolic steroid and growth hormone use. Adverse effects of anabolic steroids and other PES are characterized as well as the clinical changes seen in individuals using anabolic steroids and growth hormone. Adverse effects of anabolic and androgenic steroids include acne, hirsuitism, psychosis, aggression, and premature closure of epiphyseal growth plates. The use of anabolic steroids among adolescents has been linked to other substance abuse, including intravenous drugs. Although the use of human growth hormone (HGH) and its analogs and releasing agents has not yet been reported in the medical literature among adolescents, its use by competitive bodybuilders and higher level athletes is prevalent. Clinically observable effects of HGH are consistent with acromegaly: behavioral changes, coarsening of facial features, growth of facial bones, enlargement in thickness of fingers and toes, increase in skull circumference, broadening of the nose, enlargement of the tongue, growth of the mandible, increase separation of the teeth, cardiovascular disease, diabetes, hypertension, and peripheral neuropathy.

Comments: Of particular interest to the pediatric dentist are the orofacial and/or dental changes that can be observed in individuals using PES such as anabolic steroids and HGH and its linkage to other substance abuse. The use of PES has grown significantly among athletes to help performance and nonathletes to improve appearance. Use of PES is widespread among adolescents and is related to pressures to excel in academics, at home, and on the athletic field. **SS**

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Koch JJ. Performance-enhancing substances and their use among adolescent athletes. *Pediatr Rev*. 2002;23:310-317.

12 references