Clin Oral Invest (2012) 16:1095–1103
DOI 10.1007/s00784-011-0613-0

ORIGINAL ARTICLE

The impact of three strains of oral bacteria on the surface and mechanical properties of a dental resin material

Karen S. Gregson · Han Shih · Richard L. Gregory

Received: 18 February 2011 / Accepted: 23 August 2011 / Published online: 6 September 2011

Abstract The purpose of this study was to determine if three strains of bacteria could impact the mechanical or surface properties of a dental resin material. Resin material specimens were incubated at 37°C in sterile saline, tryptic soy broth supplemented with sucrose (TSBS), or TSBS inoculated with *Streptococcus mutans, Streptococcus gordonii*, or *Streptococcus sanguis*. The specimens were subjected to Fourier transform infrared spectroscopy before and after incubation. The flexural strength test was performed once a week for 6 weeks. Microhardness and scanning electron microscopy (SEM) was performed on specimens at 1 and 6 weeks. Differences in the area under the carbonyl peak were statistically significant for the specimens incubated in the media inoculated with either *S. mutans* or *S. gordonii*. To determine why *S. sanguis* did not produce changes as the other bacteria did, triethylene glycol dimethacrylate, methacrylic acid, and triethylene glycol were added to bacterial cultures at increasing concentrations. Both methacrylic acid and triethylene glycol reduced the number of colony-forming units of *S. sanguis*. Specimens incubated in TSBS, saline or in culture with *S. sanguis* demonstrated a decrease in peak stress in week 1 of the flexure strength test. SEM demonstrated that surface topology changed for those specimens incubated in culture with *S. mutans* or *S. gordonii*. The changes in surface topology demonstrated here could contribute to the secondary caries and changes in esthetic properties seen clinically with the use of resin materials in dental restorations.

K. S. Gregson (✉) · H. Shih · R. L. Gregory
Department of Oral Biology,
Indiana University School of Dentistry,
1121 West Michigan Street,
Indianapolis, IN 46202, USA
e-mail: kagregso@iupui.edu

Keywords Dental materials · Biodegradation · Oral bacteria

Introduction

Due to concerns about mercury toxicity and the desire for more esthetically pleasing dental restorations, the use of amalgam as a dental restoration material has decreased. The use of dental resin composites, which are mercury free and can be color matched to the dental patient's teeth, as a restoration material has increased. Even though composite resins offer many advantages over amalgams, secondary caries is often the reason for the replacement of the composite resin restorations. Bacterial biofilm covers all surfaces of dental restorations and the enamel of the teeth. While the biofilm found on amalgams is thicker than that found on composite resins, the biofilm present on composite resins is more viable than that on amalgams [1]. Amalgams have antibacterial properties which are not present in composite resins [2]. The microbial variety of microorganisms under composite restorations is much greater than the variety found under amalgams [3]. The amount and type of bacteria were compared between amalgam and composite materials from dentin samples taken after restoration removal and caries diagnosis. There were up to eight times more bacterial species under a composite restoration than under an amalgam restoration. The variety of bacteria under the composite restoration was similar to that of an infected root canal while the bacteria found under an amalgam restoration were similar to that found in carious dentin and plaque.

Bacterial growth can influence the materials, and the materials can influence the bacteria. The products of degradation of the monomers that comprise composite resins by human salivary esterases have been shown to

increase the growth of oral bacteria [4]. In particular, the degradation products of triethylene glycol dimethacrylate (TEGDMA) were shown to influence the growth rates of three strains of bacteria, *Streptococcus mutans* NG8 and JH1005 and *Streptococcus salivarius* AT2 at both pH 7.0 and 5.5 [5]. Microorganisms can cause changes in surface topology of composite resins most likely by bacterial enzymes [6]. The streptococci were grown on the surface of resin composite disks for 1 day, 1 week, or 1 month. The disks were analyzed with atomic force microscopy. The surface roughness of the resin disks was shown to increase in a time-dependent manner. No changes in microhardness were seen in the resin composite disks. Moreover, the changes in surface topology of resin composites may cause accelerated biofilm formation by increasing the retension of the biofilm.

The purpose of the present study was to determine the interactions of the bacteria and dental resin materials. The three strains of bacteria, *S. mutans*, *S. gordonii*, and *S. sanguis*, were chosen based on their prevalence in the oral cavity and importance in biofilm formation. The hypotheses tested in this study were:

1. Exposure to bacteria results in chemical degradation of a dental resin.
2. Exposure to TEGDMA or the products of degradation of TEGDMA (methacrylic acid (MA) or triethylene glycol or (TEG)) can influence the number of the bacteria.
3. Exposure to bacteria results in a reduction of the mechanical and surface properties of a dental resin.

Materials and methods

Materials

Gentamicin was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). Mylar film was purchased from 3M (St. Paul, MN, USA). Bacteria were purchased from the American Type Culture Collection (Manassas, VA, USA). Glass slides were purchased from Kodak (Rochester, NY, USA). All of the other materials and chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Specimen preparation

Commercial resins contain unknown compounds that might interact with the bacteria. Therefore, a laboratory formulated resin was used in this study to control for all the components of the resin. A light-cured resin matrix was prepared by mixing 1:1 bisphenol A glycidyl methacrylate (bis-GMA) with TEGDMA and adding 0.5% camphorquinone, 0.5% dimethylamino ethylmethacrylate, and 0.5% ditertbutyl methyl phenol. The weight of the mixture was determined. A composite was formed by the addition of 55% by weight-treated barium glass and mixed for 3 h under vacuum at 40°C. The specimens were exposed to QTH visible light for 40 s (750 mW/cm^2, Radiometer Model 100, Demetron Research Corp, Danbury, CT, USA). For the flexural strength specimens, the resin was placed in a stainless steel split mold from the American Institute of Steel and Iron (Detroit, MI, USA). Specimens with the dimensions of 2×5×25 mm were fabricated. Excess flash was removed with a scalpel. The specimens were polished with 240 and 600 grit carbide paper on a variable speed polisher with water spray at a speed of 300 rpm. The specimens were stored overnight in distilled water to allow for the release of any un-reacted monomers. They were sterilized with ethylene oxide before placement into the various culture media. A set of specimens (n=5) was subjected to the static flexure test and hardness testing to serve as the control group. For the Fourier transform infrared spectroscopy (FTIR) specimens, a circle was cut into plain copy paper and the paper was placed on mylar film to serve as a mold. The mixture was dropped onto the mylar film in the middle of the circle. A second piece of mylar film was placed on top, and the material was compressed with a glass slide. The specimens were exposed to QTH visible light for 40 s (750 mW/cm^2, Radiometer Model 100, Demetron Research Corp, Danbury, CT, USA). The specimens were allowed to set for at least 5 min before being removed from the mold. The specimens were stored overnight in distilled water to allow for the release of any un-reacted monomers. They were sterilized with ethylene oxide before placement into the various storage solutions. The dimensions of the specimens were determined with a micrometer. Specimen height and diameter was determined by the average of three measurements on each specimen. The measurements from each specimen were then averaged. The average dimensions of the specimens were 11.11±0.51×0.18±0.04 mm.

Bacterial culture conditions

S. mutans UA159, *S. gordonii* DL1, and *S. sanguis* 10,556 were cultured for 16 h in tryptic soy broth supplemented with 1% sucrose (TSBS) without antibiotics at pH 7.3 in 5% CO_2 at 37°C. In addition, TSBS was prepared with antibiotics (100 units/ml penicillin and 50 μg/mL gentamicin).

Specimen culture conditions

Resin specimens were divided into five groups of five specimens each and cultured in 1 mL of sterile saline, 1 ml

of TSBS with antibiotics, or 1 ml of TSBS without antibiotics inoculated with 10 μl of a 16-h culture of *S. mutans*, *S. gordonii*, or *S. sanguis* in 6-well sterile tissue culture plates. The storage plates were incubated in 5% CO_2 at 37°C. The media were exchanged with fresh media or saline every 2 days for 6 weeks (4 weeks for the FTIR specimens). One set of each specimen (n=5) was removed each week and subjected to the static flexure test.

Fourier transform infrared spectroscopy

The specimens were subjected to FTIR (model 4100, Jasco Corporation, Tokyo, Japan). The FTIR spectra were acquired in absorbance mode from 64 co-added scans at four wavenumber resolution. Prior to running the samples, background spectra were collected. In the infrared spectra, esters give rise to three bands at approximately 170 (carbonyl peak), 1,200, and 1,100 cm^{-1} [7]. The carbonyl peak was the most distinct in the spectra from the composite resin in this study. Therefore, it was chosen to be analyzed. Using the software installed on the FTIR instrument, the area under the carbonyl peak (~1,700 cm^{-1}) was calculated for all the specimens before storage in the various conditions. At the end of the storage period, the specimens were removed from the storage plates, blotted dry, and their FTIR spectra taken exactly as before. The area under the carbonyl peak was calculated again and the values for each group were compared to initial values using the Student's paired test.

Addition of TEDGMA, MA, or TEG to the bacterial cultures

S. mutans UA159, *S. sanguis* 10556, and *S. gordonii* DL1 were cultured for 16 h in TSBS at pH 7.3 in 5% CO_2 at 37°C. TEGDMA, TEG, or MA at 0 (control), 1.25, 4.0, or 20 mM final concentration was added to 5 ml of TSBS and 100 μl of the 16-h bacterial cultures were transferred into the TSBS tubes and incubated for 48 h in 5% CO_2 at 37°C. This concentration range was chosen based on concentrations of degradation products previously reported in the literature [5].

Measurement of viable bacteria using colony-forming units

After the 48-h incubation, the bacterial cultures were diluted 1:10 and 1:1,000 in sterile saline. The diluted bacterial samples were then mechanically plated using a spiral plater (Spiral Biotech, Norwood, MA, USA) on anaerobic blood agar plates. The agar plates were incubated in 5% CO_2 at 37°C for 48 h. Colony-forming units (CFU) were enumerated from the spiral plating on blood agar plates with a ProtoCOL-automated colony counter from Synbiosis, Inc. (Frederick, MD, USA). The data were recorded in Excel.

Static flexure test

Every week of incubation, one set (n=5) of specimens from each culture condition was tested for flexural strength with the 3-point bending test on an Instron Model 1123 testing machine with a span length of 15 mm under a crosshead speed of 1 mm/min. The peak load required to break the specimens was averaged from five specimens.

Hardness testing

A universal indenter tester (HMV-2, Shimadzu, Tokyo, Japan) was used for Knoop hardness testing on specimens from weeks 1 and 6 (n=5). The tester was set for an automatic mode of 50g-force for 15 s. Three indentations were made for each specimen with a 1-mm distance between them, and the means were calculated for each specimen. Measurements were made with ×40 magnification. The Knoop Hardness number is provided automatically by the tester's software.

Scanning electron microscope

Specimens from weeks 1 and 6 were examined for changes in surface topology under the scanning electron microscope (SEM; JEOL JSM-5310 LV, Tokyo, Japan). The specimens were sputter-coated with gold (SCD 050; Balzers, Schaan, Leichtenstein). SEM micrographs of representative areas of the surface were taken.

Statistical analysis

Using SigmaStat for Windows 3.00© (Jandel Scientific, San Rafael, CA, USA), one-way analysis of variance (ANOVA) was performed on the initial area under the carbonyl peak for all specimens. The Student's paired t test was performed on all data sets with respect to the area under the carbonyl peak with comparison to prior to storage and after storage conditions. The Student's paired t test was used to compare the CFUs from control bacterial cultures to bacterial cultures in which TEGDMA, TEG, or MA were added in increasing concentrations. Two-way ANOVA was performed on the flexure test and on the hardness test data with time and medium as the factors. The Student's paired t test was performed on the data from the flexure test and hardness testing comparing week 1 specimens to those of week 6. When the p values were less than 0.05, the differences were considered to be statistically significant.

Table 1 Mean of the area under the carboxyl peak prior to and after storage

Condition	Area under the carbonyl peak (mean±SD)		*p* value
	Prior to storage	After storage	
Streptococcus mutans	2.85±1.16	0.86±0.37	0.012*
Streptococcus gordonii	2.75±0.60	1.02±0.55	0.01*
Streptococcus sanguis	2.13±0.88	1.15±0.22	0.099
TSBS	3.24±1.29	1.80±1.24	0.170
Saline	2.46±1.12	2.01±0.87	0.418

p values from differences in the area under the carbonyl peak prior to and after storage

*$p<0.05$, statistically significant differences

Results

FTIR prior to and after storage

The values of the area under the carbonyl peak were analyzed with one-way ANOVA prior to and after incubation (Table 1). There were no significant differences in the areas under the carbonyl peaks between the specimens prior to incubation ($p=0.475$).

Storage with either *S. mutans* or *S. gordonii* resulted in significant differences in the area under the carbonyl peak before and after incubation ($p=0.012$ and 0.01, respectively). Incubation with *S. sanguis*, TSBS media, or saline resulted in differences in the area under the carboxyl peak that were not statistically significant. Figure 1a is the FTIR spectrum representative of a specimen incubated with *S. mutans*. The FTIR spectrum of a specimen incubated with *S. gordonii* resembles the *S. mutans* spectra shown in Fig. 1a (data not shown). The FTIR spectrum in Fig. 1b is from a specimen incubated with *S. sanguis* and most closely resembles the FTIR spectra for specimens incubated in TSBS media or saline.

Effect of TEGDMA or degradation products of TEGDMA on bacterial cell growth

For *S. gordonii*, TEGDMA and MA decreased the number of CFUs while TEG increased the number of CFUs (Fig. 2a). The effects are statistically significant for all three compounds at 20 mM but statistically significant at 4 nM for TEG and at 1.25 mM for TEGDMA. For *S. sanguis*, all three compounds, TEGDMA, TEG, and MA decreased the number of CFUs in a statistically significant manner at 4 and 20 mM. MA decreased the number of CFUs statistically significant at 1.25 mM (Fig. 2b). For *S. mutans*, all three compounds decreased the number of CFUs in a statistically significantly manner at 20 and 1.25 mM. TEGDMA and MA decreased the number of CFUs at 4 mM, but TEG did not (Fig. 2c).

Static flexure test

The average peak stress for the control specimens and the specimens incubated in the various conditions and broken each week were determined (Table 2). Two-way ANOVA was performed with the factors of time and media. Average peak stress was the dependent variable. There was not a statistically significant interaction between time and media, $p=0.750$. For week 1, the average peak stress of the specimens incubated in *S. sanguis* culture, TSBS medium, or saline was generally lower but not significantly lower than that of the control. The differences in average peak stress between weeks 1 and 5 were not statistically significant for any of the conditions tested. The differences in average peak

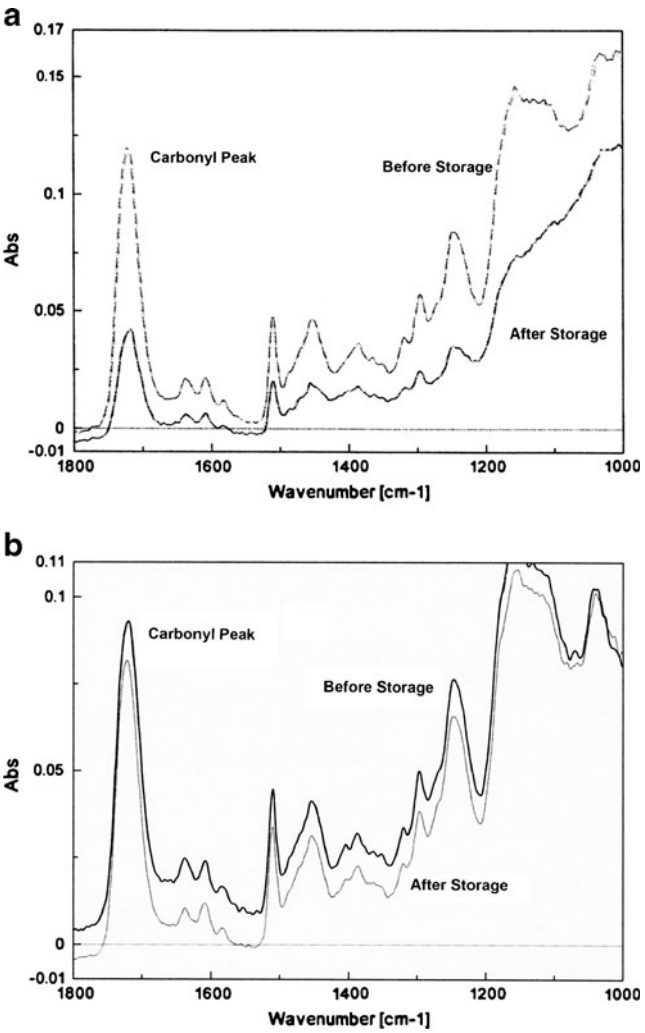

Fig. 1 FTIR spectra of the dental resin material. **a** The spectra from a specimen that was stored in media inoculated with *S. mutans*. **b** The spectra from a specimen that was stored in media inoculated with *S. sanguis*

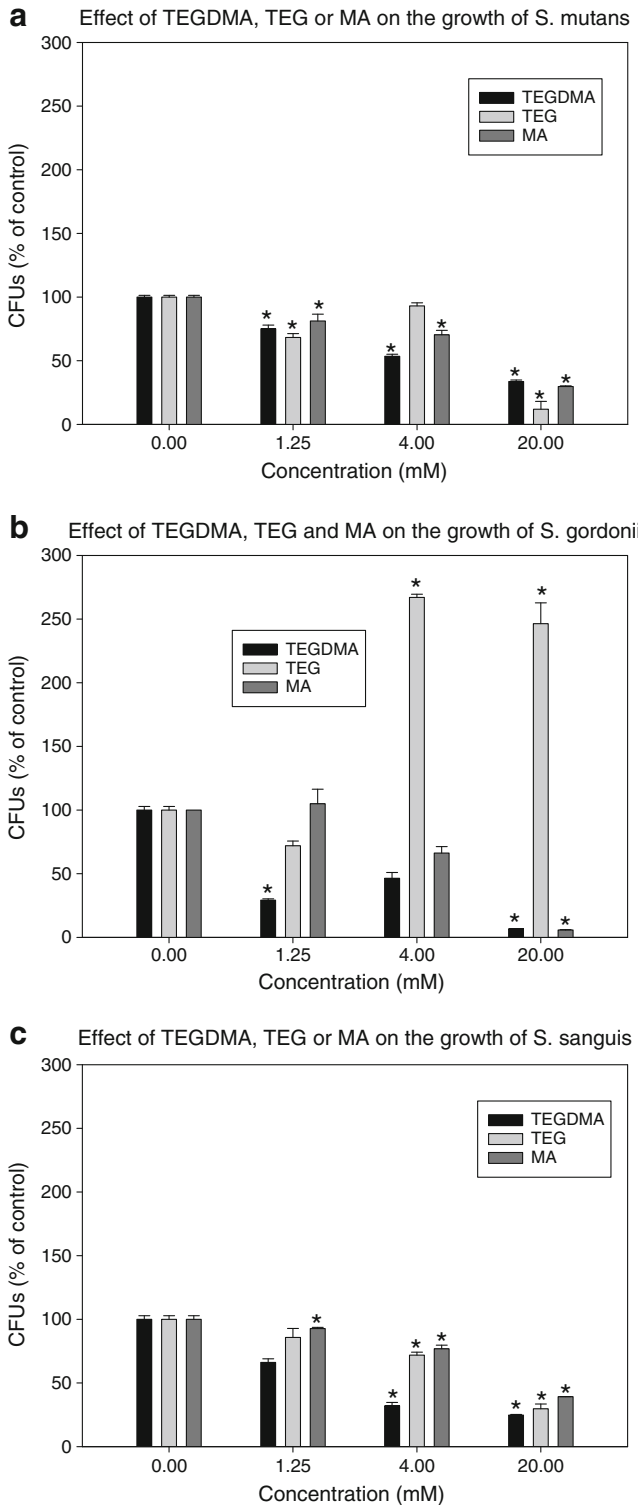

Fig. 2 Colony-forming units of *S. mutans* (**a**), *S. gordonii* (**b**), or *S. sanguis* (**c**) as a function of the concentration of TEGDMA, TEG, or MA. The *bars* represent the mean (±standard deviation) number of viable colonies on blood agar plates after 48 h of growth. *Asterisks* indicate significant differences compared with control samples

stress did not become apparent until week 6. There was a statistically significant increase in the average peak stress between weeks 1 and 6 in specimens incubated in *S. sanguis* culture, TSBS medium, or saline (p=0.032, 0.002, and 0.048, respectively). The differences in average peak stress between weeks 1 and 6 in specimens incubated in *S. mutans* or *S. gordonii* cultures were not statistically significant (p= 0.495 and 0.499, respectively).

Hardness testing

Two-way ANOVA was performed on the hardness testing data. The factors of media and time did not interact in a statistically significant manner at p=0.803. The hardness of all specimens except those incubated in saline demonstrated a statistically significant increase from weeks 1 to 6 (Table 3).

Scanning electron microscope

The surface topography from micrographs of a specimen incubated with *S. sanguis* from week 1 (Fig. 3a) appears approximately the same as a specimen from week 6 (Fig. 3b), with the groves from sanding apparent. The micrographs from specimens incubated with saline or TSBS resemble that of Fig. 3a at 1 week and that of Fig. 3b at 6 weeks (data not shown). This is in contrast to the micrographs from a specimen incubated with *S. mutans*. The groves from sanding are apparent in the week 1 (Fig. 4a) specimen. The specimen from the 6 week *S. mutans* incubation period (Fig. 4b) appears to be degraded with no apparent groves from sanding. The micrographs from specimens incubated with *S. gordonii* resemble that of Fig. 4a at 1 week and that of Fig. 4b at 6 weeks (data not shown). This indicates that the surface topography of the *S. mutans*- and *S. gordonii*-treated specimens were significantly degraded over the 6-week treatment period.

Discussion

The ability of composite resin restorations to withstand the chemical and mechanical forces in the oral cavity is directly connected to the service life of these materials. One of the challenges that restorations must endure is the presence of biofilm covering the surface of the restoration. The biofilm in the oral cavity is critical for the survival and pathogenicity of *S. mutans*, the primary bacterial species involved in the formation of dental caries in humans [4]. *S. mutans* binds to salivary agglutinin in the salivary pellicle which coats enamel and restorative surfaces. *S. gordonii* is thought to bind to α-amylase, the most common enzyme in saliva, and through this interaction the adhesion to salivary pellicles is formed [8]. A benign microorganism with regards to dental caries, *S. sanguis* has been shown to

Table 2 Peak stress of the resin specimens in the flexure test

Peak stress of resin specimens flexure test (Mpa)							
Time	24 h	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Storage conditions							
Control	66.12±17.81	N/A	N/A	N/A	N/A	N/A	N/A
Streptococcus mutans	N/A	73.24±12.61	67.76±5.20	70.50±9.78	62.82±15.45	81.86±9.84	79.64±15.54
Streptococcus gordonii	N/A	61.78±18.22	66.88±13.32	62.74±18.73	48.82±16.65	78.96±7.63	70.14±16.91
Streptococcus sanguis	N/A	53.52±25.69	67.38±17.05	78.44±10.63	62.30±23.50	68.00±18.38	78.85±22.89[a]
TSBS	N/A	56.10±10.02	65.10±17.00	64.80±15.55	78.48±10.17	75.88±10.22	83.32±9.06[a]
Saline	N/A	47.74±25.78	64.88±15.72	73.88±24.05	66.74±22.53	72.18±18.76	78.76±14.86[a]

[a] Significant increase compared to week 1 specimens

antagonize *S. mutans* at the ecological level by competitively binding to salivary agglutinin and represents another major component of dental biofilm [9].

The purpose of this study was to examine the interactions between bacteria typically found in biofilm and a resin composite material. One of the hypotheses was that exposure to bacteria would result in chemical degradation of the dental resin. The ester bonds in the dental resin are important for maintaining the chemical integrity of the resin. The esters of dental resins are measurable by FTIR. We hypothesize that bacteria degrade dental resin. For both *S. mutans* and *S. gordonii*, this hypothesis was true. Both bacteria demonstrated significant degradation of the ester bond when compared with media alone, TSBS, saline, or *S. sanguis*. The hypothesis was not true for *S. sanguis*.

The reason that *S. sanguis* did not cause as much degradation as did *S. mutans* and *S. gordonii* could be that *S. sanguis* is more sensitive to the potential toxic effects of TEGDMA and the products of degradation of TEGDMA (TEG and MA) than *S. mutans* or *S. gordonii*. Alternatively, TEGDMA and the products of degradation of TEGDMA (TEG and MA) could cause increased growth of *S. mutans* and *S. gordonii*. Increasing the numbers of bacteria would cause more degradation. TEGDMA was chosen because it is relatively small and hydrophilic and would more likely be released into the medium as degradation products than would bis-GMA. These compounds released from the resin could be influencing either the number of or the metabolic capacity of the bacteria as the bacteria interact with the resin. These hypotheses were tested by the addition of increasing concentrations of TEGDMA, MA, and TEG to bacterial cultures for 16 h followed by counting the CFU from each strain of bacteria.

The three compounds did effect the growth of the bacteria. The growth of *S. sanguis* was decreased in a statistically significant manner by MA at the lowest concentration tested, 1.25 mM. At 4 and 20 mM TEGDMA, TEG and MA decreased the growth of *S. sanguis*. The growth of *S. sanguis* was affected at lower concentrations of TEG and MA than the growth of either *S. gordonii* or *S. mutans*. The hypothesis that the growth of *S. sanguis* was decreased by the compounds most likely released from the resin holds true and could be a reason that *S. sanguis* did not degrade the resin as extensively as *S. mutans* and *S. gordonii* did.

The growth of *S. gordonii* was significantly increased by TEG. At 4 and 20 mM TEG, there was a statistically significant increase in the growth of *S. gordonii*. The effect

Table 3 Microhardness values of resin material after storage for 1 and 6 weeks

Microhardness of resin material (KHN)				
Time	24 h	1 week	6 weeks	
Storage conditions				*p* value (week 1 vs. 6
Control	13.79±3.45	N/A	N/A	N/A
Streptococcus mutans	N/A	19.38±6.41	27.7±3.26	0.032
Streptococcus gordonii	N/A	22.35±3.26	26.32±1.71	0.042
Streptococcus sanguis	N/A	21.76±4.55	27.63±1.80	0.028
TSBS	N/A	21.23±4.29	28.17±2.67	0.015
Saline	N/A	24.14±7.42	21.03±3.37	0.548

p values of <0.05 indicate statistical significance

KHN Knoop hardness number

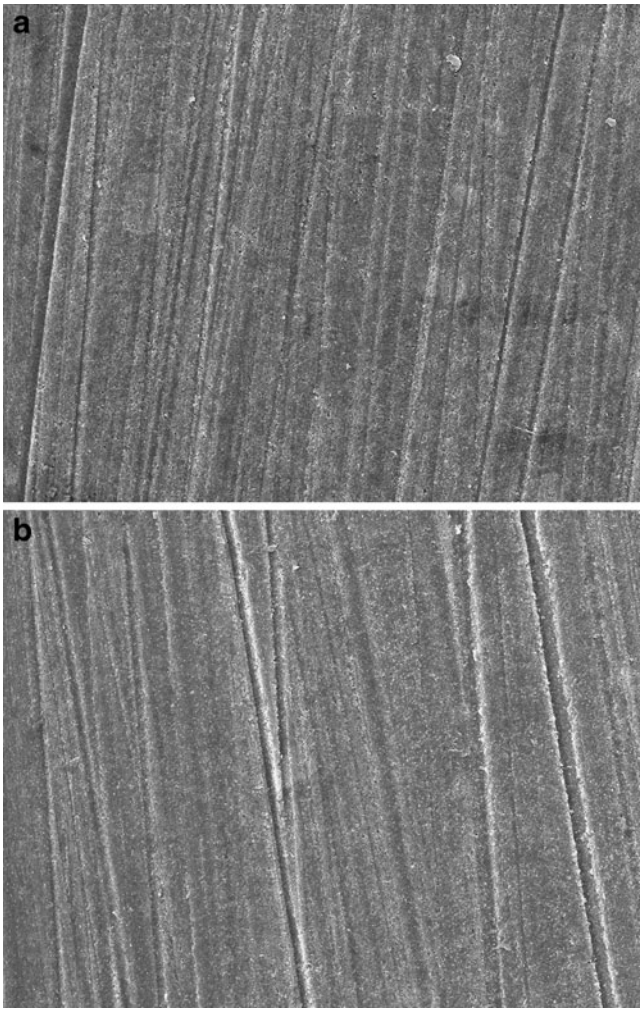

Fig. 3 SEM micrographs of a dental resin material at ×500 magnification after 1 (**a**) or 6 weeks (**b**) in culture with *S. sanguis*, indicating no effect on the surface topography after 6 weeks of treatment

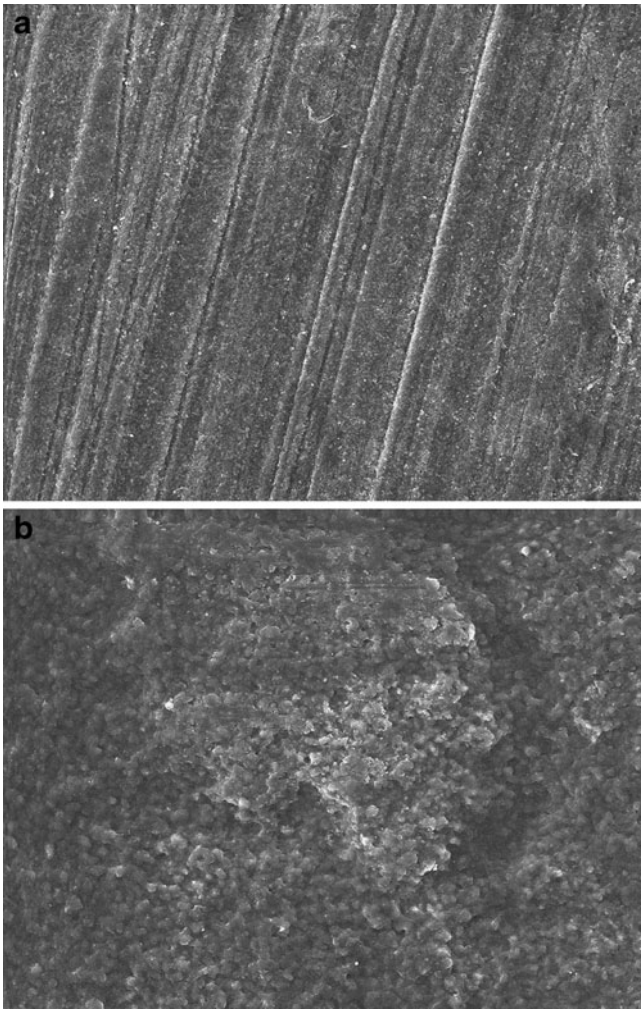

Fig. 4 SEM micrographs of a dental resin material at ×500 magnification after 1 (**a**) or 6 weeks (**b**) in culture with *S. mutans*, indicating a significant loss of surface topography after 6 weeks of treatment

of TEGDMA on the growth of *S. gordonii* was inconsistent. TEGDMA caused a statistically significant decrease in the growth of *S. gordonii* at 1.25 mM but a smaller decrease in growth at 4 mM when compared with controls. At 20 mM of TEGDMA, the decrease in growth in *S. gordonii* was significant. MA did not have a significant impact on the growth of *S. gordonii* until concentrations reached 20 mM. Lower concentrations of TEGDMA decreased the growth of *S. gordonii*, higher concentrations of MA decreased the growth of *S. gordonii*, and at concentrations above 4 mM, the growth of *S. gordonii* was greatly increased. The concentration of each of the compounds that the bacteria were exposed to during the degradation experiment is unknown, and therefore, the hypothesis that the compounds affected the growth of *S. gordonii* cannot be proven.

The growth of *S. mutans* was also affected by the three compounds. TEGDMA and MA demonstrated a significant decrease in the growth of *S. mutans* which became larger as the concentration of either TEGDMA or MA increased. The effect of TEG on the growth of *S. mutans* was inconsistent with a significant decrease in growth at 1.25 mM, an increase in growth comparable to control at 4 mM, and a large decrease at 20 mM. The hypothesis that the compounds most likely released from the resin increased the growth of *S. mutans* holds true for only TEG and at concentrations below 20 mM but above 1.25 mM. The concentrations of TEGDMA, TEG, or MA that the bacteria were exposed are unknown therefore the hypothesis that the compounds affected the growth of *S. mutans* cannot be proven.

Another hypothesis tested in this study is that exposure to bacteria results in a reduction of the mechanical and surface properties of a dental resin. The effects of the bacteria on the materials were examined with the flexural strength test, hardness testing and the SEM.

The mechanical strength of the material was measured with the static flexural test. After the materials were exposed to the media for 1 week, the flexural strength test decreased for the materials incubated in saline, TSBS, and in culture with *S. sanguis* when compared with the control specimens which were incubated in distilled water for 24 h prior to breakage. The specimens incubated in culture with *S. mutans* or *S. gordonii* did not exhibit a decrease in the flexural strength test after 1 week of incubation when compared with controls. The decrease in strength of a material after incubation in aqueous media can be attributed to plasticization of the material. Water softens the polymer and swells the material by binding to the polymer chain through hydrogen bonding. As the polymer becomes plasticized, the chains can relax and ends of the monomers that were not involved in the initial polymerization can be incorporated into the polymer matrix [10]. The polymer then shows an increased strength. This could be the process through which the specimens incubated in saline, TSBS, or in culture with *S. sanguis* became stronger at the 2-week period. The peak stress in the flexural strength test was not significantly different for all specimens at the end of the 6-week period regardless of the incubation medium. It is not clear why the specimens incubated with *S. mutans* or *S. gordonii* did not undergo the initial plasticization seen in the other specimens. The biofilm coating the specimens from these two strains of bacteria could have been sufficiently hydrophobic to keep water from entering the specimens. In this regard, *S. mutans* is extremely hydrophobic and depends on hydrophobicity to attach to tooth surfaces. Knockout mutants of *S. mutans* antigen I/II, a cell surface adhesin, significantly reduces surface hydrophobicity and attachment of *S. mutans* cells (manuscript in preparation). The current results do correlate with the previous data in which specimens incubated in culture with *S. mutans* and *S. gordonii* were chemically different than those incubated in either saline, TSBS, or in culture with *S. sanguis*.

The specimens from weeks 1 and 6 were subjected to hardness testing. The specimens from week 1 were not statistically different from the control specimens. They were also not statistically different from each other. The incubation media did not make a difference in hardness at week 1. The specimens from week 6 are statistically different from the control specimens. However, they are not statistically different from each other. As in week 1, the incubation media does not make a difference in the hardness of the specimens. Within the same incubation medium, there are statistical differences between weeks 1 and 6 for all incubation media except for saline. All other specimens exhibited an increase in hardness over the 6-week period. The hardness of those specimens incubated in saline decreased slightly over the 6-week period. The hardness data do not correlate with the previous chemical data. It was expected that the hardness in specimens cultured with *S. mutans* and *S. gordonii* would decrease since these specimens demonstrated the most chemical degradation in the previous experiment.

The specimens from weeks 1 and 6 were analyzed with SEM. The micrographs from this analysis indicate that at week 1, the appearance of all of the specimens look the same with the groves from sanding apparent. However, at week 6 the specimens cultured with either *S. mutans* or *S. gordonii* are degraded with no groves from sanding seen. This result correlates with the previous data in which chemical degradation was observed in specimens cultured with either *S. mutans* or *S. gordonii*. The surfaces of week 1 specimens appear smoother than those from week 6.

The SEM results are consistent with other studies. Indirect composite resins were polished using the four techniques by Aykent et al. [11]. After polishing, surface roughness was measured with a profilometer. The composite resins were covered with pellicle and *S. mutans* was allowed to attach. A positive relationship was observed between the surface roughness of the resin composites and the vital *S. mutans* adhesion. In another study in which *S. mutans* biofilm was grown on the surface of four types of materials for 30 days [12], the materials were a glass ceramic, a composite resin, a resin-modified glass ionomer, and a conventional glass ionomer. The growth of the biofilm had an impact on the surface morphology of the composite resin. *S. mutans* biofilm was grown on composite resin materials, and the change in roughness was measured with an atomic force microscope [6]. Surface roughness increased in a time-dependent manner. Increasing surface roughness implies that chemical changes are occurring on the surface of the composite resin restorations. The changes in the surface chemistry of the resin composites presented in the current research may represent one of the means by which the bacteria increase the surface roughness of the material. The bacterial degradation of the resin may lead to the physical changes observed. Conversely, changes in the surface roughness increase the biofilm content of the restoration, which in turn can lead to restoration failure and secondary caries. Surface topography is an important feature of resin composite materials. Often esthetic properties are the reason the patient will request a resin composite restoration over an amalgam restoration. Surface roughness can compromise esthetic properties. With an increase in surface roughness, staining, discoloration, and loss of gloss can occur [13, 14].

The clinical impact of the degradation of resin composite was illustrated by other studies. In the first, the selection of dental materials and longevity of replaced restorations were compared [15]. Restorations (n=1,536) were placed in permanent teeth in patients older than 15 years old. Three materials were compared: amalgam, resin composite, and glass ionomer. The median longevity of the materials were

16 years for amalgam, 6 years for resin composite, and 11 years for glass ionomer. Findings from the New England Children's Amalgam Trial demonstrated that in both permanent and primary teeth, the mode of failure of composite resins was predominately secondary caries while that of amalgam was new caries [16]. In that trial, children ages 6–10 years were randomized into two groups: those that received amalgam restorations in primary or permanent teeth (n=267) and those that received composite resin restorations in primary or permanent teeth (n=267). They were followed up for 5 years [16]. Composite resin restorations required seven times as many repairs as amalgam restorations.

Other factors could contribute to the degradation of composite resins. Human saliva esterases degrade the composite resin materials [17]. Human saliva esterases can cause marginal bacterial microleakage. Resin composites were bonded to human dentin and exposed to esterase enzymes found in human saliva. After 90 days, the specimens were subjected to confocal laser scanning microscopy. The depth of bacterial cell penetration between the resin and the dentin was greater in specimens exposed to esterases than in those specimens that were not exposed. The authors concluded that esterase activity and biodegradation can contribute to secondary caries [18]. In addition, other compounds can elute from composite resins and have an impact on biofilm. The amount of glucan synthesized by *S. mutans* glucosyltransferase was shown to increase when exposed to TEGDMA but decrease when exposed to camphorquinone [19]. Camphorquinone was shown to be released from a composite resin [20]. It is possible that camphorquinone or other compounds released from the composite resin had a negative impact on the growth of *S. sanguis*.

Composite resin restorations typically do not have the antibacterial properties that amalgam restorations have. These results indicate that they are susceptible to a roughening of the surface of the resin by bacterial biofilm. This could be another explanation for the increase in secondary caries and restoration failure seen in resin restorations but less commonly seen in amalgam restorations.

Conflict of interest The authors have no conflict of interest.

References

1. Auschill TM, Arweiler NB, Brecx M, Reich E, Sculean A, Netuschil L (2002) The effect of dental restorative materials on dental biofilm. Eur J Oral Sci 110:48–53
2. Beyth N, Domb AJ, Weiss EI (2007) An in vitro quantitative antibacterial analysis of amalgam and composite resins. J Dent 35:201–206. doi:10.1016/j.jdent.2006.07.009
3. Splieth C, Bernhardt O, Heinrich A, Bernhardt H, Meyer G (2003) Anaerobic microflora under class I and class II composite and amalgam restorations. Quintessence Int 34:497–503
4. Senadheera D, Cvitkovitch DG (2008) Quorum sensing and biofilm formation by *Streptococcus* mutans. Adv Exp Med Biol 631:178–188. doi:10.1007/978-0-387-78885-2_12
5. Khalichi P, Cvitkovitch DG, Santerre JP (2004) Effect of composite resin biodegradation products on oral streptococcal growth. Biomaterials 25:5467–5472. doi:10.1016/j.biomaterials.2003.12.056
6. Beyth N, Bahir R, Matalon S, Domb AJ, Weiss EI (2008) Streptococcus mutans biofilm changes surface-topography of resin composites. Dent Mater 24:732–736. doi:10.1016/j.dental.2007.08.003
7. Smith B (1999) Infrared spectral interpretation: a systematic approach, 1st edn. CRC Press, Boca Raton, p 265
8. Rogers JD, Palmer RJ Jr, Kolenbrander PE, Scannapieco FA (2001) Role of *Streptococcus gordonii* amylase-binding protein A in adhesion to hydroxyapatite, starch metabolism, and biofilm formation. Infect Immun 69:7046–7056. doi:10.1128/IAI.69.11.7046-7056.2001
9. Kreth J, Merritt J, Shi W, Qi F (2005) Competition and coexistence between *Streptococcus* mutans and *Streptococcus sanguinis* in the dental biofilm. J Bacteriol 187:7193–7203. doi:10.1128/JB.187.21.7193-7203.2005
10. Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR et al (2006) Water sorption/solubility of dental adhesive resins. Dent Mater 22:973–980. doi:10.1016/j.dental.2005.11.020
11. Aykent F, Yondem I, Ozyesil AG, Gunal SK, Avunduk MC, Ozkan S (2002) Effect of different finishing techniques for restorative materials on surface roughness and bacterial adhesion. J Prosthet Dent 103:221–227. doi:10.1016/S0022-3913(10)60034-0
12. Fucio SB, Carvalho FG, Sobrinho LC, Sinhoreti MA, Puppin-Rontani RM (2008) The influence of 30-day-old *Streptococcus mutans* biofilm on the surface of esthetic restorative materials—an in vitro study. J Dent 36:833–839. doi:10.1016/j.jdent.2008.06.002
13. Lu H, Roeder LB, Lei L, Powers JM (2005) Effect of surface roughness on stain resistance of dental resin composites. J Esthet Restor Dent 17:102–108, discussion 109
14. Barucci-Pfister N, Gohring TN (2009) Subjective and objective perceptions of specular gloss and surface roughness of esthetic resin composites before and after artificial aging. Am J Dent 22:102–110
15. Sunnegardh-Gronberg K, van Dijken JW, Funegard U, Lindberg A, Nilsson M (2009) Selection of dental materials and longevity of replaced restorations in Public Dental Health clinics in northern Sweden. J Dent 37:673–678. doi:10.1016/j.jdent.2009.04.010
16. Soncini JA, Maserejian NN, Trachtenberg F, Tavares M, Hayes C (2007) The longevity of amalgam versus compomer/composite restorations in posterior primary and permanent teeth: findings from the New England Children's Amalgam Trial. J Am Dent Assoc 138:763–772
17. Santerre JP, Shajii L, Leung BW (2001) Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. Crit Rev Oral Biol Med 12:136–151
18. Kermanshahi S, Santerre JP, Cvitkovitch DG, Finer Y (2010) Biodegradation of resin-dentin interfaces increases bacterial microleakage. J Dent Res 89:996–1001. doi:10.1177/0022034510372885
19. Kawai K, Tsuchitani Y (2000) Effects of resin composite components on glucosyltransferase of cariogenic bacterium. J Biomed Mater Res 51:123–127. doi:10.1002/(SICI)1097-4636(200007)51:1<123::AID-JBM16>3.0.CO;2-7
20. Gregson K, Beiswanger AJ, Platt J (2008) The impact of sorption, buffering, and proteins on leaching of organic and inorganic substances from dental resin core material. J Biomed Mater Res A 84:256–264. doi:10.1002/jbm.a.31470