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Evaluation of three different decontamination techniques on biofilm formation, and on physical and chemical properties of resin composites

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Abstract

Objectives: This study evaluated three different sterilization/disinfection techniques for resin composites on bacterial growth and surface modification after decontamination.

Methods: Two resin composites were sterilized/disinfected with three different techniques: UV light, 1% chloramine T, and 70% ethanol. Four different times were used for each technique to determine the shortest time that the solution or UV light was effective. The influence of sterilization/disinfection technique on bacterial growth was evaluated by analyzing the metabolic activity, using the AlamarBlue™ assay, bacterial viability, and SEM images from biofilms of *Streptococcus mutans*. The surface change, after the process, was analyzed with ATR/FTIR and SEM images. The solutions used for decontamination (1% chloramine-T and 70% ethanol) were analyzed with ^{1}H-NMR to identify any resin compounds leached during the process.

Results: One minute of decontamination was efficient for all three methods tested. Chloramine-T increased the surface porosity on resin composites, no changes were observed for UV light and 70% ethanol, however, ^{1}H-NMR identified leached monomers only when 70% ethanol was used. No chemical change of the materials was found under ATR/FTIR analyses after the decontamination process. Chloramine-T, with no previous wash, increased the bacterial viability for both resin composites and increased the bacterial metabolism for the resin composite without fluoride.

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Conclusion: UV light had no interference on the resin composites properties tested using 1 min of exposure compared to the other decontamination methods.

Keywords

resin composite; sterilization; biofilm; surface analysis

INTRODUCTION

Composite restorations are still challenged by gap formation from curing shrinkage or degradation after polymerization.[1,2] Gap formation allows bacteria to infiltrate the resin-interface, potentially leading to secondary caries development.[3] To address this problem, restorative materials with antibacterial activity are being proposed.[4–6] To elucidate the effectiveness of these materials, many methods have been used to test the restorative materials effect on biofilm formation, bacterial inhibition or bacterial viability.[7–9] Prior to bacteria growth in studies, specimens must be sterilized and several techniques, including thermal, chemical, gas and ionizing radiation, have been used to prevent contamination. However, in achieving this objective, it is important that the sterilization methods do not modify the surface of the material or leave a residue that may interfere with the assessment of the antibacterial effect.

Some techniques use high temperatures during the sterilization process, such as steam sterilization in an autoclave. The high temperature can increase the mechanical properties of cured composites with improved degree of conversion and reduction of unreacted monomers[10,11] and, in conjunction with pressure and humidity, can lead to hydrolysis and degradation of the polymer.[12,13] Chemical sterilizer and disinfectant, such as glutaraldehyde, chloramine-T, and 70% ethanol, are also used frequently for decontamination of specimens. However, it is possible that these solutions may leave a residue on the surface that can lead to reduced bacterial activity, alter biofilm formation, or kill bacteria.

Glutaraldehyde has biocidal activity from its alkylation of sulfhydryl, hydroxyl, carboxyl, and amino groups, which alters RNA, DNA, and protein synthesis within microorganisms. In addition, glutaraldehyde vapors are irritating to the eyes, nose, and throat, requiring careful handling.[14] Chloramine-T (*N*-chloro-*p*-toluenesulfonamide sodium salt) is bactericidal and virucidal.[15] Chloramine-T disrupts bacterial metabolism, inhibiting growth and destroying the DNA structure via oxidation.[16] According to Stief (2003), chloramine-T is well used for disinfection of drinking water, dialysate, or ice cream machines due to its lack of toxicity and antimicrobial power.[15] *N*-chloramines exhibit low toxicity and skin irritation.[15,17] It is believed that alcohols can cause membrane damage leading to cell lysis and a denaturation of proteins.[18] However, alcohols have a lack of sporicidal activity and are considered disinfectants, and are not recommended for sterilization.[19]

Sterilization with ethylene oxide (EtO) is also used for heat-sensitive materials,[16] however, the temperature of this process can vary from 40 to 55°C and may increase the degree of conversion of restorative materials.[10,20] Its micro-biocidal activity comes from alkylation of protein, DNA and RNA, preventing cell metabolism and replication. This technique needs an aeration time to remove EtO residue, which is toxic and carcinogenic.[16] Farrugia et al. stated

that EtO affects the chemical and physical characteristics of some restorative materials.[13] Gamma radiation can destroy or damage living cells,[16] causes degradation of materials by increasing the temperature of the polymer, and leads to increase of resins degree of conversion.[13,20]

UV light has biocide effects (UVC: wavelength between 200 and 280 nm)[21,22] and is also used for the sterilization process, especially for heat sensitive materials.[22] Unlike chemical products, chemical residues or byproducts are avoided, and light is nonpolluting and environmentally friendly.[21] The inactivation of many microorganisms by UV light is established from the formation of dimers in RNA and DNA.[21] When polymers are exposed to UV light, chemical and physical changes can occur,[23,24] and UV has been used to induce photochemical changes resulting in oxidation of the exposed surface, modification of the hydrophobic nature of polymers, and increase in surface energy.[24]

The hypotheses to be tested in this study were: (1) the three techniques (UV light, 1% chloramine-T, and 70% alcohol) would require 10 min to have complete effect, (2) any of the techniques used would not interfere with the growth of Streptococcus mutans biofilms on the surface of the material, and (3) none of the techniques would alter the chemical and physical properties of the resin composites.

MATERIALS AND METHODS

Sterilization/disinfection techniques and time

Two experimental resin composites were made to analyze the difference between the sterilization/disinfection techniques (Table I). One resin composite (RC) did not contain fluoride, and the second resin (RCF) had 250 ppm of fluoride (sodium fluoride—J.T. Baker Chemical Co., Philipsburg, NJ). The resin composites were placed in a 10 mm diameter × 2 mm thick mold and polymerized between two glass slides using a curing unit (Valo—Ultradent, South Jordan, UT) with radiant exposure of 30 J/cm^2 (20 s each side with light curing tip directly on slide) to obtain disk-shaped specimens. The specimen preparation procedure was performed with clean materials (not sterilized) and the disks were maintained in a sterile 24-well plate.

Three commonly used sterilization and disinfection techniques: UV light radiation (Mercury vapor lamp with >90% radiation in 253.7 nm), 1% chloramine-T (Sigma Aldrich, St. Louis, MO), and 70% ethanol, were chosen for evaluation. For chloramine-T and ethanol, each specimen was immersed in 5 mL of solution ($n = 4$). For all methods, 1 min, 5 min, and 10 min of decontamination were used. UV light was also tested for 15 min, 1% chloramine-T for 24 h, and 70% ethanol was also tested for an initial 5 min, with a second immersion in a fresh solution for 5 more min. A control group was also used with no decontamination to confirm if the sterilization was even mandatory before performing antimicrobial tests.

After the decontamination process, each sample was placed into a 24-well plate with 2.8 mL of Trypticase soy broth (TSB—BBL™ Trypticase™ Soy Broth, BD diagnostics, MD) supplemented with 3 wt % sucrose (Fischer Science Education, Hanover Park, IL) and kept in an incubator at 378C, 5% CO_2 for 24 h. After this period, the turbidly was visually

analyzed and 5 μL of each well was plated in Brain Heart Infusion Agar (BHA—Hi-Media Laboratories, Mumbai, India) and Brain Heart Infusion Agar + 5% blood Agar plate at 37°C, 5% CO_2 for 48 h to confirm the efficiency of the decontamination process.

Metabolic activity and proliferation of Streptococcus mutans (AlamarBlue™ and bacterial viability)

To analyze the influence of the sterilization/disinfection techniques on *Streptococcus mutans* (strain UA159) biofilm formation, the decontamination with 1% chloramine-T and 70% ethanol was divided into two groups: one with no wash and one with a wash in sterile deionized water. After the decontamination, the samples from the wash group were dipped three times in a well containing 2.8 mL of sterilized deionized water (one well for each sample). The lowest efficient time, 1 min (Table II), was used for all techniques.

Overnight cultures of *S. mutans* grown in brain heart infusion (BHI) at 37°C in an incubator with 5% CO_2 were measured for optical density at 600 nm (OD_{600}) and then diluted to an OD_{600} of 0.4 to 0.6. A 1:10 dilution of the stock solution was incubated for 3 h in new BHI medium to obtain $OD_{600} = 0.3$, which represents a bacterial concentration of 9×10^7 CFU/mL based on previous calibration studies. TSB with 3 wt % sucrose was used as the culture media. The final dilution of 1:100 subculture of *S. mutans* in TSB was placed in each well using a sterile pipette (2.8 mL). The resin composite disks ($n = 6$), after the decontamination process, were placed at the bottom of the wells of 24-well culture plates containing the culture media with bacteria. The specimens were incubated in 5% CO_2 at 37°C for 5 days, with the culture media being removed every 24 h and replaced with 2.8 mL of fresh culture media using a sterile pipette. After 5 days of growing the biofilm, the disks ($n = 5$) were removed and washed in PBS 1× (dipped once in a well with 2 mL) and 1 mL of 10% AlamarBlue (Thermo Fisher Scientific, Waltham, MA) in TSB + 3% sucrose, was added to each sample in a new 24-well plate and incubated for 1 h. Subsequently, 200 μL of each sample was placed (in triplicate) in a 96-well plate and read using a 96-well plate spectrophotometer (fluorescence with peak in 585 nm).

After this procedure, each disk was placed in a 15 mL conical centrifuge tube with 5 mL of 0.89% physiological solution (NaCl). The biofilm was scraped from the disk using a sterilized spatula and the disk was removed from the tube. The biofilm in solution was sonicated for 15 s to obtain a dispersed solution. A serial dilution was performed and the dilutions of 10^{-4} and 10^{-5} were plated on BHA to count the viable cells after 48 h.

One disk of each group, after the biofilm growth, was analyzed under scanning electron microscopy (SEM—Quanta 200, FEI Company, Hillsboro, OR) using 100× magnification. The 115 h old biofilms were washed in sterile 0.89% NaCl then fixed with a 4% glutaraldehyde solution (v/v) in phosphate buffered saline PBS, pH 7.4) for 24 h. Biofilms then were dehydrated in ascending ethanol concentration (50, 70, 90, and 100%), dried for 24 h, and sputter coated with gold-palladium (Denton Vacuum Desk II—Denton Vacuum Inc., Moorestown, NJ).

Nuclear Magnetic Resonance Spectroscopy (NMR)

The chloramine-T and ethanol solutions used for the decontamination process were collected, the solvent from these solutions was removed under rotary evaporation to constant mass, and ^{1}H NMR (600 MHz, Varian, Palo Alto, CA) spectra was obtained (in DMSO-d6) to determine the composition of the sol fraction, and to identify any extracted monomers. Also, spectra were obtained from the ethanol, chloramine T, BisGMA, and TEGDMA itself in DMSO-d6.

Attenuated total reflectance Fourier transform infrared analysis

Disks of resin composites ($n = 3$) were obtained by polymerizing the material between two glass slides in a 9 mm diameter and 2 mm thick mold. The disks were decontaminated using UV light, 1% chloramine-T, and 70% ethanol. A control group with no sterilization process was included. Attenuated total reflectance Fourier transform infrared (ATR/FTIR) spectroscopy was performed. The spectrophotometer (Nicolet Nexus 6700, Thermo Scientific) used transmitted infrared spectroscopy with frequencies between 650 and 4000 cm^{-1} (wavenumbers).

After ATR/FTIR the samples were sputter coated with gold (Denton Vacuum Desk II—Denton Vacuum Inc., Moor-estown, NJ) and the surface analyzed with a SEM at 500× magnification to determine if any changes occurred on the surface of the resin composites after the decontamination process.

RESULTS

Sterilization/disinfection techniques and time

For the plates, any growth was considered negative (not sterilized), and no growth was considered positive (sterilized). The same result was obtained for both plates (BHIA and BHIAB), as described in Table II.

The control group showed growth on both plates, which confirm the necessity to perform decontamination prior to antimicrobial tests. For the three techniques, all tested times were sufficient to sterilize the resin composites, and the shortest time, 1 min, was selected no analyze the effect of the decontamination process on the chemical and physical properties of resin composite and the subsequent biofilm formation.

Metabolic activity and proliferation of *S. mutans* (AlamarBlue and bacterial viability)

Metabolic activity and bacterial viability for the biofilms grown on the decontaminated surfaces were statistically analyzed using one-way ANOVA and Tukey's test ($\alpha = 0.05$). For the RC both methods (metabolic activity and bacterial viability) showed significant differences ($p < 0.0001$), however, for RCF only the bacterial viability showed significant differences ($p < 0.0001$). The metabolic activity was higher when the chloramine-T with no wash was used [Figure 1(A)] for the RC and a higher colony count was obtained for the chloramine-T with no previous wash for both resin composites [Figures 1(B) and2(B)]. No statistical difference was observed between the other groups for bacterial viability. A higher value of metabolic activity was also obtained for the chloramine-T with no wash for the RCF

[Figure 2(A)] with no statistical difference ($p = 0.7503$) from the other groups. No statistical difference was found comparing the resin composites for colony counting ($p = 0.1750$) and for metabolic activity ($p = 0.4676$).

SEM images show a more highly dense biofilm on top of both resin composites when sterilized with UV light and chloramine-T with no wash (Figures 3 and 4). No difference is observed between the other techniques under SEM analysis.

Nuclear Magnetic Resonance Spectroscopy

The ^{1}H NMR spectra from the monomers (BisGMA and TEGDMA), from the solvents used for decontamination (ethanol and chloramine-T) and their sol fractions from the resin composite and resin composite + fluoride, are shown in Figure 5(A–H). Major ^{1}H NMR signals are assigned to BisGMA and TEGDMA. The sol fraction from the 70% ethanol solution [Figure 5(E,F)]: at δ(CH-aromatic) = 6.98 ppm and 6.62 ppm, δ(CH_2-methacrylate) = 6.06 ppm and 5.68, δ(CH_2-pendant) = 4.31 ppm to 3.56 ppm, δ(CH_3-methacrylate) = 1.89 ppm and δ(CH_3-aromatic) = 1.52 ppm. Figure 5(G,H), shows sol fractions from the 1% chloramine-T, with identified protons in the NMR signal attributed to chloramine-T: at δ(CH-aromatic) = 7.51 ppm and 7.17 ppm, and δ(CH_3) = 2.13 ppm.

Attenuated total reflectance Fourier transform infrared analysis

The ATR/FTIR spectra of RC and RCF samples decontaminated with UV light, 70% ethanol, and 1% chloramine-T, and also a control group (with no sterilization process), confirmed the presence of different bonds in the structure of monomers, and the presence of fillers, as shown in Figure 6(A,B). The monomers (converted to polymers), BisGMA and TEGDMA, show a series of characteristic infrared bands around 2950 cm^{-1} assigned to C-H stretching, 1720 cm^{-1} indicating C=O bond and C=C bending bonds, and aliphatic and aromatic C=C at 1640 cm^{-1} and 1610 cm^{-1}, respectively. Additionally, CH_3 deformation was observed between 1450 cm^{-1} and 1380^{-1} (asymmetric and symmetric stretching). The presence of Si-O-Si and Si-O bonds of the fillers was detected at 1100 cm^{-1} and 1030 cm^{-1}.

Representative SEM images from the resin composites are shown in Figures 6 and 7. A surface modification was observed when the samples were decontaminated with 1% chloramine-T [Figures 7(C) and8(C)] compared to the other groups. No difference was observed when the decontamination was performed with UV light or 70% ethanol, compared to the control group for both resins tested.

DISCUSSION

Restorative materials containing antimicrobial components or antibacterial monomers are being developed to inhibit secondary caries formation and restoration replacements.[4,5,25] To test the efficiency of these antibacterial materials, restorative materials are being challenged against bacterial growth.[5,7,26] Prior to these types of tests, several sterilization techniques have been reported, with different sterilization process and duration. UV light sterilization was shown to be effective with 1 min of exposure, which can decrease the deleterious effect of chemical and physical changes induced in polymers by the sterilization process. For 70% ethanol and 1% chloramine-T, 1 min in both solutions was effective against contamination,

and the first null hypothesis was rejected. However, aqueous media are more likely to leach unreacted monomers, or to solubilize some of the restorative material compounds from the organic matrix.[27] Resin composite materials are more prone to chemical alteration compared to inert metal or ceramic restorations.[28]

The qualitative analysis of the ATR/FTIR showed peaks characteristic of the monomers and fillers of the composites as expected. All samples had similar spectra before and after the different decontamination techniques. Further, the formation of new absorption bands was not observed, nor was there any loss of signal, which suggests that there was no chemical change of the material's surface structure.[29] Farrugia et al.[13] found some band flattening for ATR/FTIR using a longer period of decontamination (10 min) and sonication with 70% ethanol. Therefore, it may be that the time of decontamination used was not long enough (1 min) in this study to cause changes in the chemical structure of the molecules of the resins tested.

Sol fractions, analyzed qualitatively by ^{1}HNMR, showed that the composite sterilized with 70% ethanol leached more components compared to that decontaminated with chloramine-T, rejecting the third null hypothesis. There was a chemical shift, when 70% ethanol was used, that is related to the spectra from the original monomers. The products identified from the ethanol disinfection suggests a mechanism involving the formation of hydroxyl radicals (OH·) released by methacrylate resin composites (BisGMA/TEGDMA), which react with ethanol molecules to produce free secondary radicals ethoxy (EtO·).[30,31] This hypothesis of formation of hydroxyl radicals, could explain the observed chemical degradation of resin composites disinfected with 70% ethanol solution. Lee et al. (1995) states that the hydroperoxidation of the methyl group of a methacrylate, in the presence of oxygen and unreacted C=C double bonds, that decomposes to hydroxyl and acyl radicals.[32,33] The sol fractions from the resins decontaminated with chloramine-T presents a chemical shift that belongs to the used solution, not to the monomers.

Despite no monomer identification in the sol fractions from the chloramine-T decontamination, SEM images from the resin composites after the decontamination with chloramine-T showed an increase in the surface porosity, which suggests that the mechanism of surface degradation was different than when ethanol was used. SEM images showed an increase in surface porosity for both resin composites decontaminated with the 1% chloramine-T, suggesting an attack of the silicon fillers. Chloramine-T behaves as an oxidizing agent[34] and hydrogen peroxide, widely used in dentistry for tooth bleaching, is also considered a strong oxidizing agent.[35,36] Cengiz et al. (2016) noted an increase in surface roughness for resin composites when hydrogen peroxide was applied, accompanied by an increase in surface porosity under SEM analysis.[35] The increase in surface roughness and porosity by oxidizing agents may be a result from the oxidative cleavage of polymer chains in resin matrix, which may cause a debonding failure between the resin matrix and inorganic fillers, leading to a loss of filler particles.[35,37]

Considering the greater irregularity on the resin surface after decontamination with chloramine-T, which would have the potential to increase the adherence of a biofilm,[38] it was expected that both techniques (with wash and no wash) would have a higher bacterial

viability. However, this increase is observed only when the chloramine-T had no wash previously to inserting the resin composite disk in media with bacteria, rejecting the second null hypothesis. The AlamarBlue assay gives a measurement of bacterial metabolic activity, since the active bacterial cells reduce the non-fluorescent resazurin (blue) to the fluorescent resofurin (pink), which can be further reduced to hydroresorufin.[39,40] Specimens decontaminated with chloramine-T with no previous wash had a biofilm with higher bacterial metabolism for the RC. The average from the same technique for RCF had a higher value, but with no significant difference from the other groups. Considering the increase in bacterial viability and the higher average of fluorescence, it can be suggested that the residue remaining on the composite surface after chloramine-T decontamination can interfere with bacterial metabolism.

Bedran et al. described the increase of biofilm formation of *S. mutans* when biofilm was treated with triclosan at a sub-MIC concentration. The upregulation of *comC* and *luxS* genes when *S. mutans* were grown in the presence of sub-MICs triclosan concentration has also been reported.[41] Competence activation, controlled by a quorum-sensing mechanism in *S. mutans* UA159, has been shown to influence biofilm formation, stress tolerance and virulence.[42] Also, Dong et al. (2012) reported that chlorhexidine, tea polyphenols, and sodium fluoride, in sub-MICs, may upregulate the expression of some *S. mutans* genes related to biofilm formation and promote *S. mutans* biofilm development.[43] Despite this possibility, an increase in biofilm formation was not confirmed in the SEM images in this study. The biofilm from the chloramine-T treated specimens did not differ from that on the composite sterilized with UV light. However, the biofilm images grown on disk disinfected with 70% ethanol (with and without wash) and chloramine-T with wash presents a structure modification, which suggest that the solutions may change the biofilm organization (supporting the rejection of second null hypotheses), but as chloramine-T with no wash had a higher bacterial viability, the biofilm was able to present a structure similar to the biofilm from UV light group. Also, fluoride addition to the composites did not show an antimicrobial activity in this study. The amount of fluoride release from composite resin is smaller than from glass ionomer cements and may be too small to present an antibacterial effect.[25]

CONCLUSION

The sterilization with UV light for 1 min resulted in the least deleterious effects, as no chemical and physical degradation of the surface was detected, and no interference in subsequent biofilm formation.

REFERENCES

1. Sarrett DC. Clinical challenges and the relevance of materials testing for posterior composite restorations. Dent Mater 2005;21:9–20. [PubMed: 15680997]
2. Demarco FF, Correa MB, Cenci MS, Moraes RR, Opdam NJ. Longevity of posterior composite restorations: Not only a matter of materials. Dent Mater 2012;28:87–101. [PubMed: 22192253]
3. Zivkovic S, Bojovic S, Pavlica D. Bacterial penetration of restored cavities. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 91:353–358. [PubMed: 11250635]

4. Cocco AR, Rosa WL, Silva AF, Lund RG, Piva E. A systematic review about antibacterial monomers used in dental adhesive systems: Current status and further prospects. Dent Mater 2015;31: 1345–1362. [PubMed: 26345999]
5. Andre CB, Gomes BP, Duque TM, Stipp RN, Chan DC, Ambrosano GM, Giannini M. Dentine bond strength and antimicrobial activity evaluation of adhesive systems. J Dent 2015;43:466–475. [PubMed: 25624119]
6. Miki S, Kitagawa H, Kitagawa R, Kiba W, Hayashi M, Imazato S. Antibacterial activity of resin composites containing surface pre-reacted glass-ionomer (S-PRG) filler. Dent Mater 2016;32:1095–1102. [PubMed: 27417376]
7. Salehi S, Davis HB, Ferracane JL, Mitchell JC. Sol-gel-derived bio-active glasses demonstrate antimicrobial effects on common oral bacteria. Am J Dent 2015;28:111–115. [PubMed: 26087578]
8. Yang Y, Huang L, Dong Y, Zhang H, Zhou W, Ban J, Wei J, Liu Y, Gao J, Chen J. In vitro antibacterial activity of a novel resin-based pulp capping material containing the quaternary ammonium salt MAE-DB and Portland cement. PLoS One 2014;9:e112549. [PubMed: 25389975]
9. Xie D, Weng Y, Guo X, Zhao J, Gregory RL, Zheng C. Preparation and evaluation of a novel glass-ionomer cement with antibacterial functions. Dent Mater 2011;27:487–496. [PubMed: 21388668]
10. Soares CJ, Pizi EC, Fonseca RB, Martins LR. Mechanical properties of light-cured composites polymerized with several additional post-curing methods. Oper Dent 2005;30:389–394. [PubMed: 15986961]
11. Santana IL, Lodovici E, Matos JR, Medeiros IS, Miyazaki CL, Rodrigues-Filho LE. Effect of experimental heat treatment on mechanical properties of resin composites. Braz Dent J 2009;20: 205–210. [PubMed: 19784465]
12. Ahmed M, Punshon G, Darbyshire A, Seifalian AM. Effects of sterilization treatments on bulk and surface properties of nanocompo-site biomaterials. J Biomed Mater Res B Appl Biomater 2013;101: 1182–1190. [PubMed: 24039066]
13. Farrugia C, Cassar G, Valdramidis V, Camilleri J. Effect of sterilization techniques prior to antimicrobial testing on physical properties of dental restorative materials. J Dent 2015;43:703–714. [PubMed: 25913142]
14. Rutala WA, Weber DJ. Disinfection of endoscopes: Review of new chemical sterilants used for high-level disinfection. Infect Control Hosp Epidemiol 1999;20:69–76. [PubMed: 9927274]
15. Stief TW. The physiology and pharmacology of singlet oxygen. Med Hypotheses 2003;60:567–572. [PubMed: 12615524]
16. McKeen LW. The effect of sterilization on plastics and elastomers. William Andrew (Elsevier); 2012.
17. Selk SH, Pogany SA, Higuchi T. Comparative antimicrobial activity, in vitro and in vivo, of soft N-chloramine systems and chlorhexidine. Appl Environ Microbiol 1982;43:899–904. [PubMed: 6805433]
18. McDonnell G, Russell AD. Antiseptics and disinfectants: Activity, action, and resistance. Clin Microbiol Rev 1999;12:147–179. [PubMed: 9880479]
19. Ganavadiya R, Chandra Shekar BR, Saxena V, Tomar P, Gupta R, Khandelwal G. Disinfecting efficacy of three chemical disinfectants on contaminated diagnostic instruments: A randomized trial. J Basic Clin Pharm 2014;5:98–104. [PubMed: 25316989]
20. Carvalho FG, Fucio SB, Pascon FM, Kantovitz KR, Correr-Sobrinho L, Puppin-Rontani RM. Effect of gamma irradiation on fluoride release and antibacterial activity of resin dental materials. Braz Dent J 2009;20:122–126. [PubMed: 19738944]
21. Vatansever F, Ferraresi C, de Sousa MV, Yin R, Rineh A, Sharma SK, Hamblin MR. Can biowarfare agents be defeated with light? Virulence 2013;4:796–825. [PubMed: 24067444]
22. Aeran H, Sharma S, Kumar V, Gupta N. Use of clinical UV chamber to disinfect dental impressions: A comparative study. J Clin Diagn Res 2015;9:ZC67–ZC70.
23. Pashkuleva I, Marques AP, Vaz F, Reis RL. Surface modification of starch based biomaterials by oxygen plasma or UV-irradiation. J Mater Sci Mater Med 2010;21:21–32. [PubMed: 19639265]
24. Loyaga-Rendon PG, Takahashi H, Iwasaki N, Reza F. Effect of ultraviolet light irradiation on bonding of experimental composite resin artificial teeth. Dent Mater J 2007;26:805–813. [PubMed: 18203485]

25. Imazato S Antibacterial properties of resin composites and dentin bonding systems. Dent Mater 2003;19:449–457. [PubMed: 12837391]
26. Pietrokovski Y, Nisimov I, Kesler-Shvero D, Zaltsman N, Beyth N. Antibacterial effect of composite resin foundation material incorporating quaternary ammonium polyethyleneimine nanoparticles. J Prosthet Dent 2016;116:603–609. [PubMed: 27157602]
27. Polydorou O, Konig A, Hellwig E, Kummerer K. Long-term release of monomers from modern dental-composite materials. Eur J Oral Sci 2009;117:68–75. [PubMed: 19196321]
28. Hannig C, Duong S, Becker K, Brunner E, Kahler E, Attin T. Effect of bleaching on subsurface micro-hardness of composite and a polyacid modified composite. Dent Mater 2007;23:198–203. [PubMed: 16546248]
29. Yavuz C, Oliaei SNB, Cetin B, Yesil-Celiktas O. Sterilization of PMMA microfluidic chips by various techniques and investigation of material characteristics. J Supercrit Fluids 2016;107:114–121.
30. Lamblin G, Leprince J, Devaux J, Mestdagh M, Gallez B, Leloup G Hydroxyl radical release from dental resins: Electron paramagnetic resonance evidence. Acta Biomater 2010;6:3193–3198. [PubMed: 20206719]
31. Sideridou ID, Karabela MM. Sorption of water, ethanol or ethanol/water solutions by light-cured dental dimethacrylate resins. Dent Mater 2011;27:1003–1010. [PubMed: 21783239]
32. Lee SY, Greener EH, Mueller HJ. Effect of food and oral simulating fluids on structure of adhesive composite systems. J Dent 1995;23:27–35. [PubMed: 7876413]
33. Lee SY, Greener EH, Menis DL. Detection of leached moieties from dental composites in fluids simulating food and saliva. Dent Mater 1995;11:348–353. [PubMed: 8595834]
34. Veena MS, Prashanth MK, Kumar KY, Muralidhara HB, Nayaka YA. Kinetics and mechanistic study of oxidation of amoxicillin by chloramine-T in acid medium. J Chil Chem Soc 2015;60:3063–3068.
35. Cengiz E, Kurtulmus-Yilmaz S, Ulusoy N, Deniz ST, Yuksel-Devrim E. The effect of home bleaching agents on the surface roughness of five different composite resins: A SEM evaluation. Scanning 2016;38:277–283. [PubMed: 26854422]
36. Eimar H, Siciliano R, Abdallah MN, Nader SA, Amin WM, Martinez PP, Celemin A, Cerruti M, Tamimi F. Hydrogen peroxide whitens teeth by oxidizing the organic structure. J Dent 2012; 40(Suppl 2):e25–e33. [PubMed: 22925924]
37. Rattacaso RM, da Fonseca Roberti Garcia L, Aguilar FG, Consani S, de Carvalho Panzeri Pires-de-Souza F. Bleaching agent action on color stability, surface roughness and microhardness of composites submitted to accelerated artificial aging. Eur J Dent 2011; 5:143–149. [PubMed: 21494380]
38. Park JW, Song CW, Jung JH, Ahn SJ, Ferracane JL. The effects of surface roughness of composite resin on biofilm formation of Streptococcus mutans in the presence of saliva. Oper Dent 2012; 37:532–539. [PubMed: 22339385]
39. Elshikh M, Ahmed S, Funston S, Dunlop P, McGaw M, Marchant R, Banat IM. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnol Lett 2016;38:1015–1019. [PubMed: 26969604]
40. O'Brien J, Wilson I, Orton T, Pognan F. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur J Biochem 2000;267:5421–5426. [PubMed: 10951200]
41. Bedran TB, Grignon L, Spolidorio DP, Grenier D. Subinhibitory concentrations of triclosan promote Streptococcus mutans bio-film formation and adherence to oral epithelial cells. PLoS One 2014;9:e89059. [PubMed: 24551218]
42. Galvao LC, Rosalen PL, Rivera-Ramos I, Franco GC, Kajfasz JK, Abranches J, Bueno-Silva B, Koo H, Lemos JA. Inactivation of the spxA1 or spxA2 gene of Streptococcus mutans decreases virulence in the rat caries model. Mol Oral Microbiol 2016;32:142–153. [PubMed: 27037617]
43. Dong L, Tong Z, Linghu D, Lin Y, Tao R, Liu J, Tian Y, Ni L. Effects of sub-minimum inhibitory concentrations of antimicrobial agents on Streptococcus mutans biofilm formation. Int J Antimicrob Agents 2012;39:390–395. [PubMed: 22421330]

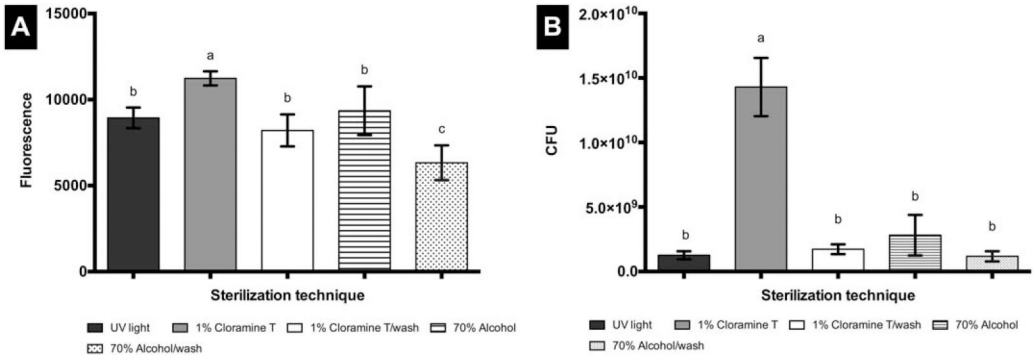

FIGURE 1.

Biofilm of *S. mutans* for resin composite (RC). (A) Metabolic activity. (B) Bacterial viability. Groups having similar letters are not significantly different ($p > 0.05$).

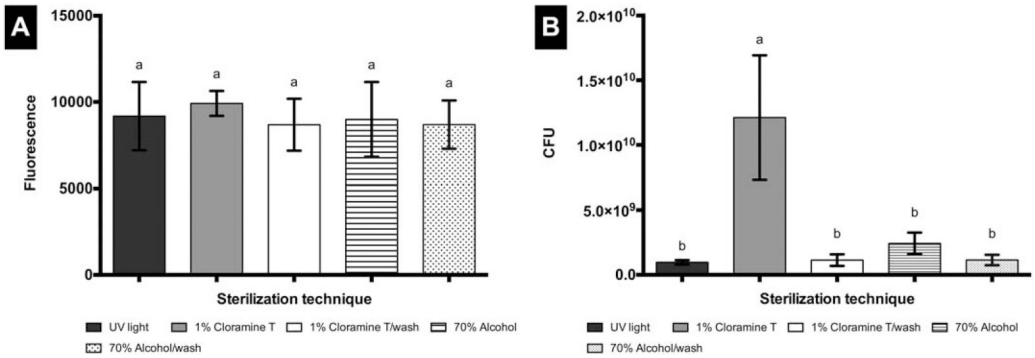

FIGURE 2.

Biofilm of *S. mutans* for resin composite + fluoride (RCF). (A) Metabolic activity. (B) Bacterial viability. Groups having similar letters are not significantly different ($p > 0.05$).

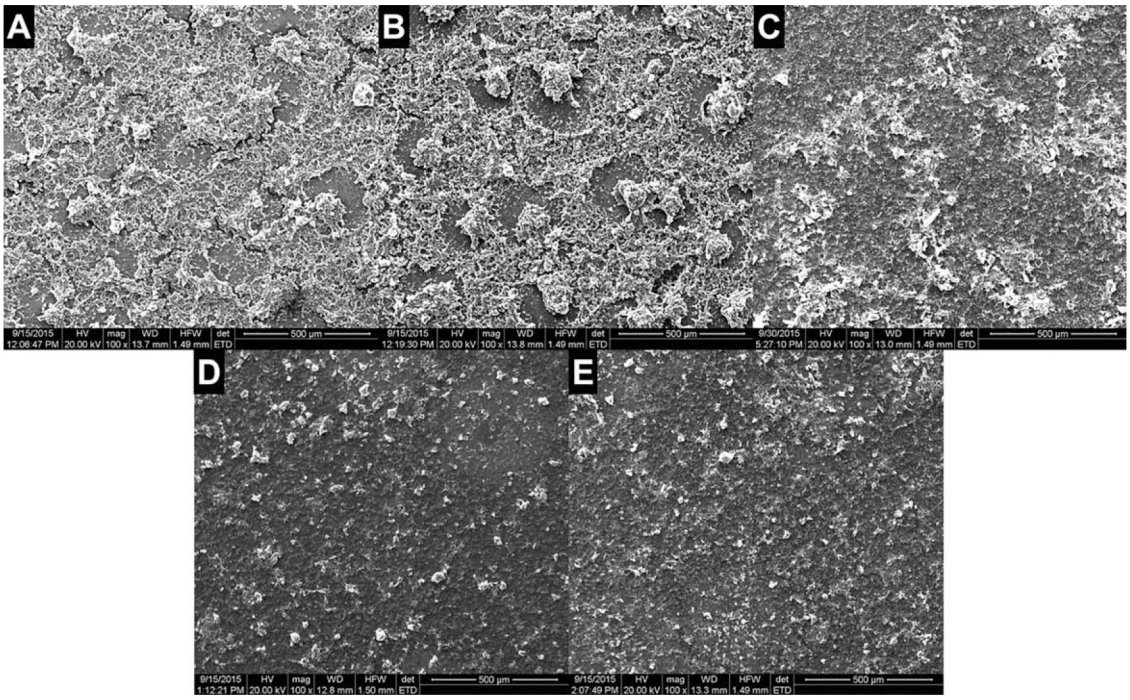

FIGURE 3.

SEM images of biofilm of *S. mutans* formed on top of the resin composite (RC). (A) UV light. (B) 1% chloramine-T. (C) 1% chloramine-T with wash. (D) 70% ethanol. (E) 70% ethanol with wash.

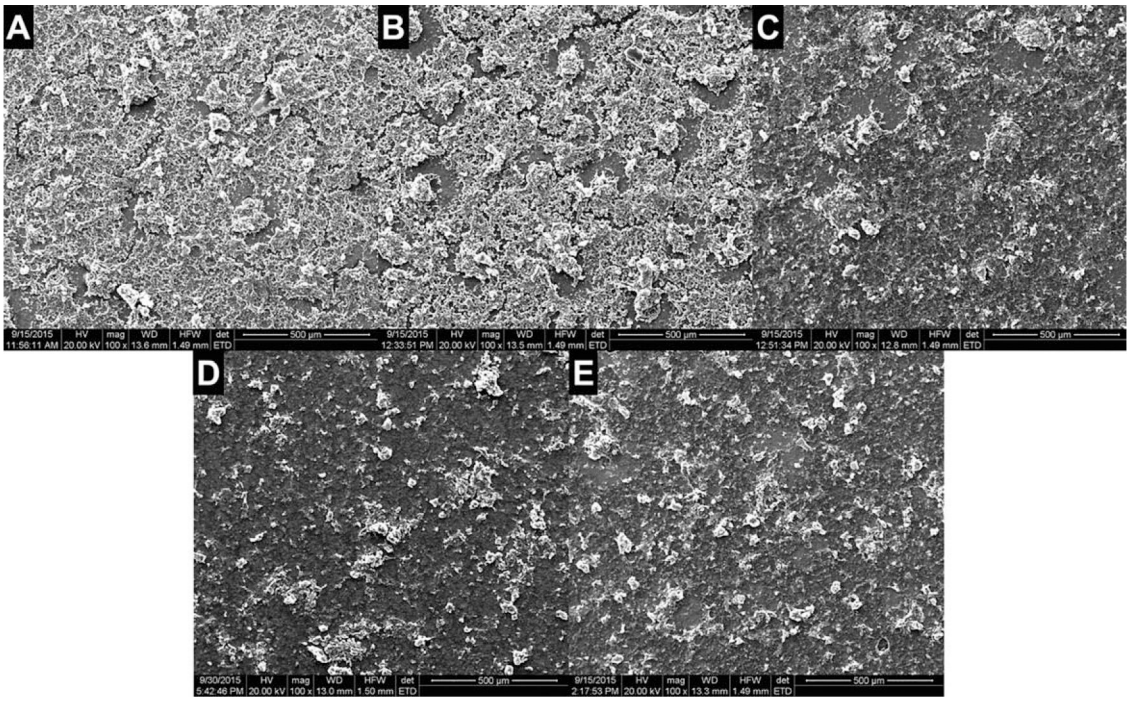

FIGURE 4.

SEM images of biofilm of *S. mutans* formed on top of the resin composite + fluoride (RCF). (A) UV light. (B) 1% chloramine-T. (C) 1% chloramine-T with wash. (D) 70% ethanol. (E) 70% ethanol with wash.

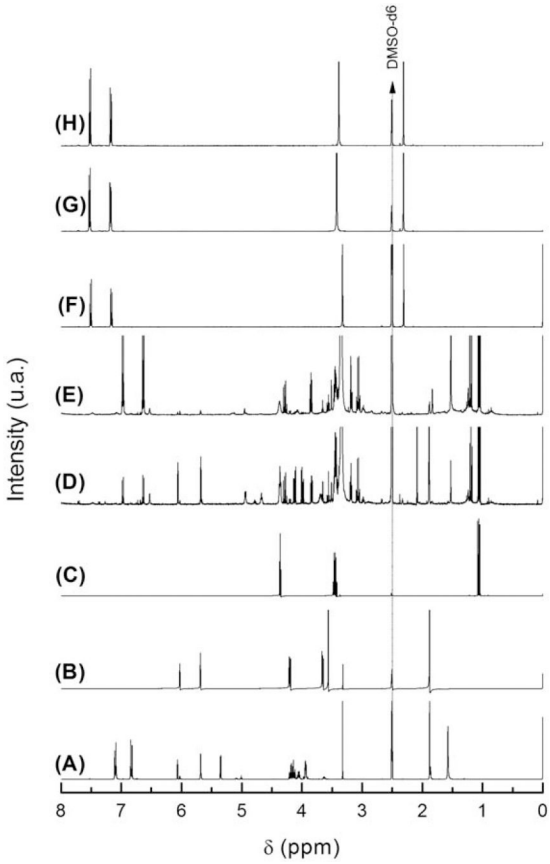

FIGURE 5.

^{1}H NMR spectra of (A) BisGMA, (B) TEGDMA, (C) ethanol,(D) sol fraction for resin composite from 70% ethanol solution, (E) sol fraction for resin composite + fluoride from 70% ethanol solution, (F) chloramine-T, (G) sol fraction from resin composite from 1% chloramine-T solution and (H) sol fraction from resin composite + -fluoride from 1% chloramine-T solution.

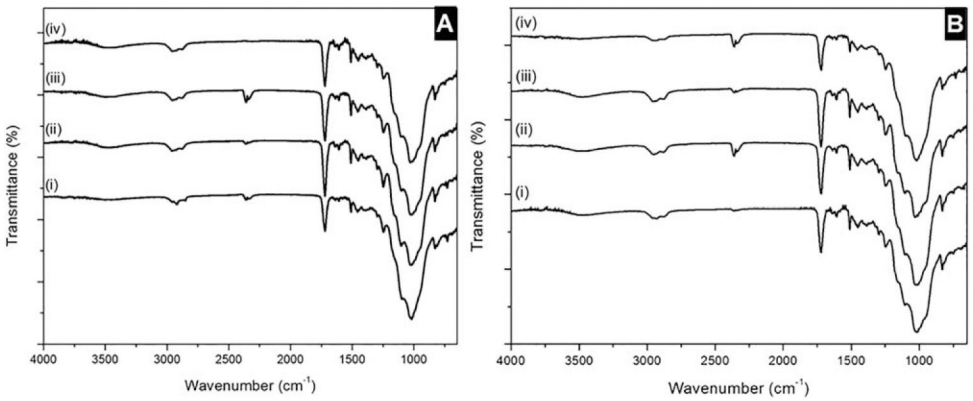

FIGURE 6.

ATR/FTIR spectra. (A) Resin composite. (B) Resin composite + fluoride. (i) control group, (ii) UV light, (iii) 70% ethanol, and (iv) 1% chloramine-T.

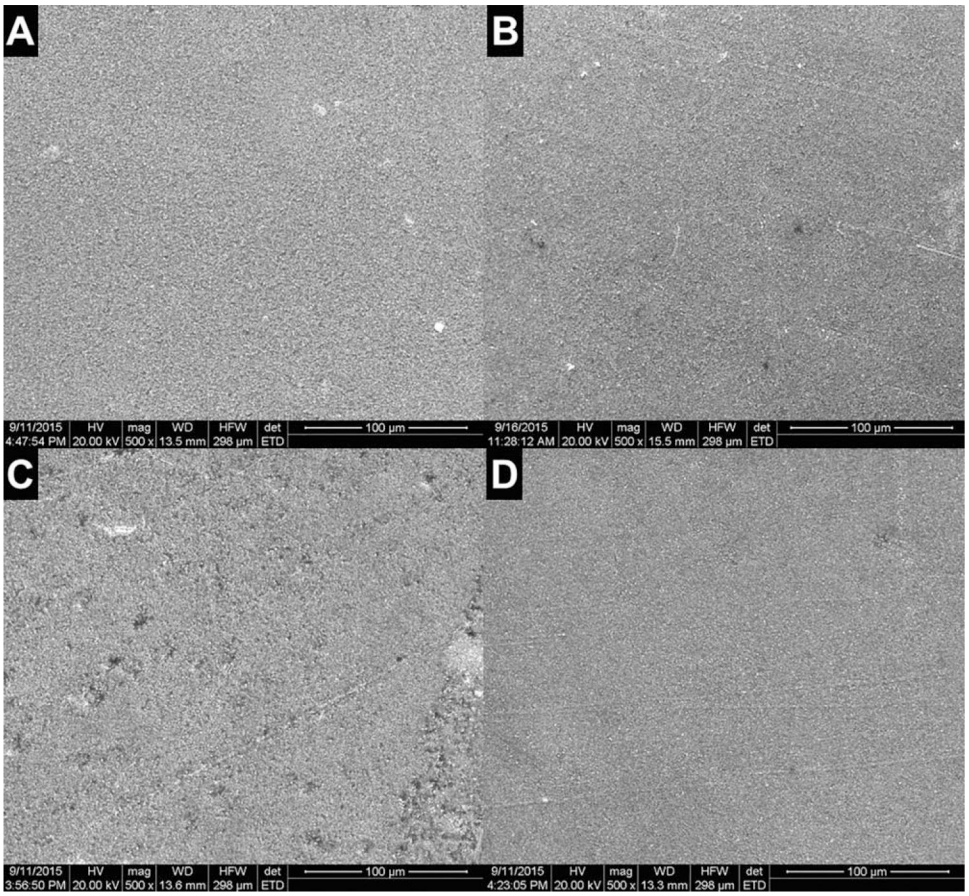

FIGURE 7.

SEM images of resin composite surface after decontamination. (A) No sterilization. (B) UV light. (C) 1% chloramine-T. (D) 70% ethanol.

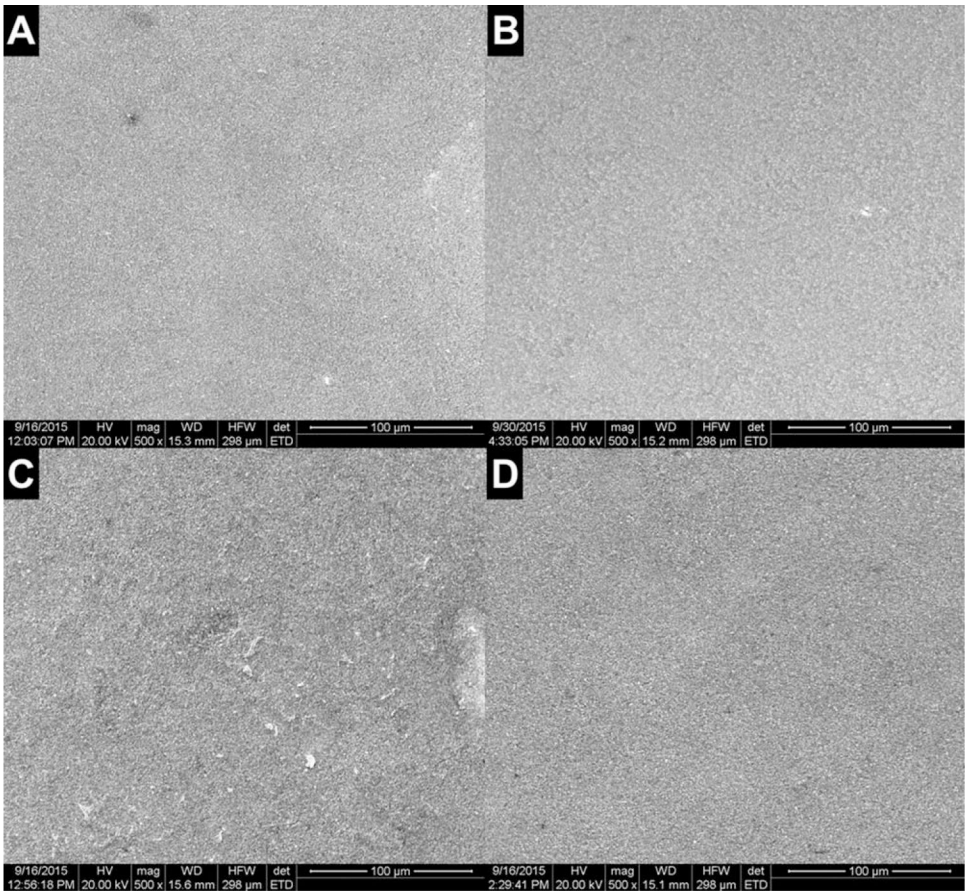

FIGURE 8.

SEM images of resin composite surface after decontamination. (A) No sterilization. (B) UV light. (C) 1% chloramine-T. (D) 70% ethanol.

TABLE I.

Resin Composite Composition

Material	Concentration
Bisphenol A Glycidyl Methacrylate (BisGMA)—Esstech, Inc., Essington, Pennsylvania, USA. Lot: 688-51	15 wt %
Tetraethylene Glycol Dimethacrylate (TEGDMA)—Esstech, Inc., Essington, Pennsylvania, USA. Lot: 736-51-04	15 wt %
Camphorquinone—Sigma Aldrich, St. Louis, MO, USA. Lot: 10726H0	0.6 wt %
Ethyl 4-dimethylaminobenzoate (EDMAB)—Across Organics/Thermo Fisher Scientific, New Jersey, US. Lot: A0237872	1.2 wt %
Butylhydroxytoluene (BHT)—Sigma Aldrich, St. Louis, MO, USA. Lot: 37H0294	0.05 wt %
0.7 μm barium borosilicate glass—Esstech, Inc., Essington, Pennsylvania, USA. Lot: 845-09	65 wt %
0.04 μm fumed silica—Aerosil® OX-50. Sun. Medical Co., Ltd, Japan. Lot: 1228059 3B	5 wt %

TABLE II.

Time of Exposure for Decontamination Techniques

	Decontamination technique					
	Resin composite (RC)			Resin composite with fluoride (RCF)		
	No sterilization	–		No sterilization	–	
Time	**UV light**	**1% Chloramine-T**	**70% Ethanol**	**UV light**	**1% Chloramine-T**	**70% Ethanol**
1 min	+	+	+	+	+	+
5 min	+	+	+	+	+	+
10 min	+	+	+	+	+	+
5 min + 5 min	Not tested	Not tested	+	Not tested	Not tested	+
15 min	+	Not tested	Not tested	+	Not tested	Not tested
24 h	Not tested	+	Not tested	Not tested	+	Not tested

Negative (–): growth. Positive (+): no growth.