THE JOURNAL OF PROSTHETIC DENTISTRY

RESEARCH AND EDUCATION

Material compatibility and antimicrobial activity of consumer products commonly used to clean dentures

Andreas Kiesow, MSc, PhD,[a] Sandra Sarembe, MSc, PhD,[b] Robert L. Pizzey, BSc, MSc,[c] Alyson S. Axe, BSc,[d] and David J. Bradshaw, BSc, PhD[e]

ABSTRACT

Statement of problem. Regular denture cleaning is essential to good oral health, but only limited evidence is available regarding the effects of common cleaning routines.

Purpose. The purpose of this in vitro study was to determine the compatibility of denture materials with and the antimicrobial effects of typical cleaning regimens.

Material and methods. The evaluated treatments were derived from a study of dental professional recommendations and consumer habits, including denture cleanser tablets, toothpaste, mouthwash, isopropyl alcohol (IPA), household bleach, soap, and vinegar. The material integrity of denture materials, including polymethyl methacrylate (PMMA) and metals, was evaluated by scanning electron microscopy and profilometry after treatment with laboratory regimens simulating 2 years of typical consumer use. Treatments were also evaluated in a microbial kill time assay against a range of oral microorganisms with typical treatment regimens.

Results. Alcohol-based mouthwash and IPA damaged the surface of PMMA, and brushing with toothpaste caused scratching and surface material loss. Bleach caused limited damage to PMMA, but corroded CoCr alloy (pitting) and solder (layer formation). Denture tablets caused little damage to any materials apart from the layer formation on silver solder. Vinegar and soap were compatible with all materials. In antimicrobial assays, bleach gave excellent results, and IPA and mouthwash required concentrated dilutions to be effective. Cleanser tablets were effective at 5 minutes treatment time against all organisms. Toothpaste was effective against bacteria but not *Candida albicans*. Vinegar, soaps, salt, and sodium bicarbonate were microbially ineffective.

Conclusions. Bleach was highly antimicrobial but incompatible with metal dental prosthesis components. IPA and mouthwash were antimicrobial but damaged PMMA. Specialist denture cleanser tablets gave a good combination of microbial efficacy and reasonable material compatibility. (J Prosthet Dent 2016;115:189-198)

Dentures are specifically designed devices fabricated from a range of different materials: polymers, metals, ceramics, and composite resins. Polymethyl methacrylate (PMMA) is often used as a denture base material. Metals and alloys are used to fabricate orthodontic appliances, partial denture frameworks, and clasp arms. Acrylic resins, ceramics, and composite resins are used to produce artificial teeth. Complete dentures can have a 90-cm^2 surface area (Fraunhofer study: Quantitative determination of the surface area of full dentures. Data on file, 2014).

Denture hygiene is recognized as an important part of oral hygiene in that dentures can harbor both oral bacterial and fungal microorganisms such as streptococci, *Candida spp*,[1,2] and other organisms, including potential respiratory pathogens.[3] Poor cleaning may also lead to problems such as denture stomatitis.[4] Despite the importance of effective denture cleaning, there is a paucity of evidence-based national or international guidelines for dental health care professionals (DHCPs) on the most appropriate methods of thoroughly cleaning dentures. Instead, many relatively informal recommendations are available, which are often inconsistent with one another. A review of

R.L.P, A.S.A., and D.J.B. are employees of GlaxoSmithKline Consumer Healthcare; and A.K. and S.S. are employed by the Fraunhofer Institute which was funded by GlaxoSmithKline Consumer Healthcare.
[a]Head, Group Characterization of Medical and Cosmetic Care Products, Fraunhofer Institute for Mechanics of Materials IWM, Halle, Germany.
[b]Project Manager, Group Characterization of Medical and Cosmetic Care Products, Fraunhofer Institute for Mechanics of Materials IWM, Halle, Germany.
[c]Microbiologist, GlaxoSmithKline Consumer Healthcare, Surrey, United Kingdom.
[d]Principal Medical Affairs Scientist, GlaxoSmithKline Consumer Healthcare, Surrey, United Kingdom.
[e]Principal Scientist, GlaxoSmithKline Consumer Healthcare, Surrey, United Kingdom.

Clinical Implications

While bleach, IPA, and some mouthwashes may be microbially effective, they are often incompatible with the metal or PMMA components of dental prostheses. Specialist denture cleanser tablets provide a good combination of microbial efficacy and reasonable material compatibility.

interventions for cleaning dentures was published in 2009 by the Cochrane Collaboration.[5] This study assessed clinical trials. Later, a task force of the American College of Prosthodontics reviewed a wider range of clinical and in vitro studies in order to provide evidence-based guidelines for the care and maintenance of complete dentures.[6] Both reviews reported a lack of clear evidence on the comparative effectiveness of denture cleaning methods and discussed the need to remove biofilm from dentures. A combination of mechanical and chemical cleaning is usually recommended. Mechanical methods generally comprise brushing with water but can also include soap or toothpaste,[7-9] because insufficient cleaning and disinfection have been observed by brushing only with water.[10] However, using dentifrices (which almost always contain abrasives) may lead to irreversible surface changes and to an increase in surface roughness.[11] Material loss and abrasion have been examined on acrylic resins in a number of studies.[10,12]

A number of specialist denture tablets, generally based on peroxide-generating chemistry are available for the chemical cleaning of dentures. These tablets were found in our own studies[7] and by a number of other authors[13-15] to be among the most commonly used remedies. Axe et al[7] also found a range of other household products that were used in different parts of the world for denture cleaning/disinfection, with many such regimens apparently recommended by dentists, prosthodontists, and other DHCPs. These products included mouthwashes, liquid handsoaps, vinegar, dishwashing detergents, salt, sodium bicarbonate, vinegar, and plain water. Consumers also use household bleach (sodium hypochlorite). These potentially aggressive solutions may risk chemically damaging denture materials. Household bleach is known to damage acrylic resins and metal alloys[16,17] by corroding (pitting) and tarnishing (discoloration) the surface, and these corrosion effects can also change the flexibility of the material.[17] Further, vinegar[16] and mouthwashes[18] are potentially incompatible with some denture materials. However, in many published studies,[17,18] treatments are not well aligned with real-world patterns of consumer use and deriving evidence-based cleaning guidelines is difficult. Furthermore, few studies systematically compared different cleaning treatments.[5,6]

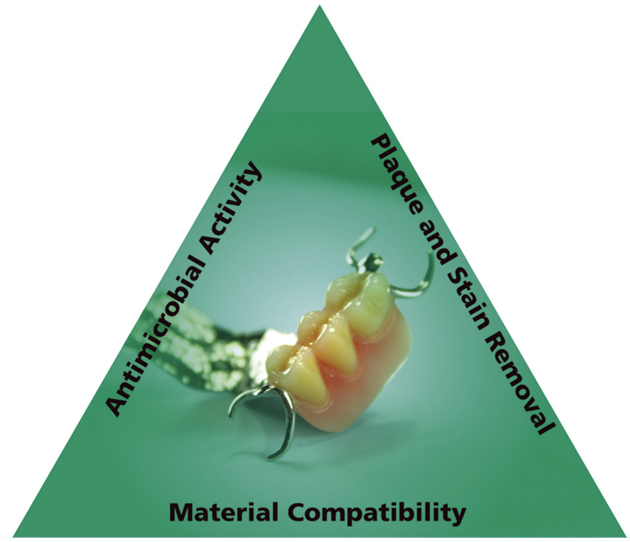


Figure 1. Denture care—requirements of denture cleansers and cleansing methods.

In making recommendations on cleaning, there is an interlocking triangle of requirements (Fig. 1). Cleansing regimens must effectively remove the biofilm. However, studies have indicated that denture materials may have microscopic-scale surface defects or pores that can serve as reservoirs for microbes, thus potentially rendering useless the mechanical aspects of hygiene routines.[19] The effective disinfection (killing) of any residual microbial biofilm is thus also essential. Denture surfaces can also harbor stains, which cleansing should remove. The cleaning regimen must not, however, damage the denture material itself.

The purpose of the present study was to address the lack of systematic research evidence on the risk/benefit profile of the most widely used denture cleaning products and cleansing regimens. The cleaning products and regimens assessed were based on data from the connected study[7] of DHCP recommendations and consumer behaviors in several countries. This risk/benefit profile was determined by assessing the effects of different cleaning products (with or without brushing) on denture material integrity and the antimicrobial effects of those cleaning products. In both studies, typical, realistic consumer behaviors were replicated in terms of times of treatment, temperatures, and in-use concentrations/dilutions.

MATERIAL AND METHODS

Representative products were selected for the compatibility tests. Orthodontic metal wires of stainless steel, cobalt chromium- (CoCr) based casting alloy, 3 different solders varying in metal composition, a heat-polymerizing resin, and an autopolymerizing acrylic resin were investigated. A full list of test denture materials is provided in Supplemental Table 1. Regarding the different solders, due to the inertness of the gold-containing solders, studies focused on one silver-based solder (Dentaurum) to simulate the worst case scenario. Materials were fabricated according to the manufacturer's instructions. Polymer specimens were fabricated by a dental laboratory from disks of the material (both autopolymerized and heat-polymerized) by sectioning them into blocks of appropriate size (10×10 mm, thickness 3 mm). Specimens were processed according to the manufacturer's instructions and mechanically finished with powder (Poliresin and pumice powder; Ernst Hinrichs Dental GmbH), polishing stones (Omnident Dental-Handelsgesellschaft mbH), and a leather buff (Polirapid).

The stainless steel wires and the 3 solder materials were prepared by cutting the wires into lengths of about 15 mm. Specimens were fixed on an acrylic resin matrix material (VersoCit-2 Powder and VersoCit-2 Liquid, size 15 mm×15 mm; Struers A/S) using UHU plus endfest 300. Specimen surfaces were exposed for the test; checks were made that the embedding process had not contaminated the exposed specimen surface. Acrylic resin blocks with the exposed wire specimens were then fixed on a glass slide used as a specimen holder. Orthodontic metal wires and solders were used as provided from the supplier. All specimens were cleaned in an ultrasonic bath before the treatment experiments.

A full list of test agents is provided in Supplemental Table 2. Two cycling treatment procedures were performed: soaking and combined soaking and brushing. For soaking experiments and to ensure consistent treatments, an automated linear soaking device with 14 beakers and a specimen holder with a dip mechanism was used (Cellstain 15; Tharmac). To remove residues from the surfaces, the specimens were stored before and after the experiments in an ultrasonic bath. A tablet cleanser was prepared in accordance with usage instructions. Material specimens were immersed in the water beaker immediately after adding the tablet for a 15-minute soak time (without brushing), after which specimens were removed and immersed in water. This was repeated for 104 cycles (total treatment time of 1560 minutes), representing 2 years of cleaning assuming 1 application per week or 3.5 months of cleaning assuming 1 application per day (Supplemental Fig. 1). For household bleach (sodium hypochlorite), vinegar, mouthwash, and isopropyl alcohol (IPA) treatments, the experimental procedure was identical except that the materials were soaked for 10 minutes in the relevant solution, resulting in a total treatment time of 1040 minutes (representing 2 years of cleaning assuming 1 application per week). For bleach treatment, a 6%w/v sodium hypochlorite solution was used. Vinegar, mouthwash, and alcohol were used as provided. Solutions were renewed after 6 cycles (60 minutes). For brushing and soaking, the soaking treatment was identical; after the 52nd cycle, specimens were brushed with an automatic brushing device for 20 minutes. The total brushing time represented a cleaning time of 1 year (23 seconds of brushing time/week or 3.25 seconds/day per 2 cm^2 area of the denture and equivalent to 2 minutes of brushing for a whole denture.[1] A contact force of 2 N was applied on each brush.[20] Soaking tests were repeated (52 cycles) and specimens were brushed again (20 minutes). Oral-B Indicator 35, medium hardness toothbrushes (Procter & Gamble) were used. Toothpaste was mixed with distilled water to obtain 1:3 w/w slurries. Soap was mixed with distilled water 1:1 w/w.

Characterization methods are listed in Supplemental Table 3. To visualize the surface effects, material surfaces were investigated by scanning electron microscopy (SEM Quanta 3D FEG). The surfaces of the specimens were measured with mechanical profilometry (Ambios XP2; Ambios Technology) to quantify changes in surfaces roughness. Average roughness differences (ΔRa, ΔRz) for different treatments were compared using 1-way analysis of variance (ANOVA) and the Tukey HSD-test.

For antimicrobial activity testing, strains were subcultured from −80°C stocks. *Klebsiella pneumoniae* NCIMB 13291 and *Streptococcus mutans* NCTC 10449 were maintained on Tryptone Soya Agar (Oxoid) in an aerobic incubator at between 35°C and 37°C. *Candida albicans* NCPF 3179 was maintained on Sabouraud Dextrose Agar (Oxoid) in an aerobic incubator at between 22°C and 25°C. *Porphyromonas gingivalis* ATCC 53978 and *Fusobacterium nucleatum* NCTC 10562 were maintained on Columbia agar supplemented with 5% v/v horse blood (Oxoid) in an anaerobic incubator at 37°C.

Mouthwashes, vinegar, bleach, and IPA were tested at 10% v/v, 50% v/v, and 80% v/v dilutions (80% dilution corresponded to being used undiluted in the test, with the allowance of 20% for addition of bacterial suspension). Soaps and toothpaste were tested at 50% (w/v for toothpaste, v/v for soap) dilution. Dilutions reflect final concentration "in test." Denture cleanser tablets (Polident Triplemint 3 minute) were tested as per pack instructions. A full list of products tested for antimicrobial effects are presented in Supplemental Table 4.

Test agents with the exception of denture cleanser tablets were tested for antimicrobial activity using the European suspension test method,[21] modified as described previously.[22] Testing was performed at 35°C. Contact times of 30, 120, and 300 seconds were used to simulate in-use denture cleaning times. The test mixtures were allowed to neutralize for 10 minutes (see Supplemental Table 5 for specific product neutralizer information). The dilutions were plated onto suitable agar. Incubation times and conditions were appropriate for the test organism.

Denture cleanser tablets were also tested at 40°C for antimicrobial activity using a method derived from the

European suspension test[21] that reflected home use conditions and pack instructions. At the start of the test, the denture cleanser tablet was added and 1 mL aliquots were removed at 30, 120, and 300 seconds and neutralized for 10 minutes. Plating and incubation conditions were as previously stated.

The magnitude of the kill in these tests is more biologically significant than whether statistically significant differences occur compared with the untreated control. Thus, the data are presented as $\log_{10}$ reductions (kill), with standard errors provided to describe the in-test variability.

RESULTS

The results of the compatibility tests are described for each test product or regimen. An overview, showing all treatment experiments and summarizing the results, is provided in Supplemental Figure 2.

Only negligible effects of bleach were observed for stainless steel. In contrast, for solder, surface tarnishing was observed; the modified surface showed an increased roughness and porosity. Pitting corrosion was detected on CoCr casting alloy. PMMA polymer materials were comparatively less affected by bleach, with insignificant roughness changes seen.

Relatively good material compatibility was found for treatments with vinegar. Only a slight increase of surface porosity was observed on the silver solder. Weak brushing traces were found on all materials except for stainless steel. Soap was only tested in the "soaking plus brushing" regimen. The material compatibility was comparable with the vinegar treatment, with no significant changes observed.

Listerine mouthwash treatment of PMMA polymers showed the greatest interaction of the pure soaking experiments, with surfaces significantly damaged. Morphologic changes were characterized by a large increase in surface roughness (Fig. 2). The microscopic-scale effects of these changes can be seen in in Figures 3 and 4. For stainless steel, no significant surface alterations were detected, whereas for the casting alloy, negligible or moderate surface effects were observed. Only a slight increase of surface porosity was observed on the silver solder.

Relatively good material compatibility was found for the tablet treatments; only with 1 material, the silver solder, was clear incompatibility observed. On silver solder, the surface damage was characterized by layer formation, which showed some similarities to the layer generated by the bleach solution (Fig. 5).

Colgate Total toothpaste was only tested in the "soaking plus brushing" regimen. Massive damage characterized by significant material abrasion was found on all PMMA surfaces (Fig. 4). In contrast, the surface

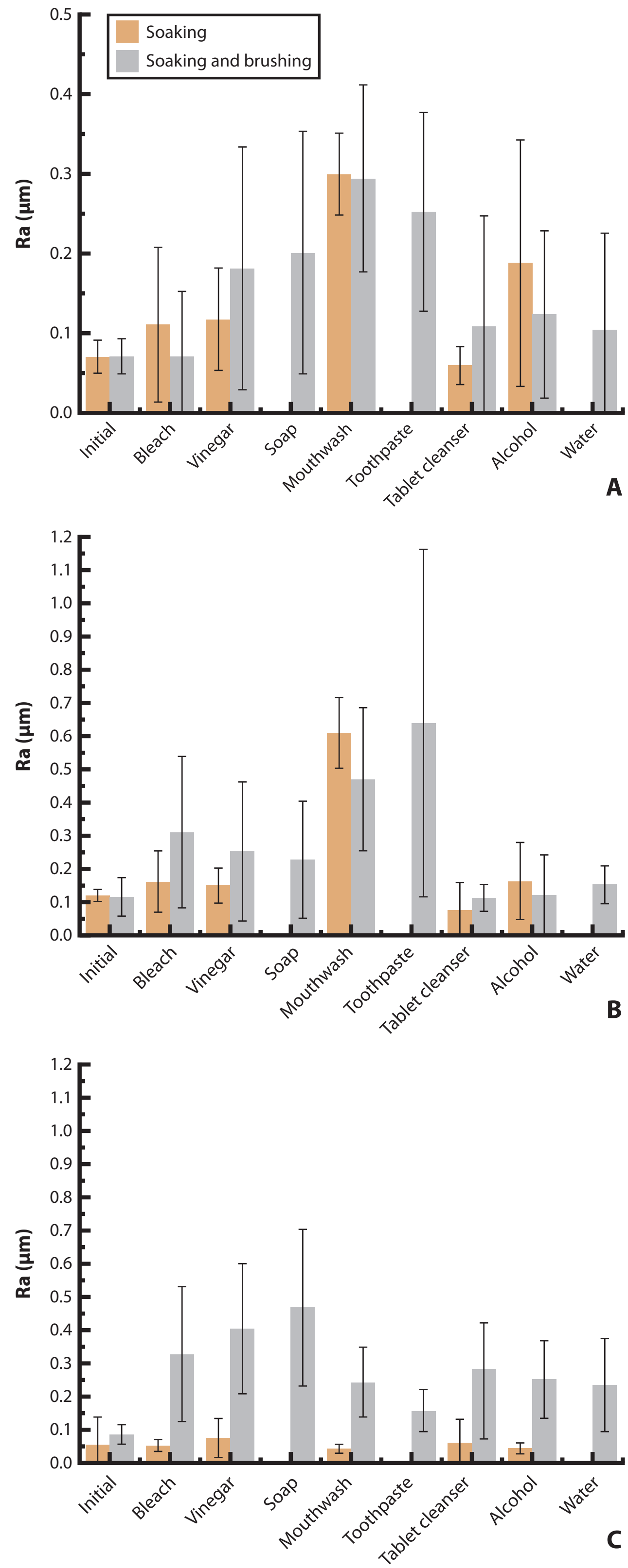


Figure 2. Surface roughness (Ra) values with standard deviations (n=8) of all tested materials after soaking or soaking and brushing with different cleansing products. A, Heat-polymerized denture polymer. B, Autopolymerized denture polymer. C, Casting alloy.

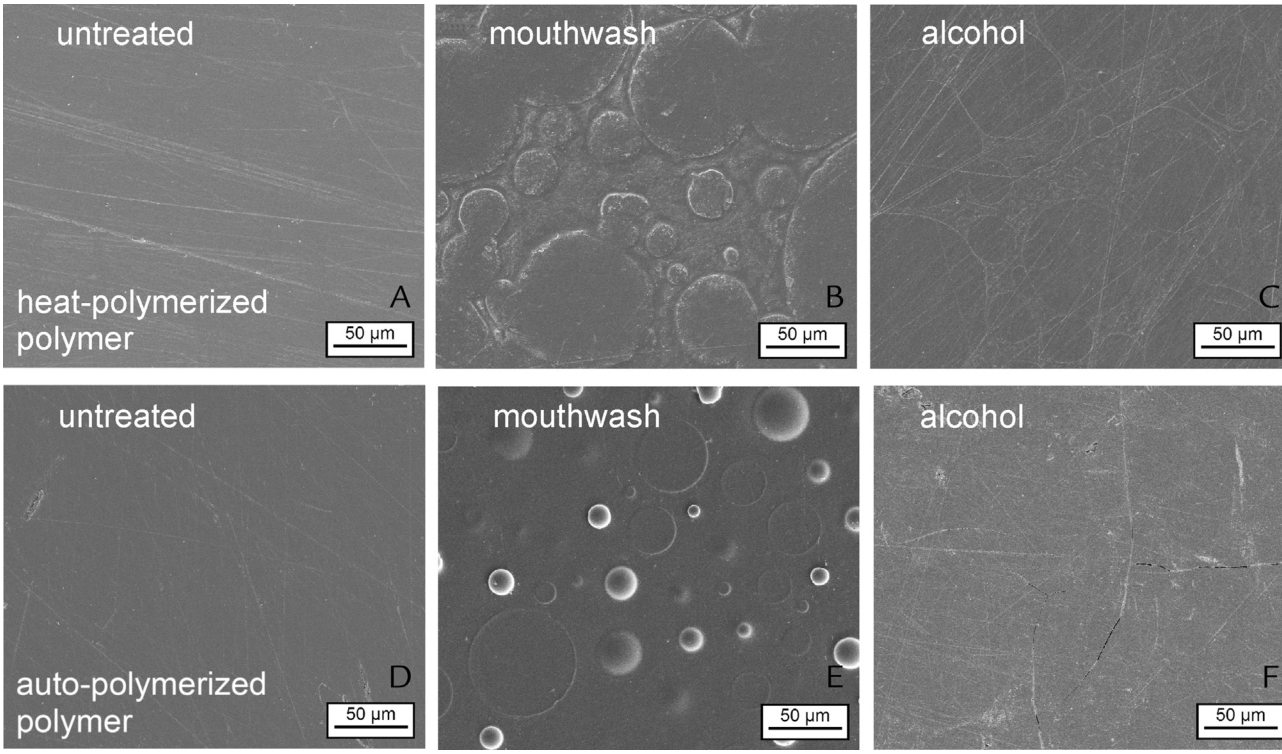


Figure 3. Scanning electron micrographs of material surfaces after soaking in isopropyl alcohol (right) and mouthwash (middle) for heat-polymerized (top) and auto-polymerized (below) polymethyl methacrylate in comparison with the untreated materials (left).

alterations on metallic surfaces after brushing were comparable with the other test products. The treatment with IPA did not produce as large a roughness increase as mouthwash on denture PMMA surfaces. However, the material surface was crazed with microscopic cracks, particularly for the auto-polymerized PMMA (Figs. 3, 4). Specimens of the metallic materials were either unaffected (stainless steel) or only slightly affected (casting alloy and solder). Water was only tested in the "soaking plus brushing" regimen. As expected, no evidence of chemical attack was found on the surfaces, only weak traces from the mechanical impact of brushing. Figure 2 summarizes the results of the roughness measurements which were only performed on polymer and casting alloy specimens. The specimens of stainless steel and solder materials, available only as thin wires, were not measurable under these conditions. The effect of mouthwash on PMMA was confirmed by gloss measurements. The toothpaste treatment also significantly reduced gloss (Supplemental Fig. 3). Color changes were investigated. Supplemental Figure 4 shows the color of the PMMA materials was altered only slightly for almost all treatments.

Tables 1 and 2 show antimicrobial summary data at 5 minutes (the longest treatment time) for selected treatments. Full sets of all data are presented in Supplemental Figures 5 through 12. For Polident cleanser tablet treatment, the 3 $\log_{10}$ kill threshold was reached for all organisms at 5 minutes (Table 1); however, at earlier time points, there was differentiation based on time: at 30 seconds for *P. gingivalis* and *F. nucleatum*, and after 2 minutes of treatment for other bacterial species, there was >4 $\log_{10}$ kill. Only after 5 minutes of treatment did tests for *C. albicans* reach the 3 $\log_{10}$ threshold. For Colgate Total, 30 seconds of treatment produced >3 $\log_{10}$ kill in all cases except for *K. pneumoniae* and *C. albicans*. All 4 bacterial species were killed beyond the >3 $\log_{10}$ kill threshold at 2 and 5 minutes, see Supplemental Figure 5. The Colgate Total treatment did not reach the 3 $\log_{10}$ kill threshold for *C. albicans* at the 5 minute treatment time (Table 1, Supplemental Fig. 6). Domestos hypochlorite bleach exceeded the 3 $\log_{10}$ kill threshold at all time points (Table 1, Supplemental Fig. 7). IPA at either 50%v/v or 80%v/v dilution exceeded the 3 $\log_{10}$ kill for both *K. pneumoniae* and *S. mutans*, whereas 50%v/v dilution of IPA treatment produced a kill exceeding 5 $\log_{10}$ for both anaerobes (Table 1). Vinegar treatments resulted in approximately 1 $\log_{10}$ kill for all organisms at 10% dilution, but kill did exceed the 3 $\log_{10}$ threshold for *K. pneumoniae* and *S. mutans* at 50%v/v and 80%v/v dilutions (Table 1). No data were generated at 80%v/v against anaerobes for IPA or vinegar. For Listerine Original, only 50%v/v and 80%v/v dilutions exceeded the 3 $\log_{10}$ kill threshold at 5 minutes of treatment time for all microorganisms (Table 2). Chlorhexidine mouthwash produced >4 $\log_{10}$ kill at all dilutions at 5 minutes for all

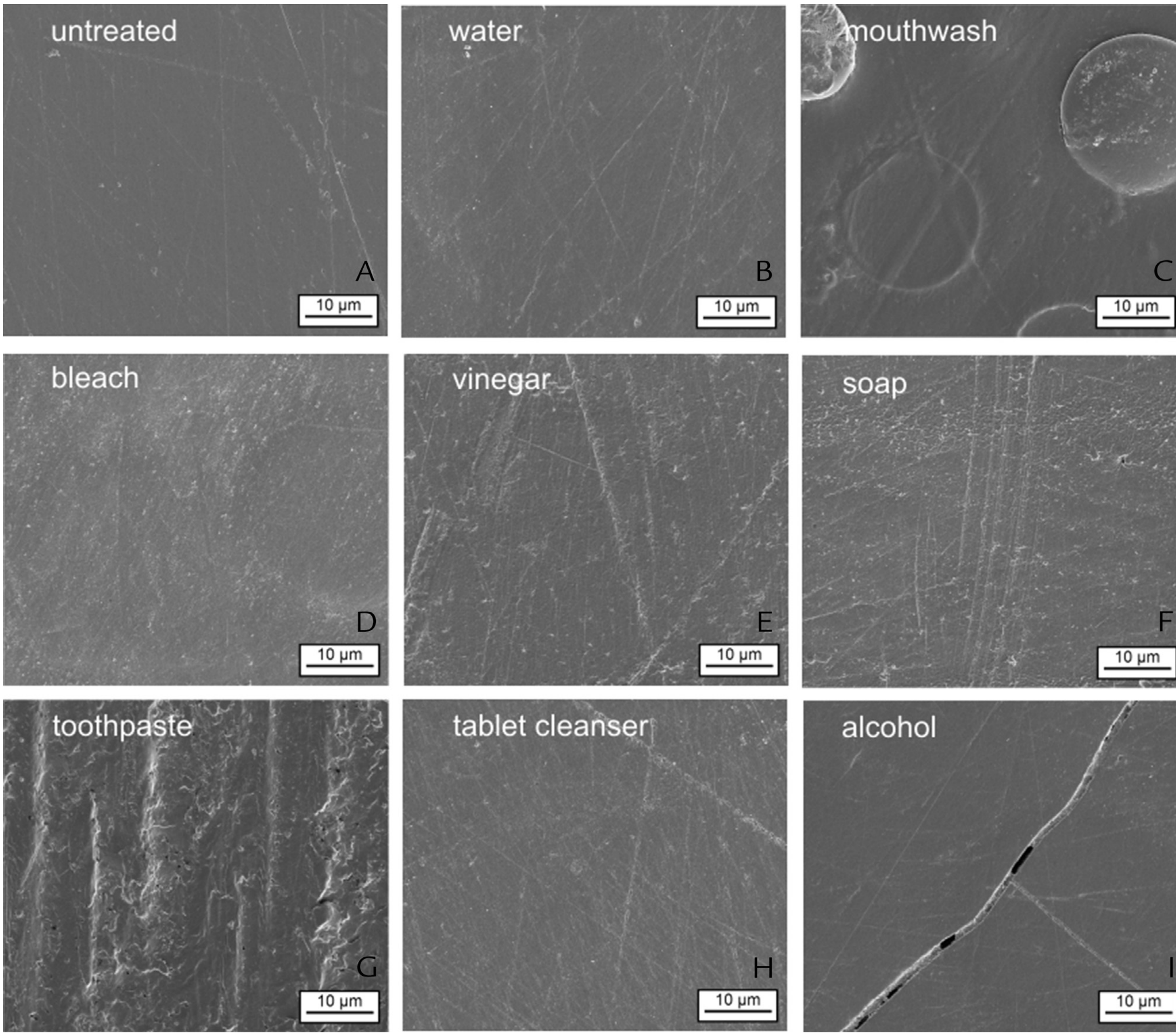


Figure 4. Scanning electron micrographs of specimen surfaces after soaking and brushing test with different test solution for auto-polymerized polymethyl methacrylate.

organisms tested (Table 2 and Supplemental Figs. 8, 9); Dentyl Active mouthwash only reached this benchmark for bacteria, but not for *C. albicans* (Table 2 and Supplemental Figs. 8, 9). Colgate Plax reached the 3 $\log_{10}$ threshold with all organisms at all dilutions except for *K. pneumoniae* at 10% and 50% (Table 2 and Supplemental Figs. 8, 9). Colgate Fluoriguard Alcohol Free did not reach the 3 $\log_{10}$ kill benchmark for *S. mutans* at 80%v/v dilution or for *F. nucleatum* with either 50%v/v or 80%v/v dilution (Table 2 and Supplemental Figs. 8, 9). DentylActive mouthwash exceeded the 3 $\log_{10}$ kill threshold at 50%v/v and 80%v/v dilutions for bacteria. For *Candida albicans*, only Chlorhexidine and Colgate Plax mouthwash produced >3 $\log_{10}$ kill at the 5 minute treatment time (Table 2 and Supplemental Fig. 9). For dishwashing liquids, none of the products reached 3 $\log_{10}$ kill for *C. albicans* or *K. pneumoniae*. Only Fairy Liquid Antibacterial met this threshold for *S. mutans*; dishwashing liquids were effective against *P. gingivalis* and *F. nucleatum* (Supplemental Fig. 10, for all dishwashing liquid data). For handsoaps, no products met the threshold 3 $\log_{10}$ kill for *C. albicans*. Additionally, Dial soap did not reach this threshold for either *K. pneumoniae* or *S. mutans*, while Carex (Table 2 and Supplemental Fig. 11) and Sanex (Supplemental Fig. 11) treatments were effective against *K. pneumoniae* and *S. mutans* (Table 2, Supplemental Fig. 11). All liquid soaps were effective against *P. gingivalis* and *F. nucleatum* (Supplemental Fig. 11). Neither sodium chloride nor sodium bicarbonate at 20% w/v resulted in more than 1 Log_{10} kill for any organism tested at any treatment time (all salt and bicarbonate data in Supplemental Fig. 12).

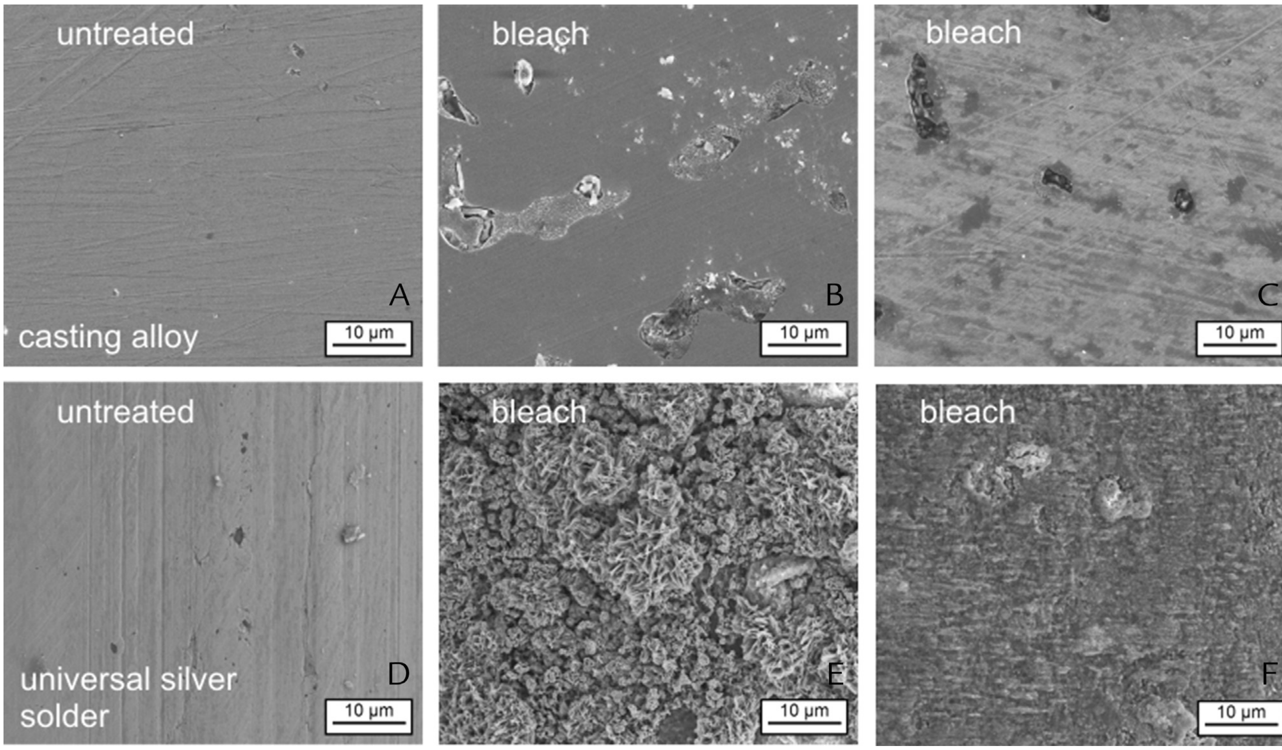


Figure 5. Scanning electron micrographs of specimen surfaces from a CoCr alloy (top) and a silver solder (below) after soaking and brushing test with different test solutions.

Table 1. Microbiology data: Mean $\log_{10}$ kill of microorganisms with treatments at 5 minutes

	Mean Log_{10} Kill										
	Polident Denture Cleanser Tablet*	Colgate Total Toothpaste 50%	Bleach			Distilled Vinegar			Isopropyl Alcohol		
Microorganism			10%	50%	80%	10%	50%	80%	10%	50%	80%
Candida albicans	4.41	1.91	4.40	4.40	4.40	0.95	0.92	1.15	1.06	4.40	4.40
Klebsiella. pneumoniae	4.56	4.01	4.44	4.44	4.44	2.07	4.59	4.38	1.30	4.44	4.44
Streptococcus mutans	5.43	5.45	5.39	5.39	5.39	1.16	5.43	5.43	0.99	5.39	5.39
Fusobacterium nucleatum	4.18	5.60	5.35	ND	ND	4.55	ND	ND	0.70	ND	ND
Porphyromonas gingivalis	8.10	8.50	8.60	ND	ND	8.60	ND	ND	5.55	ND	ND

*Used in accordance with manufacturer's instructions.

Table 2. Microbiology data: Mean $\log_{10}$ kill of microorganisms with treatments at 5 minutes

	Mean Log_{10} Kill													
	0.2% CHX	Listerine Original			DentylActive			Colgate F Alcohol Free			Colgate Plax			Carex
Microorganism	10%	10%	50%	80%	10%	50%	80%	10%	50%	80%	10%	50%	80%	50%
Candida albicans	4.01	1.17	3.89	4.54	2.14	2.07	2.01	0.99	1.00	1.09	3.32	4.44	4.44	1.31
Klebsiella pneumoniae	4.39	1.25	4.49	4.49	4.39	4.39	4.39	1.74	2.37	4.18	2.36	2.38	3.91	4.56
Streptococcus mutans	5.42	1.43	5.44	5.44	5.41	5.38	5.41	0.99	1.00	1.09	5.41	5.38	5.41	5.32
Fusobacterium nucleatum	5.50	1.30	5.67	6.53	1.57	4.90	3.57	2.47	2.90	2.57	5.17	5.67	6.53	5.70
Porphyromonas gingivalis	4.20	2.50	8.17	7.77	8.35	8.17	6.47	8.35	8.17	7.77	5.65	8.17	7.77	8.50

DISCUSSION

This study investigated specifically designed denture cleansing products and alternative and unconventional products.[7-9] These products were tested for material compatibility using "soaking" or "soaking plus brushing."

Isopropyl alcohol caused microscopic cracks in autopolymerized PMMA, whereas Listerine Original mouthwash (which apparently partially dissolved the surface), resulted in higher roughness. Surface alterations were more pronounced for auto-polymerized than for heat-polymerized polymers. For auto-polymerized PMMA treated by "soaking plus brushing," negligible morphologic changes were visible. Some scratches resulting from grinding and polishing during material preparation were visible on a microscopic scale for untreated specimens, and the number of scratches increased after the brushing treatment with bleach, vinegar, or soap. However, these morphologic changes were relatively minor compared with the brushing treatment with dentifrice.

Toothpastes typically contain abrasive particles to aid in the mechanical cleaning of teeth. On dentures, significant material was removed from the surface and relatively deep scratches were generated. Such abraded surfaces have a higher roughness and may offer improved retention opportunities for plaque and stain. For the specimens "soaked and brushed" with IPA, although the formation of cracks was observed, only a moderate roughness increase was found. The cracks, however, may significantly deteriorate the bulk properties of the material. Although neither mechanical nor thermal stress was simulated in the test model applied here, these stresses would likely enhance crack propagation and deteriorate the polymer, especially its toughness. Other studies have shown that such chemicals can lead to crazing of the polymer structure.[23] Such cracks could increase the potential for microbial colonization and may also be difficult to clean.[19,24]

Corrosion was observed for CoCr alloy, whereas silver solder showed a layer formed on the entire specimen surface, characterized by a porous, partly sponge-like structure (SEM image, Fig. 5 middle, below). After additional brushing, the surface layer was smeared due to the additional mechanical impact (Fig. 5, right). For tablet cleansers, precipitates, characterized by the crystalline growth of salt crystals with cubic (tablets) or plate (bleach) shapes were observed, together with porous structures on the surface (Fig. 6). These surface structure alterations were visible to the naked eye as tarnishing. For metallic materials, silver-based solder showed the most complex surface modifications after treatment. However, solders with high gold content showed reasonable surface integrity after treatment with tablet cleansers and bleach (data not shown).

For stainless steel, the surfaces were relatively unaffected, with only slight morphologic changes observed, and the high chemical and mechanical resistance of the orthodontic stainless steel wires was confirmed (data not shown); only minor effects of brushing were found in some regions of these surfaces.

The microbiology results presented in this report were all generated using the EN1276 suspension test,[21] modified appropriately. This method establishes pure antimicrobial effects of treatments, the data generated is quantitative, and the results are easy to convey: "2 log kill" "3 log kill," or "99.9% kill." The test is ideal for comparing the effects of different formats— for example toothpastes versus mouthwashes or bleaches versus other hard surface cleansers. The test is readily adapted to reproduce typical usage patterns and/or label instructions. It does not, however, assess the effects of products on microorganisms on surfaces, such as biofilms. Therefore, it does not replicate how the denture-associated bacteria grow. The Expert and Consumer Insight data[7] and other data,[9] indicate that consumers usually also included mechanical cleaning steps in denture cleaning routines, in addition to the use of various products. Most commonly, this was brushing with a toothbrush but included rinsing and rubbing. These mechanical cleaning methods would inevitably remove a great amount of denture plaque either before or after the chemical cleaning effect of treatments. However, dentures may have occluded areas, including microscopic-scale defects such as surface pores. These defects can harbor microbes and may be sufficiently small to shield microorganisms from direct physical cleaning.[19,24] It is thus essential that any denture cleansing methodology has the direct antimicrobial efficacy to kill residual microorganisms. In addition, cleansing products should facilitate denture cleaning through detergent or other physicochemical means.

Of the products tested (see Supplemental material), only the following reached the 3 log_{10} kill benchmark against all 5 microorganisms: bleach (at all dilutions and at all time points); Polident/Corega; chlorhexidine mouthwash; Listerine Original; IPA. Bleach, at least from a microbiological perspective, is highly effective, and only small volumes of either bleach or IPA would be required. There are, however, concerns over the suitability of bleach for use with dentures, especially with partial dental prostheses that usually contain metal components, which may be prone to hypochlorite corrosion. IPA is effective at higher concentrations but is not widely available (at least in the UK). Additionally, IPA may have deleterious effects on the PMMA (see above). Consumer data indicate that mouthwashes are often used for denture cleansing in dilution, while data suggest minimal dilutions were required to maintain efficacy for both chlorhexidine and alcohol-based mouthwash. Because daily cleansing is clearly desirable,[5,6] this implies a significant volume (for example, 75 mL/day) of mouthwash would be needed. Furthermore, Listerine Original mouthwash contains ca. 25% v/v ethanol, which also has interactions with PMMA.

All other products tested, namely, Colgate Total toothpaste, DentylActive, Colgate Fluorigard Alcohol Free, and Plax mouthwashes, Sanex, Carex, and Dial soaps, Fairy Liquid, Fairy Liquid Antibacterial, and Dawn dishwashing liquids, Sarson's Distilled Vinegar, sodium chloride (table salt), and sodium bicarbonate (baking soda) were ineffective against at least 1 microorganism

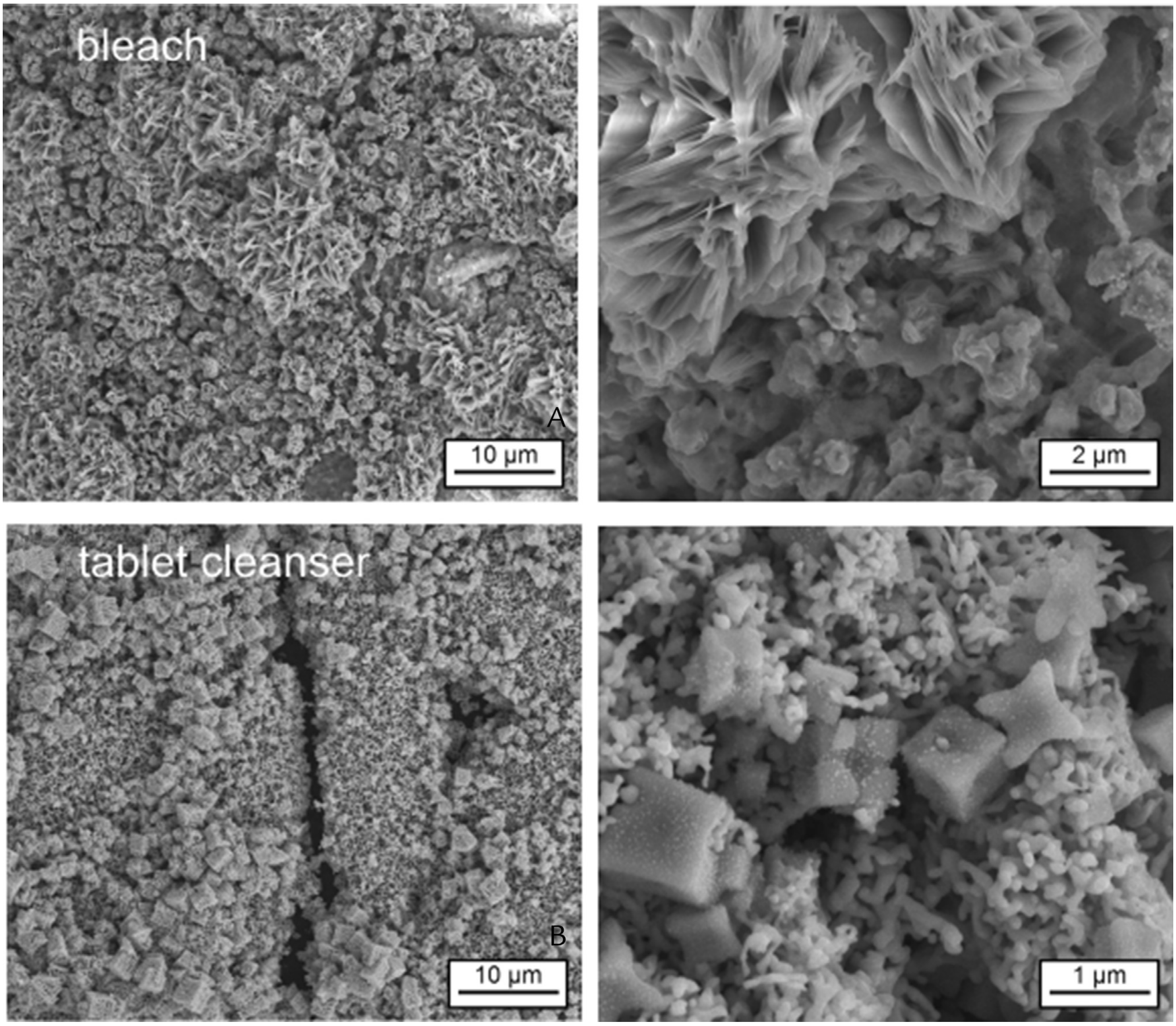


Figure 6. Scanning electron micrographs of specimen surfaces from silver solder (below) after soaking in bleach (top) and after soaking in tablet cleanser solution (below).

at the longest treatment time and at the greatest concentration tested. Two of the soap products claim antibacterial efficacy; however, all of these products, including those with antimicrobial claims, were microbiologically ineffective in our tests. Furthermore, biofilms formed on dentures are often significantly more resistant to antimicrobial treatments (data on file), in common with biofilms from many other sources.[25] The toothpaste chosen here is considered to be antimicrobial[26] and contains Triclosan; other toothpastes may be still less antimicrobially effective. Further, repeated brushing of dentures with toothpaste caused scratching of denture acrylic (see images in figures and supplemental figures). Such scratching may render the acrylic resin more prone to microbial colonization.[24,27]

CONCLUSIONS

The implications of the material compatibility and antimicrobial data for global DHCP recommendations are clear. Using the international standard EN1276 suspension test, only bleach, denture cleanser tablets, IPA, chlorhexidine, and Listerine mouthwashes were effective in killing microorganisms typically found in denture plaque to the 99.9% (3 $\log_{10}$) standard. Mouthwashes typically had to be applied undiluted or with minimal dilution to retain antimicrobial efficacy. Antimicrobial toothpaste, soaps, dishwashing liquids, many marketed non-chlorhexidine mouthwashes, table salt, sodium bicarbonate, and vinegar were ineffective against typical denture plaque microorganisms. Of these microbially effective treatments, only specialist denture cleanser tablets and chlorhexidine mouthwash were reasonably compatible from a material perspective; bleach, IPA, and Listerine Original mouthwash caused significant material damage to acrylic.

The data provide the basis for evidence-based recommendations or guidelines for denture cleaning and regimens. Further, material incompatibilities should be considered in the development of prosthodontic materials and of denture care products. In particular, the use of ignoble metals should be avoided since solder materials commonly showed surface reactions. Physical surface damage could have mechanical and esthetic effects and may increase bacterial adhesion.

The requirements for denture cleansers or cleansing methods can be summarized in a simple triangle (Fig. 1): they should effectively remove plaque and stain and kill any remaining microbes on the denture surface without affecting or damaging the material and surface properties of the denture.

REFERENCES

1. Pereira-Cenci T, da Silva WJ, Cenci MS, Cury AA. Temporal changes of denture plaque microbiologic composition evaluated in situ. Int J Prosthodont 2010;23:239-42.
2. Teles FR, Teles RP, Sachdeo A, Uzel NG, Song XQ, Torresyap G, et al. Comparison of microbial changes in early redeveloping biofilms on natural teeth and dentures. J Periodontol 2012;83:1139-48.
3. Russell SL, Boylan RJ, Kaslick RS, Scannapieco FA, Katz RV. Respiratory pathogen colonization of the dental plaque of institutionalized elders. Spec Care Dentist 1999;19:128-34.
4. Gendreau L, Loewy ZG. Epidemiology and etiology of denture stomatitis. J Prosthodont 2011;20:251-60.
5. de Souza RF, de Freitas Oliveira PH, Lovato da Silva CH, Abu-Naba'a L, Fedorowicz Z, Gurgan CA. Interventions for cleaning dentures in adults. Cochrane Database Syst Rev 2009:CD007395.
6. Felton D, Cooper L, Duqum I, Minsley G, Guckes A, Haug S, et al. Evidence-based guidelines for the care and maintenance of complete dentures: a publication of the American College of Prosthodontists. J Prosthodont 2011;20 Suppl 1:S1-12.
7. Axe AS, Varghese R, Bosma ML, Kitson N, Bradshaw DJ. Dental health professional recommendation and consumer habits in denture cleansing. J Prosthet Dent 2016;115:183-8.
8. Jagger DC, Harrison A. Denture cleansing–the best approach. Br Dent J 1995;178:413-7.
9. Jagger R. Lack of evidence about the effectiveness of the different denture cleaning methods. Evid Based Dent 2009;10:109.
10. Harrison Z, Johnson A, Douglas CW. An in vitro study into the effect of a limited range of denture cleaners on surface roughness and removal of Candida albicans from conventional heat-cured acrylic resin denture base material. J Oral Rehabil 2004;31:460-7.
11. Richmond R, Macfarlane TV, McCord JF. An evaluation of the surface changes in PMMA biomaterial formulations as a result of toothbrush/dentifrice abrasion. Dent Mater 2004;20:124-32.
12. Anusavice K. Phillips' Science of Dental Materials. 12th ed. Philadelphia: Saunders; 2012.
13. Apratim A, Shah SS, Sinha M, Agrawal M, Chhaparia N, Abubakkar A. Denture hygiene habits among elderly patients wearing complete dentures. J Contemp Dent Pract 2013;14:1161-4.
14. Baran I, Nalcaci R. Self-reported denture hygiene habits and oral tissue conditions of complete denture wearers. Arch Gerontol Geriatr 2009;49:237-41.
15. Barreiro DM, Scheid PA, May LG, Unfer B, Braun KO. Evaluation of procedures employed for the maintenance of removable dentures in elderly individuals. Oral Health Prev Dent 2009;7:243-9.
16. Backenstose WM, Wells JG. Side effects of immersion-type cleansers on the metal components of dentures. J Prosthet Dent 1977;37:615-21.
17. McGowan MJ, Shimoda LM, Woolsey GD. Effects of sodium hypochlorite on denture base metals during immersion for short-term sterilization. J Prosthet Dent 1988;60:212-8.
18. Sadaghiani L, Wilson MA, Wilson NHF. Effect of selected mouthwashes on the surface roughness of resin modified glass-ionomer restorative materials. Dent Mater 2007;23:325-34.
19. Wu T, Hu W, Guo L, Finnegan M, Bradshaw DJ, Webster P, et al. Development of a new model system to study microbial colonization on dentures. J Prosthodont 2013;22:344-50.
20. Lefever D, Perakis N, Roig M, Krejci I, Ardu S. The effect of toothbrushing on surface gloss of resin composites. Am J Dent 2012;25:54-8.
21. European Committee for Standardisation. European standard EN 1276. Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and industrial areas. Test method and requirements (phase 2, step 1). European Committee for Standardisation; 1997.
22. Pizzey RL, Marquis RE, Bradshaw DJ. Antimicrobial effects of o-Cymen-5-ol and zinc, alone and in combination in simple solutions and toothpaste formulations. Int Dent J 2011;61:33-40.
23. Arnold J. The effect of diffusion on environmental stress crack initiation in PMMA. Journal of Material Science 1998;33:5193-204.
24. Verran J, Jackson S, Coulthwaite L, Scallan A, Loewy Z, Whitehead K. The effect of dentifrice abrasion on denture topography and the subsequent retention of microorganisms on abraded surfaces. J Prosthet Dent 2014;112:1513-22.
25. Costerton JW, Lewandowski Z, Debeer D, Caldwell D, Korber D, James G. Biofilms, the customized microniche. J Bacteriol 1994;176:2137-42.
26. Furgang D, Sreenivasan PK, Zhang YP, Fine DH, Cummins D. The effects of a new therapeutic triclosan/copolymer/sodium-fluoride dentifrice on oral bacteria, including odorigenic species. Compend Contin Educ Dent 2003;24:14-9.
27. Jackson S, Coulthwaite L, Loewy Z, Scallan A, Verran J. Biofilm development by blastospores and hyphae of Candida albicans on abraded denture acrylic resin surfaces. J Prosthet Dent 2014;112:988-93.

Corresponding author:
Dr David Bradshaw
GlaxoSmithKline Consumer Healthcare
St George's Avenue, Weybridge, Surrey
KT13 0DE
UNITED KINGDOM
Email: david.j.bradshaw@gsk.com

Supplemental Table 1. Overview of tested denture materials

Product/ Materials	Brand Name (Composition)	Manufacturer	Material Class	Specimen Preparation
Heat-polymerized denture polymer	Paladon 65	Heraeus Kulzer	PMMA-based material	Polymer specimens were prepared from disks of polymer material (both auto-polymerized and heat-polymerized) by sectioning them in blocks of appropriate sample size (with dimensions of 10 mm×10 mm, thickness 3 mm). Fabrication of polymer material was conducted by dental laboratory. Specimens were processed according to manufacturer's instructions and mechanically finished using powder, polishing stones, and leather buff. Polymer specimens were fixed on glass slide used as specimen holder.
Auto-polymerized denture polymer	PalaXpress	Heraeus Kulzer	PMMA-based material	
Orthodontic metal wires	Retainer wires 14 medium >50% Fe; Cr 3-29%, Ni 3.5-42.5%,Mn 0.25 7.58%, Si 0.13-3%, Nb/Ta 0.002-1.10%	Henry Schein Inc	Stainless steel	The stainless steel wires and the three solder materials were prepared by cutting the wires into appropriate sample size (length of about 15 mm). Samples were fixed on an acrylic matrix material (VersoCit-2 Powder and VersoCit-2 Liquid, size 15 mmx15 mm) using UHU plus endfest 300. The specimen's surface was exposed for the test and examined to determine that the embedding process had not contaminated the exposed sample surface. Afterward, the acrylic blocks with the exposed wire specimens were fixed on a glass slide used as a specimen holder. Orthodontic metal wires and solders were used as provided from the supplier.
Solder	Universal silver solder (Ag 59%, Zn 25%, Cu 16%)	Dentaurum	Ag-based	
	Universal solder PKF (48.8% Au, 40.5% Ag, 7.3% Zn, In <1.0)	Ivoclar	Ag-based	
	Universal solder 1015 W (18.5% Au, 6% Pd; 72.5% Ag, 3.0% In)	Ivoclar	Ag-based	
Casting alloy	Solidur 63% Co, 29.4% Cr, 5.95% Mo, 0.6% Mn, 0.29% C, 0.05% Fe, 0.7% Si	Yeti Dental	CoCr-alloy	The fabrication of the CoCr material was conducted by a dental laboratory. The specimens were processed according to the manufacturer's instructions resulting in specimens with dimensions of 10 mmx10 mm, thickness 1 mm. For the brushing experiments, the samples were fixed on an acrylic matrix material as described above.

Supplemental Table 2. Products tested for material compatibility

Test Product	Brand Name	Manufacturer
Bleach (6% sodium hypochlorite)		Carl Roth; Germany
Vinegar (clear, 100%)	Table vinegar	Kühne, Germany
Dishwashing Soap (50%)	Fairy liquid antibacterial	Procter & Gamble
Mouthwash (100%)	Listerine Original	Johnson & Johnson
Toothpaste (33%)	Colgate Total	Colgate Palmolive
Tablet cleanser	Polident Overnight Whitening	GSK
Isopropyl alcohol (100%)	Isopropyl alcohol	Carl Roth, Germany
Water	Tap water, pH 7.65	

Supplemental Table 3. Methods applied and equipment used for material compatibility tests

Method	Device Name	Manufacturer
SEM	Quanta 3D FEG	FEI Company
Mechanical profilometry	Ambios XP2	Ambios Technology
Nanoindentation	Nanoindenter Agilent G200 with Berkovich	Agilent
Color measurements	CM 3600A	Konica Minolta
Gloss measurements	ZGM 1120	Zehntner

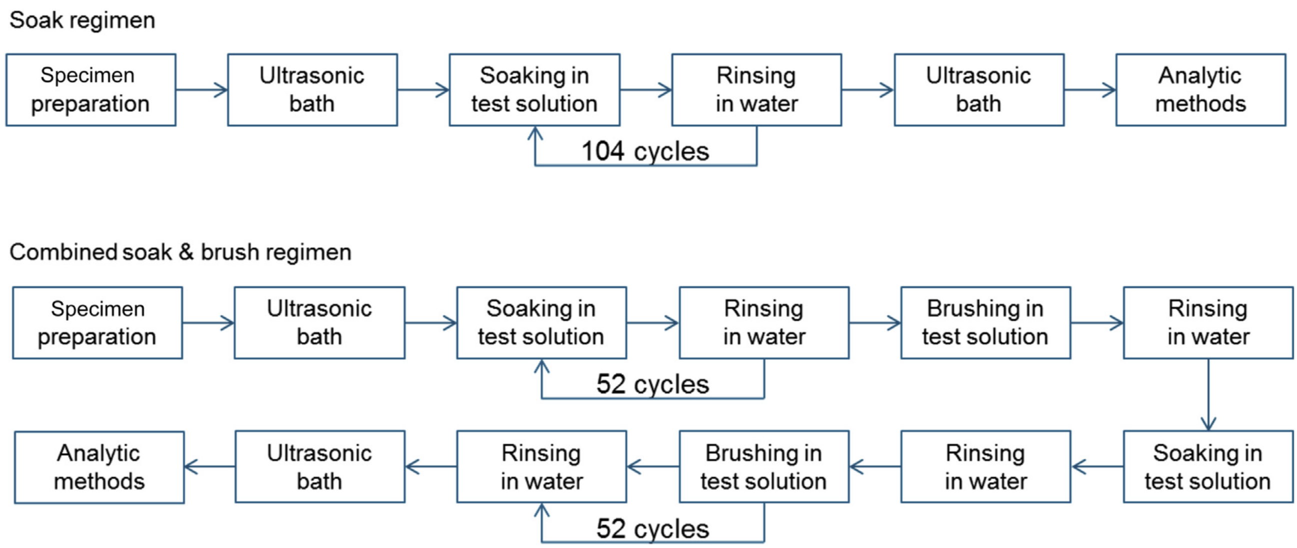
Supplemental Table 4. Products tested for antimicrobial effects

Test Product	Brand Name	Manufacturer
Denture Cleanser Tablet	Polident	GlaxoSmithKline
Toothpaste	Colgate Total	Colgate Palmolive
Mouthwash	Listerine Original	Johnson and Johnson
Mouthwash	Superdrug Chlorhexidine Mouthwash	Superdrug
Mouthwash	Colgate Fluoriguard Alcohol-Free	Colgate Palmolive
Mouthwash	Colgate Plax	Colgate Palmolive
Washing up liquid	Dawn	Proctor & Gamble
Washing up liquid	Fairy Liquid	Proctor & Gamble
Washing up liquid	Fairy Liquid Antibacterial	Proctor & Gamble
Liquid handsoap	Carex	PZ Cussons
Liquid handsoap	Sanex	Colgate Palmolive
Liquid handsoap	Dial	Dial Corp.
Table salt	N/A	Sigma
Sodium bicarbonate (baking soda)	N/A	Sigma
Vinegar	Sarson's	Mizkan Europe
Bleach	Domestos	Unilever
Isopropyl alcohol	N/A	Sigma Aldrich

Supplemental Table 5. Products and neutralizers for antimicrobial testing

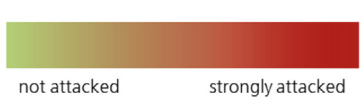
Product	Neutralizer
Superdrug Chlorhexidine Mouthwash 0.2%	TSBS
Dentyl pH mouthwash	TSBS
Colgate Plax mouthwash	TSBS
Listerine Original mouthwash	TSBS
Colgate Fluorigard alcohol-free mouthwash	TSBS
Fairy Liquid Original	TSBS
Fairy Liquid Antibacterial	TSBS
Carex Original handwash	TSBS
Sanex handwash	TSBS
Dial	TSBS
Dawn	TSBS
Colgate Total toothpaste	TSBS
Sarson's Distilled Vinegar	By dilution
Bleach	D/E Broth
Isopropyl alcohol	TSBS
Polident denture tablets	D/E broth

TSBS, Tryptone Soy Broth, Tween, Lecithin (Code BO 1084J; Oxoid); D/E broth, Dey-Engley Broth (D3435; Fluka/Sigma-Aldrich).

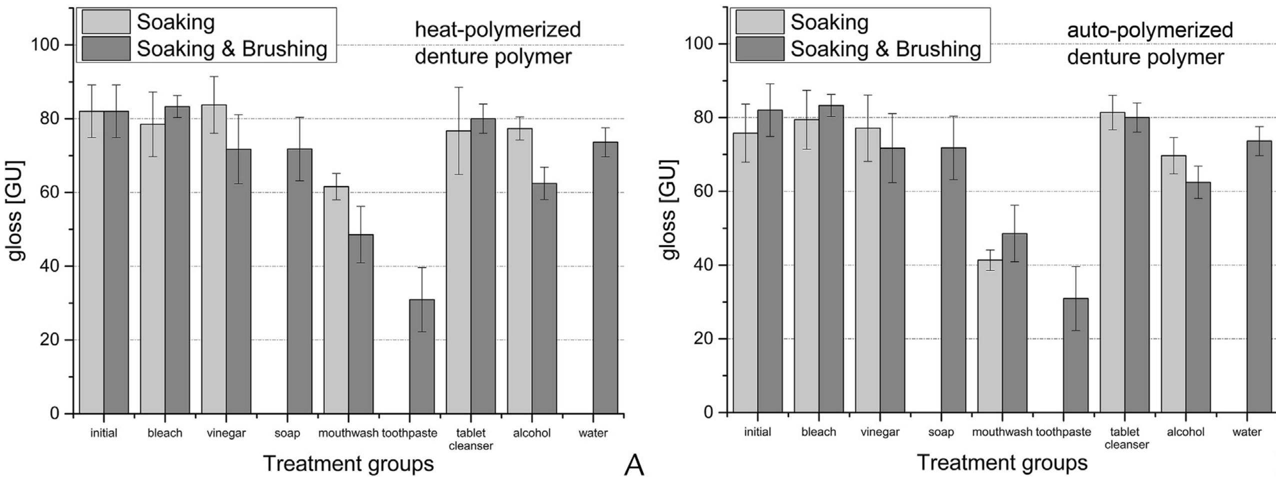


Supplemental Figure 1. Test procedure for the soaking (above) and for combined soaking and brushing (below) experiments in schematic form. To ensure that time taken per cleaning solution was constant and consistent at soaking experiments, automated linear soaking device with 14 beakers and sample holder with dip mechanism was used (Cellstain 15; Tharmac). To remove residues from surfaces, samples were stored before and after experiments in ultrasonic bath.

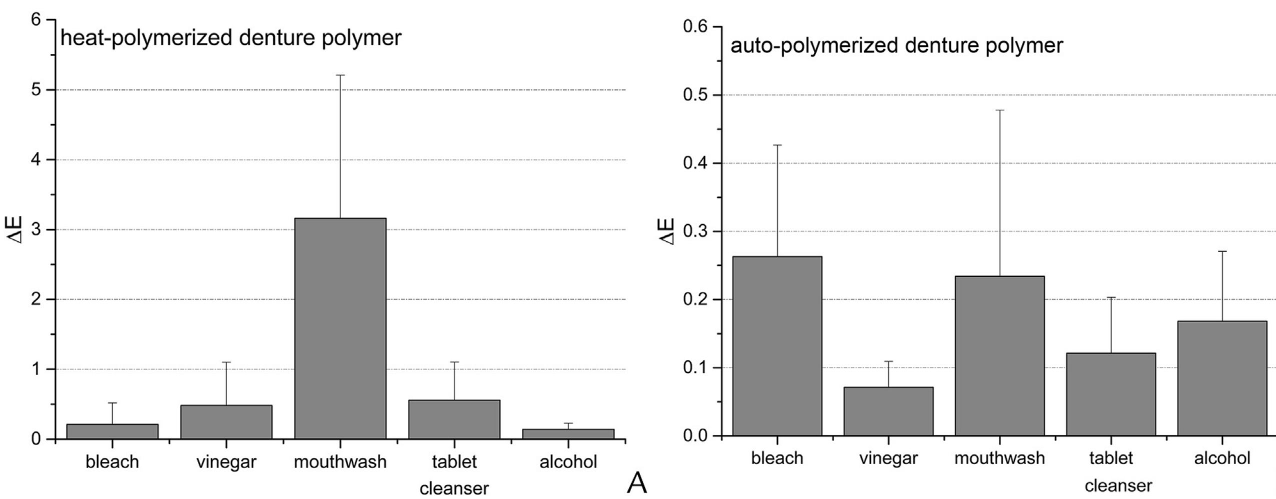
Material	Soaking					Soaking & Brushing							
	bleach	vinegar	mouthwash	tablet cleanser	isopropyl alcohol	bleach	vinegar	soap	mouthwash	toothpaste	tablet cleanser	isopropyl alcohol	water
Heat-polymerized denture material	- not damaged	- local effects	- chemical damage - R, G	- not damaged	- chemical damage - R	- brush-ing traces - R	- brushing traces - R	- brushing traces - R	- chemical damage - R, G	- brushing traces - R, G	- local defects	- chemical damage	- brushing traces, local
Cold- polymerized denture material	- local defects	- local defects	- chemical damage - R, G	- local defects	- chemical damage - cracks	- brush-ing traces - R	- brushing traces - R	- brushing traces - R	- chemical damage - - R, G	- brushing traces - R, G	- local defects	- chemical damage - cracks	-brushing traces, local
Stainless steel	- no significant changes	- no significant changes	- no significant changes	- no significant changes	- no significant changes	- no sig-nificant changes	- no significant changes	- no significant changes	- no significant changes	- no significant changes	- no sig-nificant changes	- no significant changes	- no significant changes
Casting alloy	- tarnish - pitting	- slight residuals	- slight residuals	- slight residuals	- slight residuals	- tarnish - pitting	- brushing traces - R	- brushing trace - R	- brushing traces - R	- brushing traces - R	- brush-ing traces - R	- brushing traces - R	- brushing traces - R
Silver solder	- tarnish - layer formation	- porosity increase	- porosity increase	- layer formation	- porosity increase, local	- tarnish - layer formation	- porosity increase	- porosity increase	- porosity increase	- brushing traces	- layer formation	- porosity increase, local	- brushing traces, local



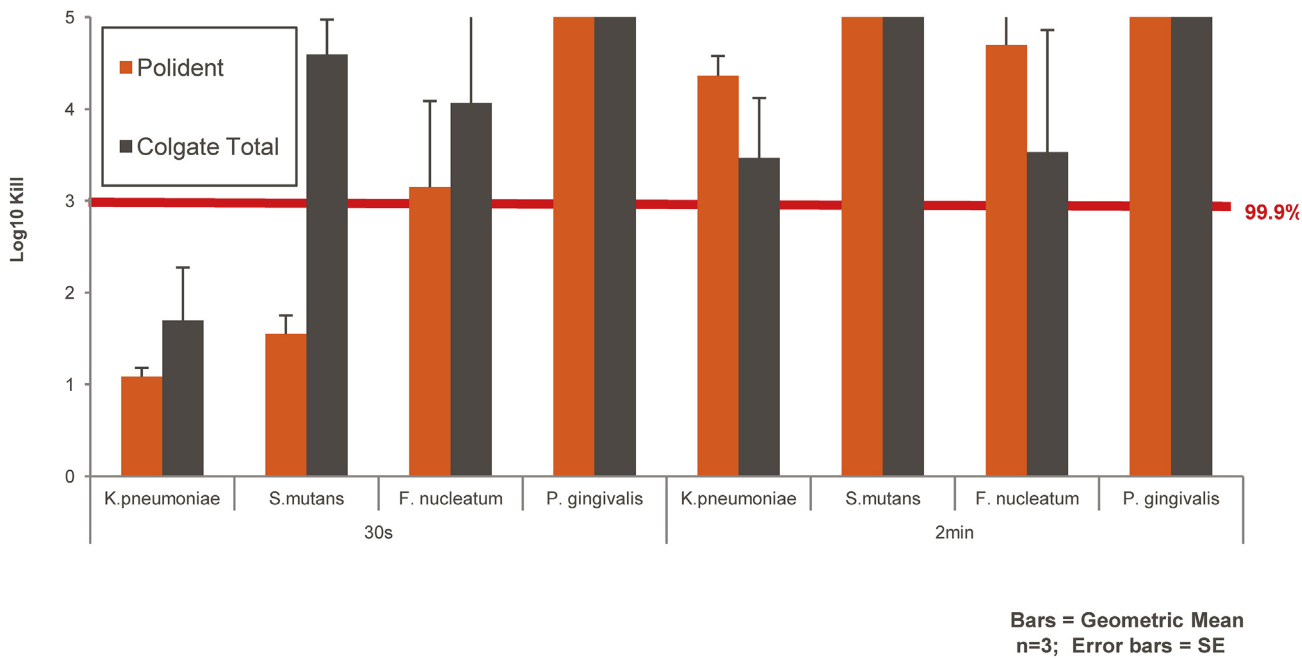
Supplemental Figure 2. Overview of denture material compatibility with different cleansers or cleansing regimens. R, roughness increase; G, gloss decrease.



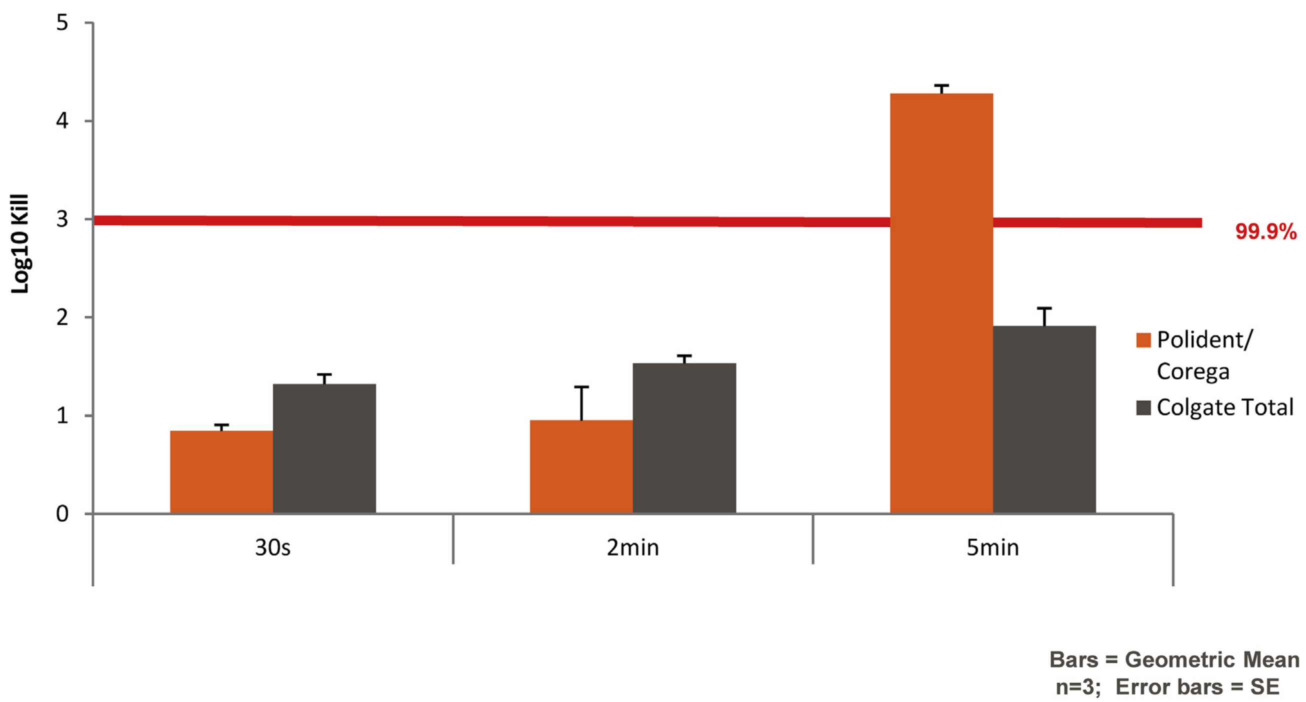
Supplemental Figure 3. Gloss measurements on denture polymethyl methacrylate material surfaces after different treatments.



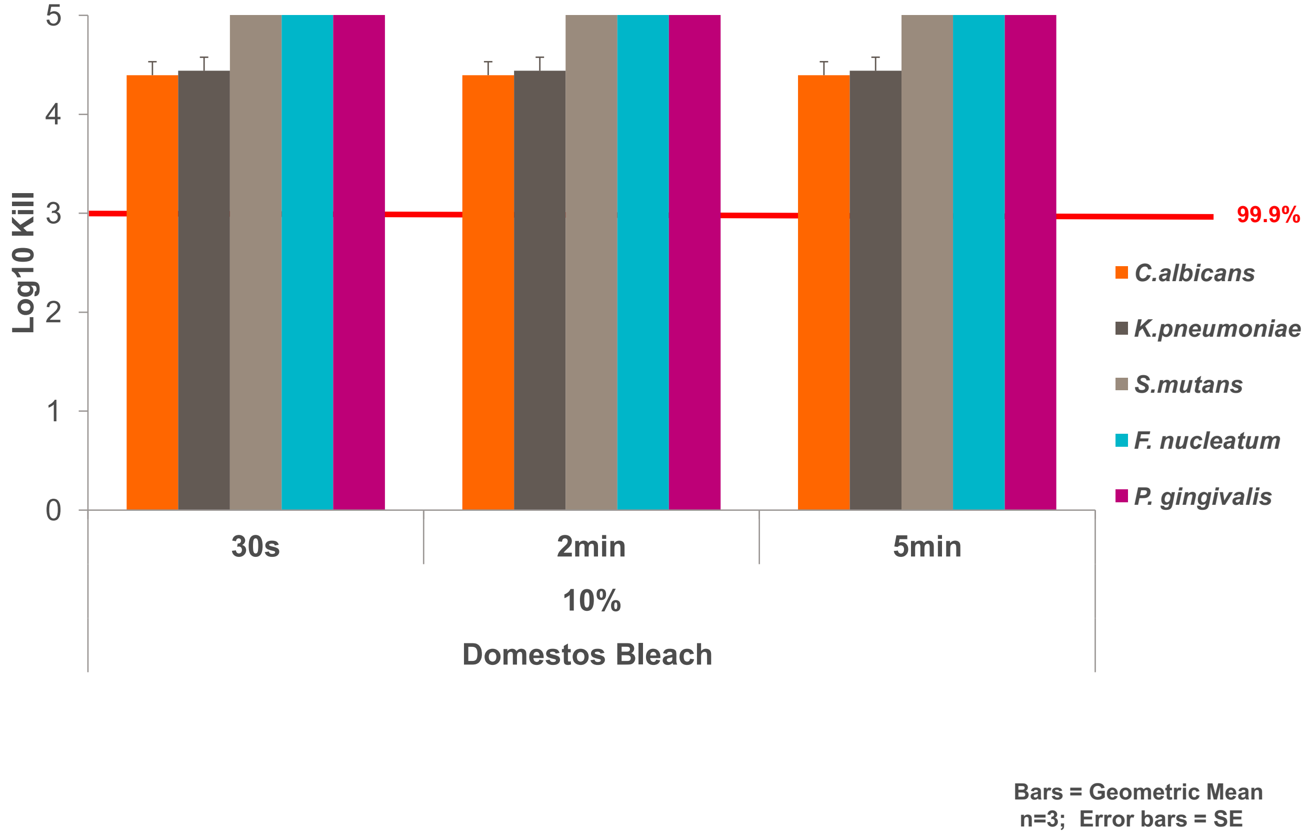
Supplemental Figure 4. Color changes of heat- and autopolymerized polymers by treatment. Color measurements were performed only on polymethyl methacrylate specimens treated according to "soaking" regimens. Color difference ΔE was calculated according to CIELab color system* using equation: $\Delta E = [\Delta L^2 + \Delta a^2 + \Delta b^2]^{1/2}$, where ΔL, Δa, and Δb are differences of L, a, and b values before and after treatment.*Commision Internationale de l'Eclairage (CIE). Colorimetry. Publication CIE No. 15.3. 3rd Edition ed. Central Bureau of the CIE; Vienna: 2004.



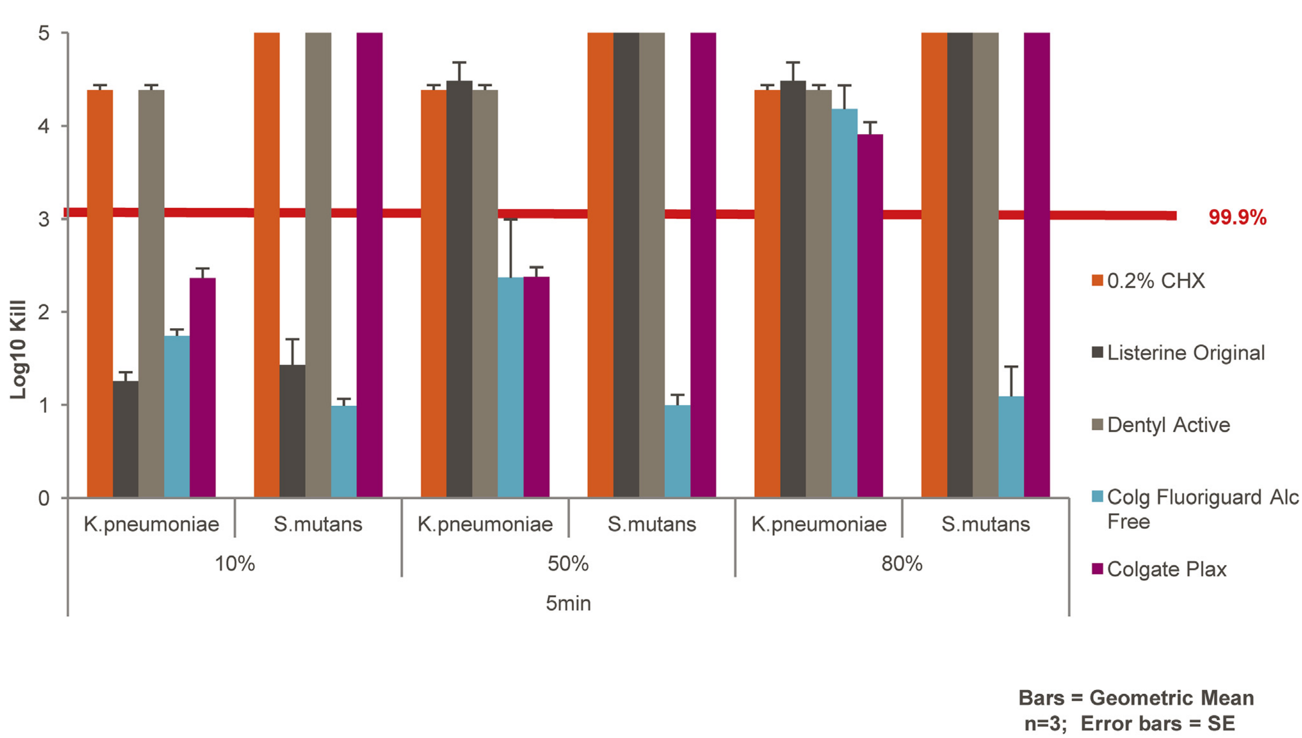
Supplemental Figure 5. Denture cleanser versus toothpaste–bacteria.



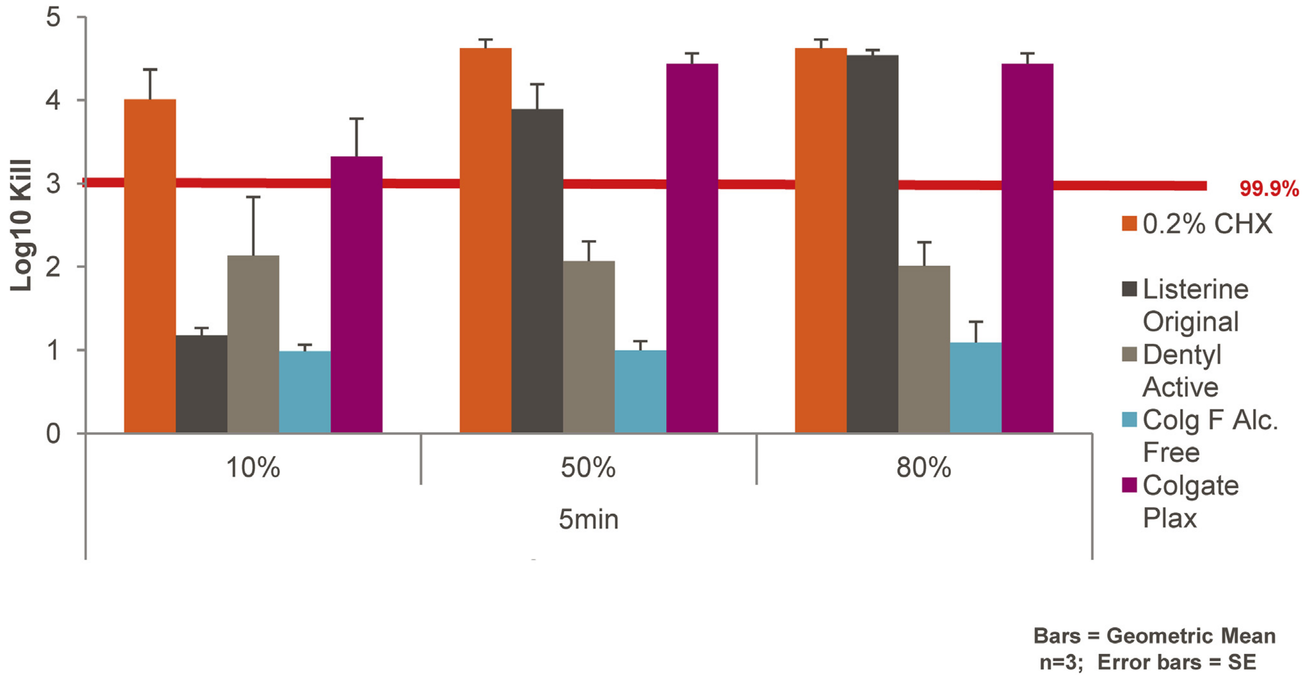
Supplemental Figure 6. Denture cleanser versus toothpaste–*Candida albicans*.



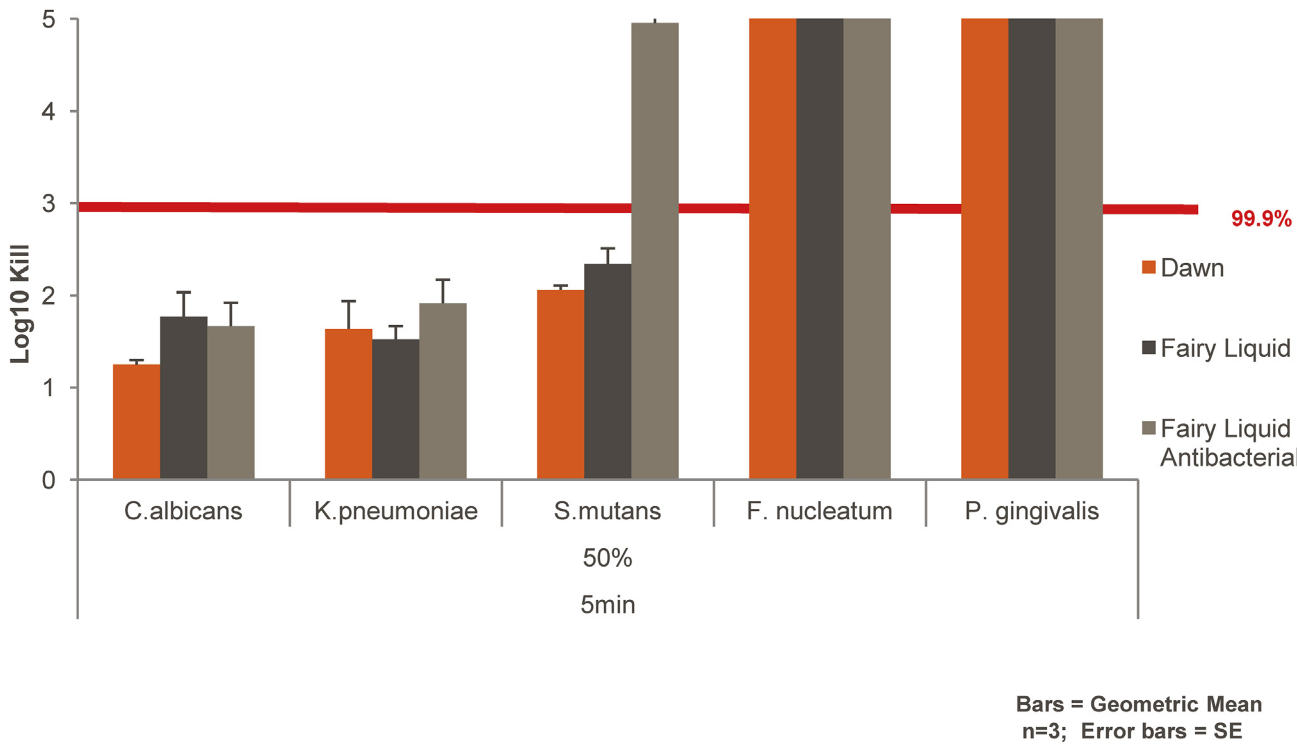
Supplemental Figure 7. Domestos bleach (hypochlorite).



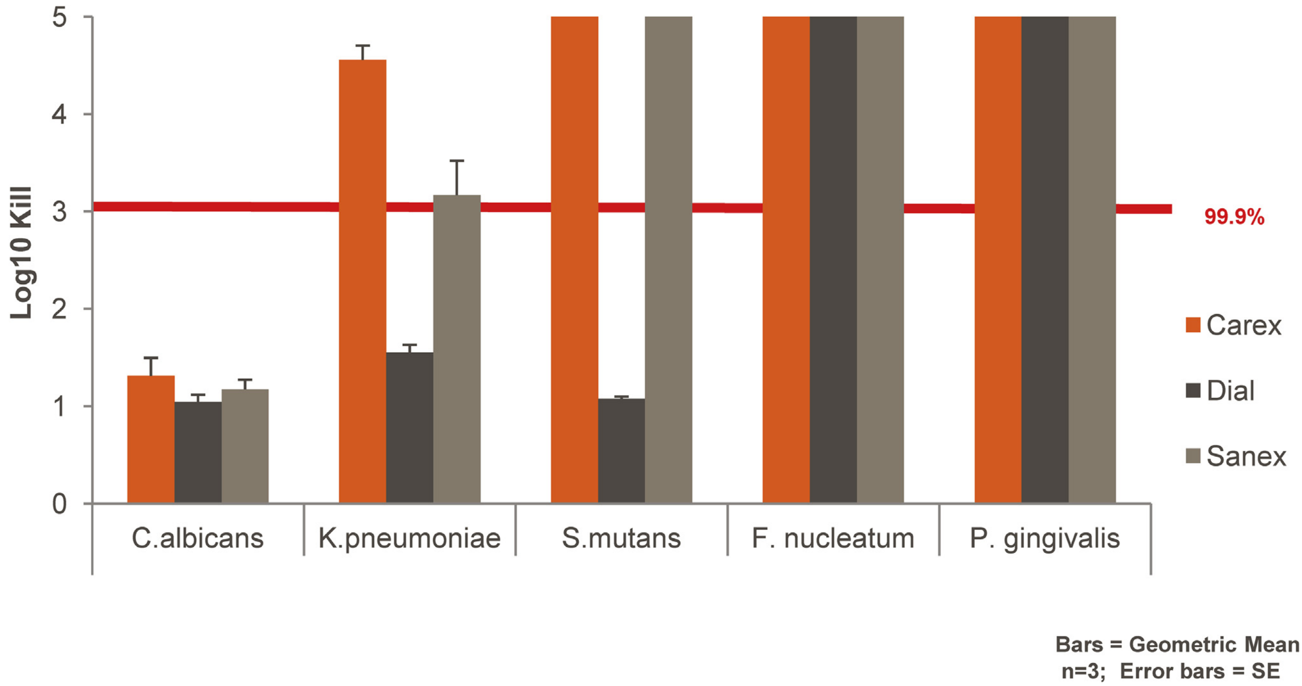
Supplemental Figure 8. Mouthwash: facultative bacteria.



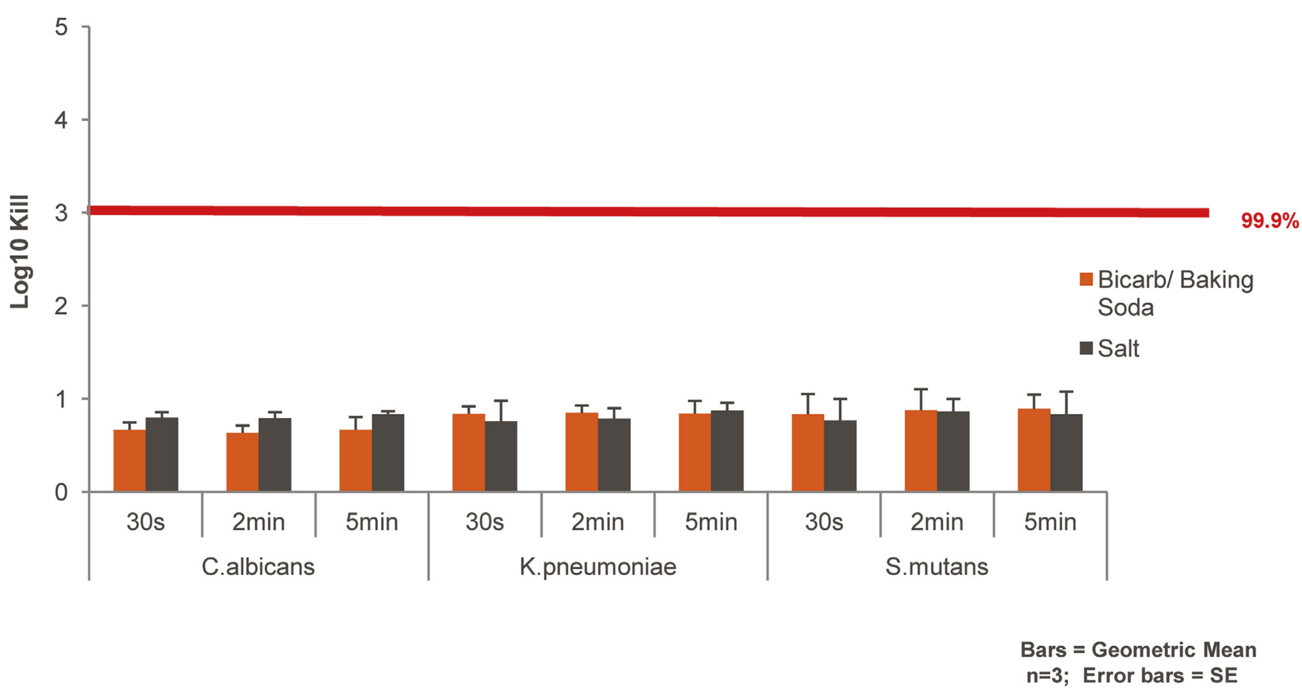
Supplemental Figure 9. Mouthwash: *Candida albicans*.



Supplemental Figure 10. Dishwashing liquids at 50%w/w dilution.



Supplemental Figure 11. Handsoaps at 50%w/w.



Supplemental Figure 12. Salt and sodium bicarbonate (at 20%w/w dilution).