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ORIGINAL ARTICLE

Streptococcal adhesion to various luting systems and the role of mixing errors

RALF BUERGERS[1], SEBASTIAN HAHNEL[1], UDO REISCHL[2], RAINER MUELLER[3], MARTIN ROSENTRITT[1], GERHARD HANDEL[1] & MICHAEL BEHR[1]

[1]*Department of Prosthetic Dentistry, University Medical Center Regensburg, Germany,* [2]*Institute of Medical Microbiology and Hygiene, University Medical Center Regensburg, Germany and* [3]*Institute of Physical and Theoretical Chemistry, University of Regensburg, Germany*

Abstract

Objective. This study aims at ranking various luting systems according to their susceptibility to adhering *Streptococcus mutans* and at evaluating the influence of incongruent mixing ratios on adhesion quantities. ***Material and Methods.*** Circular specimens measuring 8 mm in diameter were made of nine widely used dental cements—three of them mixed in different ratios—and then incubated with *S. mutans*. Adhering streptococci were quantified using a biofluorometric assay in combination with an automated plate reader for cell quantification. Surface roughness (R_a) was determined by perthometer measurements. ***Results.*** Meron plus revealed the highest R_a (0.90 μm) and glass the lowest R_a (<0.01 μm). In regular cement mixtures, the highest mean fluorescence intensities indicated the presence of many viable bacteria [Meron Plus (35,533 relative fluorescence units (rfu)), Maxcem (13,374 rfu), and Panavia F 2.0 (11,701 rfu)]. Moderate fluorescence intensities were found in Harvard (4,171 rfu), Ketac cem (3,766 rfu), Durelon (3,276 rfu), Calibra (3,259), Rely X Unicem (4,358 rfu), and Bifix SE (3,102 rfu). A medium correlation between R_a and *S. mutans* adhesion was found. Changes in regular cement proportions (powder/liquid and base/catalyst, respectively) had a significant influence on relative fluorescence intensities, which linearly increased with a higher proportion of liquid in Harvard and with a higher proportion of catalyst in Calibra and Maxcem. ***Conclusions.*** Various luting systems revealed considerable differences in their potential to adhere *S. mutans*. Variations from recommended cement proportions led to significant changes in the amount of adhering streptococci.

Key Words: *Bacterial adhesion, cements, fluorescence, mixing errors, Streptococcus mutans*

Introduction

In cemented restorations, the selection of a particular luting system is crucial, because the type of luting system may significantly influence both the survival and the success rate of indirect dental restorations. In general, luting systems for the restoration of alloys and ceramics demonstrate good long-term success rates [1–4]. With regard to this aspect, no significant differences have been found among conventional zinc oxide phosphate, glass ionomer, and resin-modified glass ionomer cements [3]. Adhesively luted restorations showed similar survival rates [5]. An analysis of the clinical studies on survival rates of luted restorations showed secondary caries as the main reason for failure, for example in 22% of cases in an investigation conducted by DeBacker et al. [1] and in 33% of cases in a study by Holm et al. [6]. The margins of dental restorations are highly vulnerable to the initiation of caries, which in turn results from the adhesion of cariopathogenic bacteria to teeth, restorations, and exposed cement surfaces [7,8]. Incidentally, the bacterium *Streptococcus mutans* (*S. mutans*) has been identified as the main etiological agent of caries [9].

Dental science is predominantly focused on selecting the “ideal cement”, but the cementation procedure *per se* is often neglected in this context [10]. Although proportioning scoops and modern automatic mixing dispensers simplify the mixing of cement components, ideal mixing conditions are seldom achieved; thus, mixing errors and inhomogeneities are normal in clinical conditions [11]. The

Correspondence: Ralf Buergers, Department of Prosthetic Dentistry, University Medical Center Regensburg, DE-93042 Regensburg, Germany. Tel: +49 941 944 6059. Fax: +49 941 944 6171. E-mail: ralf.buergers@klinik.uni-regensburg.de

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decisive influence of these unrecommended mixing ratios of powder/liquid or base/catalyst on the mechanical properties of cements is widely accepted [5,10–13]. Additionally, secondary caries is assumed to accelerate at the margin of restorations because of cementation failures caused by, for example, mixing errors. Surprisingly, the influence of variations in mixing ratios on the susceptibility of adhering microorganisms has not yet been investigated. In fact, only few investigations on the bacterial adhesion to different types of cement are available so far [14,15].

The aim of the present *in vitro* study was to examine the quantity of *S. mutans* adhesion to various luting systems and to evaluate the influence of incongruent mixing ratios. The research hypotheses were: (a) no significant differences exist in the potential to adhere *S. mutans* to nine luting systems within their regular cement proportions, and (b) low-grade variations of mixing ratios only have a negligible influence on the amount of adhering bacteria.

Material and methods

Specimen preparation and arithmetic surface roughness R_a

Table I lists the nine luting systems used in this investigation. The regular mixing ratios of three cements (Harvard, Calibra, and Maxcem) recommended by the manufacturers were varied (cf. Table II) based on the results of a former investigation by Behr et al. [5]. With a chemical balance (Sartorius, Göttingen, Germany), powder and liquid or pastes were weighed with a tolerance limit of ±0.1 mg. A veneering composite and glass disks served as reference materials. Circular specimens (diameter 8 mm, thickness 2 mm) were prepared using a custom metal mold with calibrated circular holes. The materials were inserted into the mold and immediately covered with a glass slide (Alfred Becht, Offenburg, Germany) to obtain smooth specimen surfaces. Additionally, all specimens had been polished to high gloss using a polishing machine (Motopol 8; Buehler, Coventry, UK) and wet abrasive paper (grain 4000, Buehler, Düsseldorf, Germany). The arithmetic average of surface roughness (R_a) was determined on two spots of five different specimens for each material using a profilometric stylus (Perthometer S6P; Perthen, Göttingen, Germany). All specimens were rinsed with ethanol (70%) and fixed onto 96 well-plates (Sarstedt, Newton, N.C., USA).

Adhesion testing

Adherent *S. mutans* were quantified as described previously [16]. We used the *S. mutans* strain 20523 (DMSZ, Braunschweig, Germany) as a test micro-

Table I. Overview of cement types, brand names, manufacturers, and compositions.

Cement type	Brand name	Manufacturer	Composition*
Zinc oxide phosphate	Harvard (fast luting)	Harvard, Berlin, Germany	Powder: zinc oxide, magnesium oxide Liquid: o-phosphoric acid
Glass ionomer	Ketac cem	3M ESPE, Seefeld, Germany	Powder: glass oxide, chemicals Liquid: water, maleic acid, copolymer, tartaric acid
Resin-modified glass ionomer	Meron Plus, hand-mixed	VOCO, Cuxhaven, Germany	Powder: tartaric acid, fluorsilicate Liquid: polyacrylic acid, HEMA
Carboxylate	Durelon	3M ESPE	Powder: zinc oxide, polyacrylic acid, stannous fluoride, tin dioxide Liquid: water
Dual-curing composite	Panavia F 2.0	Kuraray, Osaka, Japan	Paste A: methacryloyloxydecyldihydrogen phosphate, hydrophic/hydrophilic dimethacrylate, silanated silica, camphorquinone, initiators Paste B: sodium fluoride, hydrophic/hydrophilic dimethacrylate, Silanated barium glass fillers, initiators, accelerators, pigments
	Calibra	Dentsply DeTrey	Base: dimethycrylate resins, camphorquinone, stabilizers, glass fillers, fumed silica, titanium dioxide, pigments Catalyst: dimethacrylate resins, catalyst, stabilizers, glass fillers, fumed silica
Self-adhesive composite	Rely X Unicem	3M ESPE	Powder: silanized glass powder, silane treated silica, calcium hydroxide, substituted pyrimidine, sodium persulfate Liquid: methacrylated phosphoric acid esters, triethylene glycol dimethacrylate, substituted dimethacrylate
	Bifix SE	VOCO	Base: UDMA, glycerine dimethacrylate, initiators, catalyst Catalyst: UDMA, glycerine dimethacrylate, acid methacrylate, Bis-GMA, hydroxyl-propyl methacrylate
	Maxcem	Kerr, Orange, Calif., USA	Self-etch cement: composition not available
Reference material	Sinfony; veneering composite	3M ESPE	Glass powder, dicyclopentyldimethylene diacrylate, diurethane dimethacrylate, glass ionomer fillers, silane treated silica
	Glass	Marienfeld, Koenigshofen, Germany	

*According to material safety data sheets.

Table II. Overview of cement types, mixing ratios (powder:liquid and base:catalyst), and arithmetic surface roughness.

Brand name	Mixing variations	Mean (SD) surface roughness R_a (µm)
Harvard (fast luting)	1.8:1.8	0.58 (0.17)
	1.8:1*	0.36 (0.13)
	1.8:0.75	0.45 (0.15)
Ketac cem	3.8:1	0.09 (0.02)
Meron Plus, hand-mixed	1.8:1	0.90 (0.48)
Durelon	2:1	0.26 (0.11)
Panavia F 2.0	1:1	0.48 (0.11)
Calibra	1:2	0.08 (0.02)
	1:1*	0.09 (0.03)
	1.75:1	0.06 (0.02)
Rely X Unicem	1:1	0.53 (0.17)
Bifix SE	1:1	0.19 (0.05)
Maxcem	4:1.5	0.36 (0.07)
	4:1*	0.36 (0.05)
	4:0,6	0.52 (0.18)
Sinfony; veneering composite		0.06 (0.02)
Glass		<0.01

*Original mixing ratio according to manufacturer's instructions.

organism. On the day prior to the experiment, 1 ml of bacterial suspension was inoculated with 250 ml of sterile trypticase soy broth (BD Diagnostics) and incubated at 37°C for 12 h. The optical density of the suspensions was adjusted with a spectrophotometer (Genesys 10S; Thermo Spectronic, Rochester, N.Y., USA) to 0.3 at 540 nm. We used the oxidation-reduction fluorescence dye Alamar Blue/Resazurin (Sigma-Aldrich, 0.75 g/ml *aqua dest*) to determine the quantity of bacterial adhesion. Fluorescence intensities were recorded using an automated multi-detection reader (Fluostar optima; BMG Labtech, Offenburg, Germany) at wavelengths of 530 nm excitation and 590 nm emission. High relative fluorescence intensities indicated high streptococcal adhesion.

Statistics

Means and standard deviation (SD) were calculated. Statistical differences were investigated by one-way ANOVA and the Tukey–Kramer multiple comparison test for post-hoc analysis. The level of significance was set at $\alpha = 0.05$. The Spearman rank correlation coefficient was calculated to assess the correlation between surface roughness and relative fluorescence. Statistical software (SPSS 15.0 for Windows; SPSS, Chicago, Ill., USA) was used for all calculations.

Results

Surface roughness R_a

Table II displays means and standard deviations as gathered by perthometer measurements. One-way ANOVA revealed statistically significant differences in surface roughness between the various cements tested and the reference materials ($p < 0.001$). Post-hoc analyses Table III showed the highest significant R_a values in Meron Plus (mean: 0.90 µm) and the lowest in the reference material glass (mean: <0.01 µm). No statistically significant differences were found among the variations in the mixing ratios of Harvard, Calibra, and Maxcem ($p > 0.05$ for all post-hocs). A medium correlation between surface roughness and *S. mutans* adhesion has been calculated (Spearman's rank correlation coefficient of 0.534).

S. mutans adhesion (cf. Figure 1)

One-way ANOVA revealed statistically significant differences in relative fluorescence intensities among the various cement specimens ($p < 0.001$). Post-hoc analyses (Table III) of all cements with original mixing ratios found the highest significant fluorescence values—indicating high bacterial adhesion—in Meron Plus (mean relative fluorescence intensity 35,533 rfu). Maxcem revealed the second highest fluorescence value (13,374 rfu) with a significant difference to all other cements ($p < 0.05$) except Panavia F 2.0 (11,701 rfu). Medium fluorescence intensities were found in Harvard (4,171 rfu), Ketac cem (3,766 rfu), Durelon (3,276 rfu), Calibra (3,259), Rely X Unicem (4,358 rfu), and Bifix SE (3,102 rfu). The lowest relative fluorescence intensities were observed in both reference materials, Sinfony (552 rfu) and glass (196 rfu).

In Harvard cement, fluorescence intensity increased with a higher proportion of liquid, indicating an increasing amount of *S. mutans*. The 1.8:1.8 powder/liquid ratio revealed higher statistically significant fluorescence intensities (29,811 rfu) than the regular 1.8:1 ratio (4,171 rfu) and the 1.8:0.75 (2,471 rfu) ratio. In the dual-curing composite Calibra, fluorescence intensities decreased linearly with increasing base component, although the differences among the three mixtures of 1:2 (7,623 rfu), 1:1 (3,260 rfu), and 1.75:1 (1,691 rfu) were not statistically significant. Highly significant differences ($p < 0.001$) were found among the three mixing ratios of Maxcem, in which fluorescence intensities decreased linearly with a decreasing proportion of catalyst. The 4:1.5 mixture with the highest amount of catalyst showed the highest significant fluorescence intensity (29,876 rfu). No statistical difference ($p = 0.331$) was found between the regular 4:1 ratio (13,374 rfu) and the 4:0.6 (8,122 rfu) ratio.

A medium correlation between surface roughness and *S. mutans* adhesion has been calculated (Spearman's rank correlation coefficient of 0.534).

Table III. Statistical analysis (Tukey-Kramer) of surface roughness R_a (right and up) and fluorescence intensities (left and down); level of significance $\alpha = 0.05$.

	Harvard (1.8:1.8)	Harvard (1.8:1)	Harvard (1.8:0.75)	Ketac cem	Meron plus	Durelon	Panavia F 2.0	Calibra (1:2)	Calibra (1:1)	Calibra (1.75:1)	Rely X Unicem	Bifix SE	Maxcem (4:1.5)	Maxcem (4:1)	Maxcem (4:0.6)	Sinfony	Glass
Harvard (1.8:1.8)	—	.236	.984	<.001	.001	.009	1.000	<.001	<.001	<.001	1.000	<.001	.341	.264	1.000	<.001	<.001
Harvard (1.8:1)	<.001	—	.996	.050	<.001	.999	.933	.030	.042	.011	.695	.595	1.000	1.000	.727	.006	<.001
Harvard (1.8:0.75)	<.001	1.000	—	.001	<.001	.522	1.000	<.001	<.001	<.001	1.000	.032	.999	.997	1.000	<.001	<.001
Ketac cem	<.001	1.000	1.000	—	<.001	.712	<.001	1.000	1.000	1.000	<.001	.999	.050	.073	<.001	1.000	.998
Meron plus	.806	<.001	<.001	<.001	—	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Durelon	<.001	1.000	1.000	1.000	<.001	—	.230	.595	.703	.383	.069	1.000	.998	.999	.078	.314	.045
Panavia F 2.0	<.001	.291	.078	.253	<.001	.168	—	<.001	<.001	<.001	1.000	.007	.969	.939	1.000	<.001	<.001
Calibra (1:2)	<.001	.998	.907	.994	<.001	.979	.989	—	1.000	1.000	<.001	.996	.031	.046	<.001	1.000	1.000
Calibra (1:1)	<.001	1.000	1.000	1.000	<.001	1.000	.109	.964	—	1.000	<.001	.999	.034	.064	<.001	1.000	.996
Calibra (1.75:1)	<.001	1.000	1.000	1.000	<.001	1.000	.033	.759	1.000	—	<.001	.968	.012	.018	<.001	1.000	1.000
Rely X Unicem	<.001	1.000	1.000	1.000	<.001	1.000	.293	.998	1.000	1.000	—	.001	.805	.720	1.000	<.001	<.001
Bifix SE	<.001	1.000	1.000	1.000	<.001	1.000	.092	.951	1.000	1.000	1.000	—	.573	.671	.001	.950	.493
Maxcem (4:1.5)	1.000	<.001	<.001	<.001	.820	<.001	<.001	<.001	<.001	<.001	<.001	<.001	—	1.000	.830	.007	<.001
Maxcem (4:1)	<.001	.060	.011	.052	<.001	.030	1.000	.800	.015	.004	.058	.012	<.001	—	.830	<.001	<.001
Maxcem (4:0.6)	<.001	.981	.753	.965	<.001	.913	.995	1.000	.862	.536	.985	.829	<.001	.844	—	.007	<.001
Sinfony	<.001	.993	1.000	.999	<.001	1.000	.004	.359	1.000	1.000	.983	1.000	<.001	<.001	.163	—	1.000
Glass	<.001	.980	1.000	.995	<.001	.999	.002	.274	.998	1.000	.960	.999	<.001	<.001	.112	1.000	—

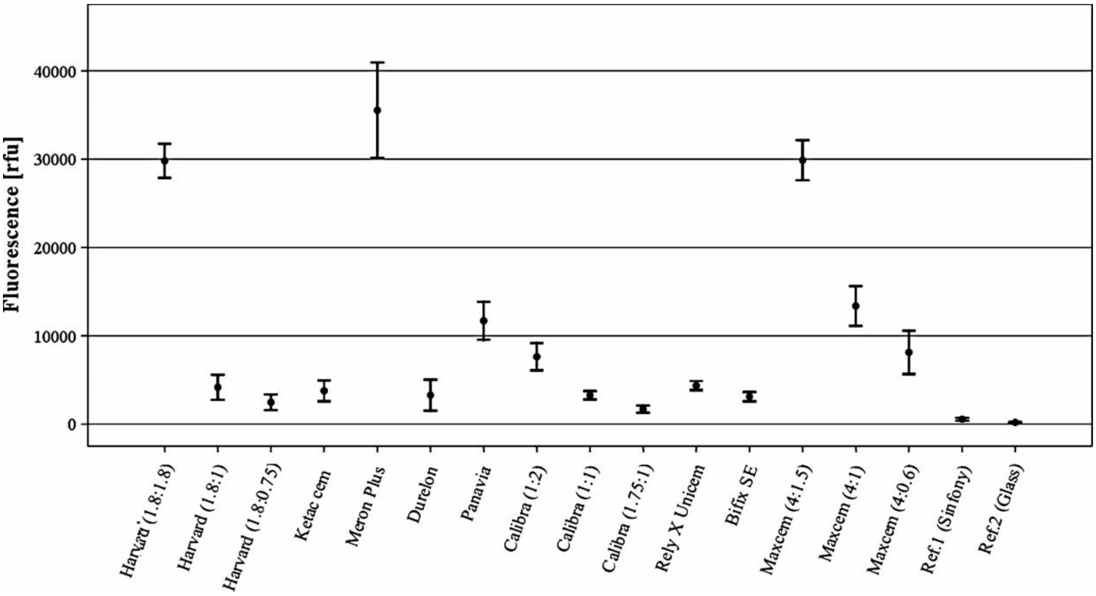

Figure 1. Mean (SD) fluorescence [rfu] of nine test cements and different mixing variations ($n = 3$).

Discussion

The present study investigates the adhesion of *S. mutans* to nine luting systems and the influence of incongruent mixing ratios on adhesion quantities. Despite the existence of other species, *S. mutans* has been considered the major microbiological factor in the pathogenesis of caries and has therefore been used as a test organism in this investigation [9]. Numerous methods have been described for the quantification of bacterial adhesion, including scanning electron microscopy, radiolabeling, and direct plate counting [17,18]. Fluorometric quantification techniques have gained increasing importance; the Resazurin fluorescence dye, which was used for quantifying viable bacterial cells in this study, has proved to be highly sensitive and reproducible [16,19–22].

Although secondary caries is the main reason for the failure of luted restorations, only a few investigations into bacterial adhesion to dental cements are available so far [14,15]. Consequently, one of the main concerns of this study was to include a range of different cements and cement types in one investigation and subsequently to rank them according to their potential to adhere *S. mutans*. Despite proportioning scoops and automatic dispensers, mixing errors occur frequently and are known to reduce the mechanical properties of luting systems significantly [5,10–13]. Therefore, the influence of incongruent mixing ratios on specific adhesion properties should also be investigated.

The presence of a salivary pellicle is known to affect the adhesion process, but there is conflicting evidence whether it reduces or enhances bacterial accumulation [23]. No conditioning film was used in this investigation in order eliminate the leveling effect of the pellicle and in order to simplify interpretation of the results. Therefore, differences in the quantities of bacterial adhesion could be attributed to varying material properties and not to the influence of pellicle coating. In general, high surface roughness values are associated with extensive plaque accumulation on both oral tissues and dental materials [23,24]. All test materials—except Ketac cem, Calibra, and Bifix SE—revealed surface roughness values above a threshold of 0.2 μm. According to Bollen et al., a direct influence on the quantity of adhering bacteria should be expected for such values [24]; this is an assumption that was supported in our study by the correlation of the highest surface roughness value and the highest amount of adhering streptococci on Meron Plus. Nevertheless, no general correlation between surface roughness and adhesion quantities could be observed; only a medium correlation between surface roughness and *S. mutans* adhesion has been calculated (Spearman rank correlation coefficient of 0.534). Interestingly, in Harvard, Calibra, or Maxcem, variations in mixing ratios (powder/liquid or base/catalyst) did not lead to significant changes in surface roughness.

There are considerable differences in the potential to adhere *S. mutans* among the tested luting systems. In contrast, the highest adhesion quantities were found in resin-modified glass ionomer Meron Plus, self-adhesive composite Maxcem, and dual-curing composite Panavia F 2.0. However, in our opinion, these findings do not allow the conclusion that specific cement types have superior properties with regard to the adhesion of micro-organisms. On the other hand, each assessed cement showed a significantly higher amount of streptococci than the reference materials Sinfony and glass. This fact proves the generally high adhesion potential of luting systems and thus emphasizes the necessity of continuative investigations into this topic. Basically, the composition of a specific luting system defines the individual surface properties of the material, which in turn determine the quantity and quality of microbial adhesion. Therefore, different potentials to adhere streptococci among the tested cements may be attributed to differences in material composition such as monomer systems, filler sizes, and catalyst systems (cf. Table II). The main finding of this study is that variations in mixing ratios resulted in significant changes in the adhesion quantities of the three luting systems tested, i.e. Harvard (zinc oxide phosphate), Calibra (dual-curing composite), and Maxcem (self-adhesive composite). As these mixing errors are promoted by hand-mixed cements, modern luting systems with proportioning dispensers might also be preferential in a bacterial perspective [11]. Clinical observations showed a general tendency to use more liquid than powder in cement mixtures [10]. In Harvard cement, streptococcal adhesion increased with a higher proportion of liquid, which implies a higher content of phosphoric acid. *S. mutans* has the ability to generate acid and preferentially exists at low pH, which might be a possible explanation for its increased accumulation on specimens prepared from a liquid/powder ratio of 1.8:1.8 [9]. Behr et al. showed very sensitive reactions in the mechanical properties of the self-adhesive composite Maxcem and the degree of conversion after mixing errors [5]. Likewise, Maxcem and the dual-curing composite Calibra revealed linearly decreasing adhesion potentials after a gradual increase of the base component, which might result from increasing proportions of residual monomers and from changes in the degree of conversion [5]. Released residual monomers are oxidized to formaldehyde, which probably has an "unrequested" antibacterial effect on adhering microorganisms [25].

In conclusion, the tested luting systems revealed significant differences in their potential to adhere *S. mutans*. Variations in liquid/powder or base/catalyst ratios resulted in significant changes of adhesion quantities, and increased proportions of liquid or catalyst components led to an intensified adhesion of *S. mutans*.

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