EFFECTS OF EASY-TO-PERFORM PROCEDURES TO REDUCE BACTERIAL COLONIZATION WITH *STREPTOCOCCUS MUTANS* AND *STAPHYLOCOCCUS AUREUS* ON TOOTHBRUSHES

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It is well known that dental caries and periodontitis are the consequence of bacterial colonization and biofilm formation on the enamel surface. The continuous presence of bacterial biofilms on the tooth surface results in demineralization of the tooth enamel and induces an inflammatory reaction of the surrounding gums (gingivitis). The retention and survival of microorganisms on toothbrushes pose a threat of recontamination especially for certain patients at risk for systemic infections originating from the oral cavity, e.g., after T-cell depleted bone marrow transplantation. Thus, the effects of different decolonization schemes on bacterial colonization of toothbrushes were analyzed, in order to demonstrate their applicability to reduce the likelihood of (auto-)reinfections.

Toothbrushes were intentionally contaminated with standardized suspensions of *Streptococcus mutans* or *Staphylococcus aureus*. Afterwards, the toothbrushes were exposed to rinsing under distilled water, rinsing and drying for 24 h, 0.2% chlorhexidine-based decolonization, or ultraviolet (UV) radiation. The remaining colony forming units were compared with freshly contaminated positive controls. Each experiment was nine-fold repeated. Bi-factorial variance analysis was performed; significance was accepted at $P < 0.05$.

All tested procedures led to a significant reduction of bacterial colonization irrespective of the toothbrush model, the brush head type, or the activity state. Chlorhexidine-based decolonization was shown to be superior to rinsing and slightly superior to rinsing and drying for 24 h, while UV radiation was similarly effective as chlorhexidine. UV radiation was slightly less prone to species-dependent limitations of its decolonizing effects by bristle thickness of toothbrushes than chlorhexidine.

Reduction of bacterial colonization of toothbrushes might reduce the risk of maintaining bacterial infections of the upper respiratory tract. Accordingly, respective procedures are advisable, particularly as they are cheap and easy to perform.

Keywords: toothbrush, bacterial colonization, decolonization, *Streptococcus mutans*, *Staphylococcus aureus*, oral hygiene

Introduction

The most important and most frequently applied tool for everyday dental care is the toothbrush. In this context, electric toothbrushes are more and more frequently used for mechanical reduction of dental plaque, because they show superior cleaning efficiency in comparison to traditional manual toothbrushes [1–3]. Clear differences of the mechanical efficiencies of the rotation/oscillation-based or the sonic-based technical approach have not yet been demonstrated [4–6].

Toothbrushes are usually used for several weeks or

even months, leading to colonization with the oral bacterial flora of the user including facultative pathogenic bacteria [7–10]. Continuous re-exposition by tooth brushing may maintain persisting oral infections or lead to (auto-)reinfections. Accordingly, changing the toothbrush at least once a month is advisable [9]. Oral infections due to tooth brushing-associated lesions of the oral mucous membrane have been repeatedly described [11, 12]. Accordingly, there might be a – however minimal – risk of infection due to tooth brushing, which could be further minimized by reducing the bacterial colonization on toothbrushes.

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Data on bacterial colonization of toothbrushes are scarce, particularly for electric toothbrush heads. Effects of automated brushing and sonic-based toothbrushes on the bacterial colonization of the brush heads are unknown. It is further unclear, whether easy-to-perform methods like rinsing, rinsing and drying, application of disinfectants, or UV radiation after usage of manual and electric toothbrushes might effectively reduce their bacterial colonization.

The study assessed whether simple chlorhexidine disinfection or even rinsing with tap water might reduce bacterial colonization on electric toothbrushes. Different types of toothbrushes were included into the analysis. In a further approach, the decolonizing effects of UV toothbrush sanitizers on manual and sonic toothbrushes were assessed. UV-C radiation is known to be suitable to reduce bacterial colonization on toothbrush heads [13–16]. Finally, the effect of bristle thickness on the effects of decolonizing procedures was assessed comparing two manual toothbrushes.

Methods

Bacterial suspensions

Staphylococcus aureus (ATCC 29212; American Typ Culture Collection, Manassas, Virginia, USA) and *Streptococcus mutans* (DSM 20532; German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) were used. The strains were grown to stationary growth phase in 10 ml sterile brain heart infusion (BHI) broth (Bacto™, BD, Heidelberg, Germany). These two suspensions (adjusted to McFarland 0.5 using BHI broth) were diluted 1 : 100 in sterile 0.9% sodium chloride solution and distributed to aliquots prior to the experiments.

Comparison of bacterial colonization of three electric toothbrushes depending on toothbrush model, state of activity, brush head, as well as decolonization by rinsing, rinsing with consecutive drying, and chlorhexidine treatment

Toothbrushes

Three electric toothbrushes were included, comprising the rotation/oscillation-based Oral-B Professional Care 8000 (Braun, Kronberg/Taunus, Germany), the sonic toothbrush Oral-B Sonic Complete (Braun, Kronberg/Taunus, Germany), and the sonic toothbrush Philips Sonicare Flex-Care (Philips Deutschland GmbH, Hamburg, Germany) (Fig. 1).

Contamination procedure

The electric toothbrushes were dipped into the bacterial suspensions and activated for 2 min. Both activated and inactivated states were assessed. Each bacterial suspension volume was used for one experiment only (Fig. 1).

Experimental procedures to reduce the bacterial load

Four different experimental settings were assessed with 9-fold repeats. The positive controls were analyzed directly after the above described contamination procedure. The tested procedures comprised rinsing under distilled water, rinsing and consecutive air-drying for 24 h at ambient temperature, and applying 0.2% chlorhexidine (Fig. 1).

Quantification of bacterial colonization

Each contaminated toothbrush was shaken and sonicated (Bandelin Electronic UW2200) in 20 ml brain heart infusion broth to remove adhesive bacteria. 10-fold dilution steps to a dilution of 1 : 10⁸ were pipetted, and 100 µl of each dilution was streaked onto Columbia sheep blood agar plates (supplemented with 5% sheep blood, BD) and incubated at 36 °C in a CO₂-enriched atmosphere for 24 h. Colonies were counted, and bacterial load was calculated. Bacterial load was assessed as percentage of the number of bacterial concentration in colony forming units (cfu) per 20 ml (corresponding to one brush head) in relation to the cfu per milliliter of the initial bacterial suspension used for contamination (Fig. 1).

Statistical analysis

All experiments were performed with 9-fold repeats. The freely available (www.r-project.org) open-source software R (version 2.6) was used for statistical analysis. The influences of the toothbrush models and the modes decolonization on the density of isolated bacteria were analyzed by bi-factorial variance analysis. Significance was accepted at $P < 0.05$.

Decolonizing effects of storage in UV toothbrush sanitizers in comparison with chlorhexidine treatment and rinsing with consecutive drying

Toothbrushes

A manual toothbrush Oral B Flex plus intermediate (Braun, Kronberg/Taunus, Germany) and a sonic toothbrush Philips Sonicare (Philips Deutschland GmbH, Hamburg, Germany) were chosen for these experiments.

Experimental decolonization settings

Artificial contamination with *S. mutans* and *S. aureus* as well as decolonization by chlorhexidine treatment and rinsing with consecutive drying was performed as described above. The Philips Sonicare sonic toothbrush was activated during the contamination procedure. In addition, two further decolonization protocols based on UV radiation were added, including exposure to UV-C radiation for 10 min of the Philips Sonicare in a Philips UV toothbrush sanitizer (Philips Deutschland GmbH,

Fig. 1. Flow chart of the experiments

Hamburg, Germany) and of the Oral B Flex plus intermediate manual toothbrush in a VIO light UV toothbrush sanitizer (Violife Inc., New York, USA) according to the manufacturers' instructions. Assessment of bacterial growth including nine-fold repeats and statistical analysis were performed as described above. Residual bacterial growth after decolonization was assessed in percent of growth of the positive control samples without decolonization.

Comparison of bristle thickness of toothbrushes on decolonizing effects of rinsing with consecutive drying, chlorhexidine treatment, chlorhexidine spraying, and UV-C radiation

Toothbrushes

The manual toothbrushes Dr. Best Flex Plus intermediate (GlaxoSmithKline Consumer Healthcare Ltd., Bühl,

Germany) and Dr. Best Flex Plus hard (GlaxoSmithKline Consumer Healthcare Ltd., Bühl, Germany) were chosen for these experiments.

Experimental decolonization settings

Artificial contamination with *S. mutans* and *S. aureus* as well as decolonization by chlorhexidine treatment, rinsing with consecutive drying, and UV-C radiation using a VIO light toothbrush sanitizer (Violife Inc., New York, USA) according to the manufacturers' instructions were performed as described above. In addition, decolonization using five sprays of Chlorhexamed Forte 0.2% spray (GlaxoSmithKline Consumer Healthcare Ltd., Bühl, Germany) containing 0.2% chlorhexidine was assessed. Assessment of bacterial growth including nine-fold repeats and statistical analysis were performed as described above. Residual bacterial growth after decolonization was assessed as percentage in comparison to the initial bacterial load of the broth used for contamination as well as in comparison to the maximum contamination rate of the positive control samples without decolonization.

Results

Comparison of bacterial colonization of three electric toothbrushes depending on toothbrush model, state of activity, brush head type, as well as decolonization by rinsing, rinsing with consecutive drying, and chlorhexidine treatment

Colonization with *S. mutans*

Toothbrush model (in active state), state of activity, and brush head type showed no significant effects on the colonization density with *S. mutans* (data not shown). In inactive state, highest colonization densities were observed on the sonic toothbrush Sonic Complete with a median of 1.3% (in comparison to the initial bacterial suspension used for contamination) lowest colonization on the rotating/oscillating Oral B Professional Care 8000 tooth-

brush with a median of 0.0% and an intermediate colonization for the sonic toothbrush Sonicare FlexCare with a median of 0.4% ($P < 0.01$). On inactive toothbrushes, highest *S. mutans* concentrations were observed directly after the contamination procedure. All decolonization schemes led to a significant reduction of bacterial density, with chlorhexidine demonstrating the most prominent effects. In detail, a median of 8.9% (in comparison to the initial bacterial suspension used for contamination) was detected after contamination (corresponding to 100% colonization). The bacterial load declined to 3.1% (corresponding to 34.8% of the maximum contamination rate) after rinsing and to 0.0% (corresponding to 0.0% of the maximum contamination rate) after rinsing and drying as well as after chlorhexidine treatment. However, a more pronounced scattering of the measured colony numbers after rinsing and drying led to a significance in favor of the chlorhexidine treatment ($P < 0.001$). Nevertheless, the latter effect is unsure, because rinsing and drying showed statistically identical decolonizing effects compared with chlorhexidine on active toothbrushes ($P = 0.72$) (Table 1).

Colonization with *S. aureus*

Toothbrush model in inactive state, state of activity, and different types of brush head did not result in significant effects on the measured *S. aureus* densities after contamination (data not shown). The sonic toothbrush Sonic Complete showed a significantly more dense colonization than the sonic toothbrush Sonicare FlexCare with a bacterial load of 3.3% vs. 0.6%, while an intermediate density of 2.0% was observed for the rotating/oscillating Oral B Professional Care 8000 toothbrush ($P < 0.01$). Highest bacterial concentrations were observed after contamination with a median of 18.9% (corresponding to 100% colonization), followed by rinsing with 7.6% (corresponding to 40.0% of the maximum contamination rate), rinsing and drying with 0.9% (corresponding to 4.8% of the maximum contamination rate), and chlorhexidine treatment with 0.3% (corresponding to 1.6% of the maximum contamination rate) ($P < 0.0001$) (Table 2).

Table 1. Summarized results of the experiments with *Streptococcus mutans*

		Chosen contaminant <i>Streptococcus mutans</i>	
State of activity of the toothbrush		Off	On
Effect of decolonization procedures	Statistics	Significant	Significant
	Effectiveness of the procedures	Chlorhexidine treatment > rinsing and drying > rinsing > no treatment	Chlorhexidine treatment = rinsing and drying > rinsing > no treatment
Toothbrush model	Statistics	Significant	Not significant
	Lowest number of isolates	Oral-B Professional Care < Sonicare FlexCare < Oral-B Sonic Complete	–
Brush heads	Statistics	Not significant	Not significant

Table 2. Summarized results of the experiments with *Streptococcus aureus*

		Chosen contaminant <i>Streptococcus aureus</i>	
State of activity of the toothbrush		Off	On
Effect of decolonization procedures	Statistics	Significant	Significant
	Effectiveness of the procedures	Chlorhexidine treatment > rinsing and drying > rinsing > no treatment	Chlorhexidine treatment > rinsing and drying > rinsing > no treatment
Toothbrush model	Statistics	Not significant	Significant
	Lowest number of isolates	–	Sonicare FlexCare < Oral-B Professional Care < Oral-B Sonic Complete
Brush heads	Statistics	Not significant	Not significant

Decolonizing effects of storage in UV toothbrush sanitizers in comparison with chlorhexidine treatment and rinsing with consecutive drying

The toothbrush model did not influence the colonization due to the contamination procedure (data not shown).

*Colonization with *S. mutans**

Compared with the positive controls without decolonization, rinsing and drying preserved a median of 0.2% *S. mutans* growth while chlorhexidine treatment and both UV toothbrush sanitizers led to a reduction of *S. mutans* growth to a median of 0.0%. While rinsing and drying showed worse decolonizing effects in comparison with all other decolonizing procedures ($P < 0.001$), there was no measurable difference between chlorhexidine treatment and UV radiation ($P > 0.38$) (Table 3).

*Colonization with *S. aureus**

Compared with the positive controls without decolonization, rinsing and drying preserved a median of 0.2% *S. aureus* growth while chlorhexidine treatment and both UV toothbrush sanitizers led to a reduction of *S. aureus* growth to a median of 0.0%. While rinsing and drying showed worse decolonizing effects in comparison with all other decolonizing procedures ($P < 0.01$), again there was

no measurable difference between chlorhexidine treatment and UV radiation ($P > 0.19$) (Table 3).

Comparison of bristle thickness of toothbrushes on decolonizing effects of rinsing with consecutive drying, chlorhexidine treatment, chlorhexidine spraying, and UV-C radiation

*Colonization with *S. mutans**

Compared with the positive controls without decolonization, rinsing and drying preserved a median of 0.1% *S. mutans* growth while chlorhexidine treatment and chlorhexidine spraying as well as UV-C radiation led to a reduction of *S. mutans* growth to a median of 0.0%. While rinsing and drying showed worse decolonizing effects in comparison with all other tested decolonizing procedures ($P < 0.01$), there was no measurable difference between either chlorhexidine-based procedures or UV radiation ($P > 0.34$). Measurable differences between toothbrushes with intermediate and with hard bristles were not detected ($P = 0.68$).

*Colonization with *S. aureus**

Compared with the positive controls without decolonization, rinsing and drying preserved a median of 0.3%

Table 3. Decolonizing effects of UV radiation, chlorhexidine treatment, and rinsing with consecutive drying on manual toothbrushes and sonic toothbrushes

		Chosen contaminant <i>Staphylococcus aureus</i> and <i>Streptococcus mutans</i>	
Type of toothbrush		Manual toothbrush	Sonic toothbrush
Effect of decolonization procedures	Statistics	Significant	Significant
	Effectiveness of the procedures	UV radiation = chlorhexidine treatment > rinsing and drying > no treatment	UV radiation = chlorhexidine treatment > rinsing and drying > no treatment
Brush heads	Statistics	Not significant	Not significant

Table 4. Effects of bristle thickness on decolonizing effects after contamination of manual toothbrushes with *Staphylococcus aureus*

	Effects of bristle thickness on decolonizing effects after contamination of manual toothbrushes with <i>Staphylococcus aureus</i>		Significance level
	Intermediate bristles	Hard bristles	
Rinsing with consecutive drying	No difference		$P = 0.4$
Chlorhexidine treatment	Better	Worse	$P < 0.01$
Chlorhexidine spraying	Better	Worse	$P < 0.01$
UV radiation	No difference		$P = 0.58$

S. aureus growth while both chlorhexidine-based procedures and UV radiation led to a reduction of *S. aureus* growth to a median of 0.0% on brush heads with intermediate bristles. In contrast, rinsing and drying of brush heads with hard bristles preserved a median of 0.4% *S. aureus* growth, chlorhexidine treatment a median of 0.2%, chlorhexidine spraying a median of 0.3%, and UV radiation a median of 0.1% compared with the positive controls without decolonization. Accordingly, for toothbrushes with intermediate bristles, rinsing and drying showed worse decolonizing effects in comparison with all other decolonizing procedures ($P < 0.05$), while there was no measurable difference between the chlorhexidine-based procedures and UV radiation ($P > 0.35$). For toothbrushes with hard bristles, in contrast, UV radiation alone scored better than rinsing with consecutive drying ($P < 0.001$), while there was no statistical difference between all other decolonizing procedures ($P > 0.05$). For *S. aureus* contaminations, both chlorhexidine-based procedures were more efficient for toothbrushes with intermediate bristles than for toothbrushes with hard bristles ($P < 0.01$), while there was no such difference for rinsing with consecutive drying or UV radiation ($P > 0.4$) (Table 4).

Discussion

Toothbrushes are colonized by a variety of different microorganisms [9, 10, 17–19]. Two species with relevant importance for dentistry were chosen for the here described *in vitro* study. *S. mutans* has previously been shown to be etiologically relevant for the initiation and progression of caries [20, 21], while the facultative pathogen *S. aureus* was repeatedly isolated from brush heads in previous studies [9, 15, 22–24].

Decolonizing effects of rinsing, rinsing and drying for 24 h, and chlorhexidine treatment could be demonstrated after artificial contamination of electric toothbrushes with *S. mutans* and *S. aureus*. Although rinsing and drying for 24 h was similarly effective as chlorhexidine treatment alone, particularly after contamination with *S. mutans*, tooth brushing several times per day will usually not allow 24-hour drying periods. Reductions of this drying period would, however, presumably reduce the decolonizing effect, because the duration of drying is positively correlated

with this effect [17]. For *S. aureus*, chlorhexidine treatment was superior to both rinsing and rinsing with consecutive drying. These data are in line with previous analyses [18, 19]. Additional trials with UV radiation for 10 min with a manual toothbrush and a sonic toothbrush did not show differences between chlorhexidine treatment and UV radiation while rinsing with consecutive drying scored worse. These data are in line with previous experiments, suggesting decolonizing effects of UV radiation on bacterial colonization of brush heads [13–16]. Exposure of used brush heads to UV radiation in manufacturer-designed devices might be a slightly more convenient procedure than chlorhexidine treatment with comparable good decolonizing effects.

Significant differences between sonic toothbrushes and rotating/oscillating toothbrushes regarding colonization with oral bacteria could not be demonstrated for the analyzed models Oral B Professional Care 8000, Oral B Sonic Complete and Philips Sonicare FlexCare. Previous data suggesting a potential superiority of sonic toothbrushes [25] could not be confirmed by our study. The brush head type did not show a significant effect either.

Potential effects of bristle thickness of toothbrushes on decolonizing procedures seem to be species dependent. Chemical decolonization by chlorhexidine-based approaches may be slightly more vulnerable to the effects of bristle thickness than mechanical- and radiation-based decolonization.

However, artificial contaminations do not reflect the mode of colonization of toothbrushes *in vivo*, a limitation of this study. Accordingly, respective studies under real-life conditions should follow.

Conclusions

Toothbrushes are a known reservoir for facultative pathogenic bacteria and should be thoroughly cleaned after each use. UV radiation and chlorhexidine-based approaches are more efficient than rinsing with consecutive drying, while chlorhexidine application is slightly more vulnerable to interfering effects of bristle thickness. Neither the brush head type nor sonic- or rotation/oscillation-based technology showed a significant effect on the colonization density.

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