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## REVIEW

# Brushing without brushing?—a review of the efficacy of powered toothbrushes in noncontact biofilm removal

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## Abstract

**Objectives** The aim of the present review was to analyze the impact of the hydrodynamic effects created by powered toothbrushes on biofilm removal in vitro.

**Materials and methods** A MEDLINE search was performed for publications published by 20 May 2012; this search was complemented by a manual search. The study selection, data preparation, and validity assessment were conducted by two reviewers.

**Results** Sixteen studies were included. The studies differed with respect to the methods of biofilm formation and brushing protocols. Eighteen different powered toothbrush models were evaluated. Toothbrushes with side-to-side action demonstrated biofilm removal without direct bristle contact to biofilms ranging from 38 to 99 %. Most studies found biofilm removal exceeding 50 %. Biofilm reduction using multidimensional toothbrushes was significantly lower than by those with the side-to-side mode. Detachment forces due to hydrodynamic phenomena, passing air–liquid interfaces, and acoustic energy transfer were suggested to cause reduction of the biofilm.

**Conclusion** Noncontact biofilm reduction was obtained by the hydrodynamic effects of some powered toothbrushes in vitro.

**Clinical relevance** Powered toothbrushes may have the potential to simplify self-performed oral hygiene. However, additional beneficial effects of higher amounts of noncontact biofilm removal in vitro have not been shown clinically, yet.

**Keywords** Powered toothbrush · Biofilm · Hydrodynamic effect · Oral hygiene · Preventive dentistry

## Introduction

Periodontitis is a multifactorial disease caused by infection with pathogenic bacteria organized in a biofilm. A biofilm is a complex microbial structure on a solid wet surface protecting the microorganisms from the host's immune system and therapeutic measures, including mouth rinsing with antiseptics or antibiotics. Mechanical means are likely to be more predictable in biofilm removal but may still leave biofilm behind [1].

Removal of the supra- and subgingival biofilm is, therefore, a main concern of both preventive and therapeutic measures. Optimal oral hygiene performed by the patient is an important goal of periodontal therapy to establish periodontal health in the long term [2].

A major problem related to self-performed oral hygiene is the cleaning of the interdental areas [3]. While different methods exist, including sticks, dental floss, and interdental brushes, the use of interdental brushes seems currently to be the most efficient method [4–9]. Noncompliance with interdental hygiene measures, however, is a key problem in self-performed oral hygiene [10]. Inadequate compliance in turn is associated with progression of periodontal diseases

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[11–13]. Time spent on oral hygiene as well as the use of interdental brushes were negatively correlated with further tooth loss in treated periodontitis patients [14].

Powered toothbrushes have been developed to improve and facilitate oral hygiene [15, 16]. Various toothbrush modes of action, including multidimensional and side-to-side action, exist and were classified in two recent systematic Cochrane reviews [17, 18].

Bristle motion at a high frequency may generate turbulent fluid flows in the oral cavity. The fluid flow causes hydrodynamic forces in terms of wall shear forces acting parallel to a surface [19, 20]. The amount of entrained air bubbles correlates with increasing fluid motions. The passage of air bubbles along a substratum creates thermodynamic surface tension forces and turbulent flow depending on the gas fraction, the velocity, and the diameter of the bubbles entrained [21–24]. The vibration of toothbrush bristles may further enable energy transfer in terms of sound pressure waves [25]. In addition, the acoustic energy is thought to increase shear forces due to an oscillation of entrapped air bubbles [26]. The interaction of these effects created by powered toothbrushes is suggested to remove biofilms without bristle contact [27].

The aim of the present review was to evaluate the current evidence regarding the efficacy of powered toothbrushes in noncontact biofilm removal in *in vitro* studies.

## Methods

### Outcome variables

The primary outcome variable was the quantification of biofilm removal following application of a powered toothbrush without contact to the surface, *i.e.*, to the biofilm, *in vitro*. The secondary outcome variables considered the viability of biofilm-associated bacteria and hydrodynamic phenomena occurring during noncontact application.

### Literature search

A MEDLINE search (PubMed) of resources published prior to 20 May 2012 was conducted using the validated keywords “powered” and “toothbrush” or “powered” and “toothbrushes” or “electric” and “toothbrush” or “electric” and “toothbrushes” or “sonic” and “toothbrush” or “sonic” and “toothbrushes” or “ultrasonic” and “toothbrush” or “ultrasonic” and “toothbrushes.” Further databases beside MEDLINE were not reviewed. Any relevant work published in the English language and presenting pertinent information about the described outcome variables was

considered for inclusion in the review. Moreover, the references of studies examined for inclusion were thoroughly analyzed searching for further studies. However, there may be other evidence but for convenience the search was limited to English publications and the grey literature was not actively searched.

### Validity assessment

One reviewer (J. S.) screened the abstracts of the search results for compliance with the inclusion criteria. The full texts of studies with questionable potential for inclusion after the abstracts were read were further analyzed. A second reviewer (C. W.) reconsidered included studies as well as exclusions. Disagreements were clarified by discussion between the two reviewers.

## Results

### Study characteristics

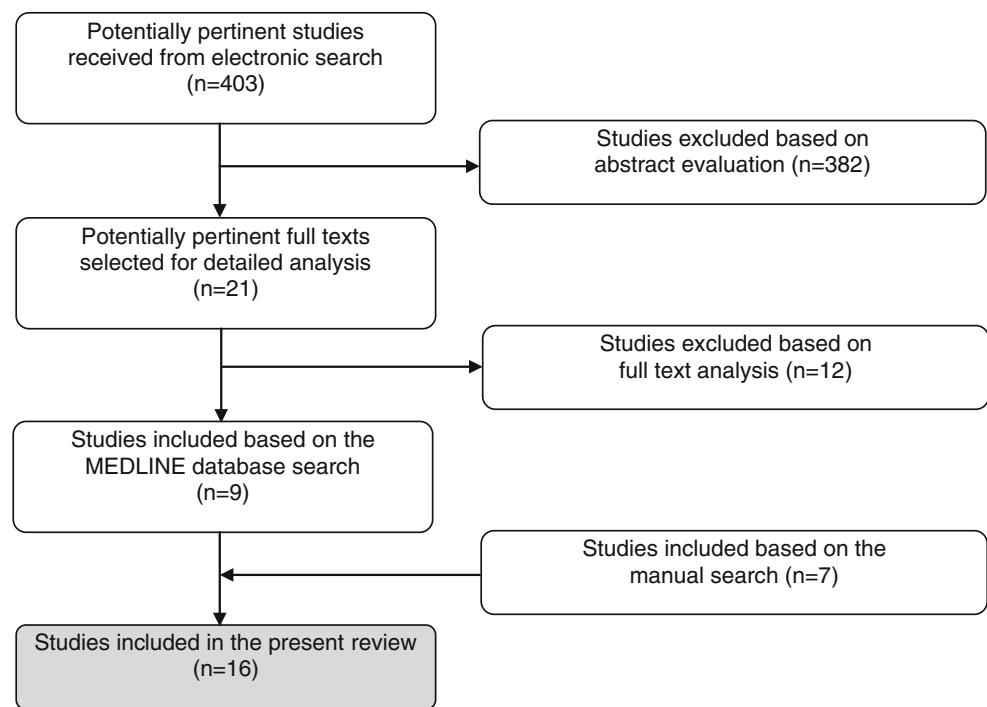
The combinations of the search terms resulted in a list of 403 titles. Abstracts were first screened to identify possible relevant publications. Of these 403 initial titles, 382 were excluded following evaluation of the abstracts. The causes of exclusion were as follows:

- Review articles (72 publications),
- Clinical studies (254 publications),
- Animal studies (five publications),
- Comments (five publications),
- Case reports (three publications),
- *In vitro* studies without data on noncontact biofilm removal (43 publications).

Full-text analysis was performed on the remaining 21 publications [15, 22, 25, 26, 28–44]. Twelve publications were further excluded for the following reasons:

- No noncontact brushing [32]
- Use of nonmicrobial substitute for biofilm [29, 31, 33, 36, 38]
- Use of planktonic bacteria [34, 35]
- No quantification of biofilm removal [30]
- No powered toothbrushes [22, 26]
- Review article [15]

Finally, a total of nine articles from the electronic search of the MEDLINE database were included [25, 28, 37, 39–44]. Seven additional publications were identified by screening the references of the studies evaluated for inclusion [27, 45–50] (Fig. 1). Finally, 16 studies were integrated in the present review.

**Fig. 1** Selection process of the studies included

## Analysis of the included studies

### Microbial aspects and biofilm formation

**Substratum** Synthetics and substrates derived from animals or humans were utilized for bacterial adhesion and biofilm formation. Titan [45], glass and quartz [25, 27, 44, 46, 49], hydroxyapatite [28, 40–43, 47], bovine enamel sections [48], and human enamel sections from noncarious molars [37, 39] were used in the experiments (Table 1).

**Bacteria** The biofilm formation was conducted in vitro [25, 27, 28, 44, 46, 48, 49], in vivo [37, 39] and ex vivo [40–43, 47]. Whereas the identity of bacteria and their applied concentrations in in vitro biofilms were known, these parameters were not precisely defined in in vivo and ex vivo biofilms.

In vitro studies primarily formed monospecies biofilms using different *Streptococcus mutans* strains (Ingbritt, UA 159, ATCC 700610, OSP 236, and NS) [27, 28, 44, 48, 50]. The bacteria used in monospecies biofilms were *Porphyromonas gingivalis* (ATCC 33277), *Actinomyces naeslundii* (T14V), *Streptococcus oralis* (J22), *Lactobacillus acidophilus* (DSP 211), *Streptococcus salivarius* (DSP 248), and *Veillonella alcalescens* (DSP 203) [48].

In vitro dual-species biofilms were created by sequential inoculation of *A. naeslundii* (T14V) and *S. oralis* (J22) or *A. naeslundii* (T14V) and *Streptococcus sanguis* (PK 1889) [25, 46, 49].

Additionally, multispecies biofilms were formed, using human whole saliva as the inoculum [40–43, 47] in an ex

vivo model. Saliva samples were collected from 10 healthy volunteers.

In contrast, Stanford et al. used biofilms formed in vivo [37, 39]. All biofilm samples were obtained from two probands who continuously wore a palatal prosthesis with fixed enamel sections overnight.

**Model biofilm system** The (acquired) pellicle is important for in vitro and ex vivo biofilms. The use of pooled human saliva is documented [25, 46, 49]. Human saliva was collected from ten healthy volunteers and incubated with the substratum for 16 h. Additionally, saliva was supplementally added to the circulating bacterial suspension.

The simultaneous flow of saliva and inoculum is described by Hope et al. [40–43]. Briefly, the flow of human saliva containing oral bacteria started concurrently with artificial saliva and was continued for 8 days. Artificial saliva consisted of hog gastric mucin without urea. Another type of artificial saliva used for pellicle formation comprised sterile 2.5 % bovine mucin [28, 48] and 10 % fetal calf serum [45]. The incubation periods lasted 0.5, 12, and 1 h. A few studies failed to include pellicle formation in their biofilm model [27, 44].

In vitro and ex vivo model biofilm systems may be differentiated into static and dynamic systems. Static biofilms were established in multiwell plates. Exclusively, monospecies biofilms were developed [45, 48]. Incubation periods varied from 1 to 48 h. Dynamic biofilm systems imitate the flow rate and shear forces caused by the dynamic flow of saliva in the oral cavity.

**Table 1** Biofilm models

First author (year of publication)	Bacteria	Substratum	Biofilm model characteristics	Pellicle formation (medium, duration)	Incubation of bacteria (medium, duration)	Specifics
Wu-Yuan et al. (1994) [45]	(a) <i>Streptococcus mutans</i> Ingbritt (b) <i>Porphyromonas gingivalis</i> ATCC 3327 (c) <i>Actinomyces naeslundii</i> T14V	(a) Titanium (b) Titanium (c) HA	In vitro static	(a) –	(a) Brain–heart infusion broth+ 2 % sucrose (48 h)	2 probands wear palatal prosthesis with fixed enamel sections 1 proband wear palatal prosthesis with fixed enamel sections 4-channel drip-flow reactor system
				(b) 10 % fetal calf serum (1 h)	(b) PG broth (1 h)	
				(c) Saliva (overnight)	(c) Carbonate buffer+0.23 mg/ml fluorescein isothiocyanate isomer I (1 h)	
Stanford et al. (1997) [37]	Individual mix of intraoral bacteria	Human enamel sections	In vivo	Human saliva	16 h	
Stanford et al. (2000) [39]	Individual mix of intraoral bacteria	Human enamel sections	In vivo	Human saliva	16 h	
Adams et al. (2002) [27]	<i>S. mutans</i> UA 159	Glass	In vitro static and dynamic	–	Brain–heart infusion broth+2 % sucrose+overnight culture of <i>S. mutans</i> (3 h attachment period (static))+48 h growth period (dynamic)) Human saliva+artificial saliva (8 days)	Constant-depth film fermenters
Hope et al. (2002) [40]	Bacteria of human saliva pool	HA	Ex vivo dynamic	Human saliva (samples from 20 volunteers, 20–40 years, good oral health, nonsmokers and smokers)+artificial saliva (hog gastric mucin without urea), during bacteria incubation (8 days)	Human saliva+artificial saliva (8 days)	Constant-depth film fermenters
Hope et al. (2003) [41]	Bacteria of human saliva pool	HA	Ex vivo dynamic	Human saliva (samples from 20 volunteers, 20–40 years, good oral health, nonsmokers and smokers)+artificial saliva (hog gastric mucin without urea) during bacteria incubation (8 days)	Human saliva+artificial saliva (8 days)	Constant-depth film fermenters
Hope et al. (2003) [42]	Bacteria of human saliva pool	HA	Ex vivo dynamic	Human saliva (samples from 20 volunteers, 20–40 years, good oral health, nonsmokers and smokers)+artificial saliva (hog gastric mucin without urea) during bacteria incubation (8 days)	Human saliva+artificial saliva (8 days)	Constant-depth film fermenters
Heersink et al. (2003) [44]	<i>S. mutans</i> ATCC 700610	Glass	In vitro static and dynamic	–	Brain–heart infusion broth+2 % sucrose+overnight culture of <i>S. mutans</i> (24 h attachment period (static))+48 h growth period (dynamic)) Adhesion buffer (10 min)	4-channel drip-flow reactor system Parallel-plate flow chamber
Busscher et al. (2003) [46]	(a) <i>A. naeslundii</i> T14V-J1 and <i>Streptococcus oralis</i> J22	Quartz	In vitro dynamic	Human saliva (samples from 10 volunteers, good oral health) dissolved at 1.5 mg/ml in adhesion buffer (16 h)		

**Table 1** (continued)

First author (year of publication)	Bacteria	Substratum	Biofilm model characteristics	Pellicle formation (medium, duration)	Incubation of bacteria (medium, duration)	Specifics
	(b) <i>A. naeslundii</i> T14V-J1 and <i>Streptococcus sanguis</i> PK1889				<i>A. naeslundii</i> (in adhesion buffer, $1 \times 10^8$ bacteria/ml) until surface coverage of $1 \times 10^6$ bacteria/cm <sup>2</sup> Adhesion buffer (10 min) (a) <i>S. oralis</i> (in adhesion buffer+ 1.5 g/l lyophilized human saliva, $3 \times 10^8$ bacteria/ml; 2 h) (b) <i>S. sanguis</i> (in adhesion buffer +1.5 g/l lyophilized human saliva, $3 \times 10^8$ bacteria/ml; 2 h)	
Yuen et al. (2004) [47]	Bacteria of human saliva pool	HA	Ex vivo dynamic	Human saliva (samples from 20 volunteers, 20–40 years, good oral health)	4–5 days, aerobic conditions	Constant-depth film fermenter
Hope et al. (2005) [43]	Bacteria of human saliva pool	HA	Ex vivo dynamic	Human saliva (samples from 20 volunteers, 20–40 years, good oral health, nonsmokers and smokers) +Artificial saliva (hog gastric mucin without urea) During bacteria incubation (8 days)	Human saliva+artificial saliva (8 days)	Constant-depth film fermenter
Brambilla et al. (2006) [48]	(a) <i>S. mutans</i> OSP 236 (b) <i>Lactobacillus acidophilus</i> OSP 211 (c) <i>Streptococcus salivarius</i> OSP 248 (d) <i>Veillonella atcalescens</i> OSP 403	Bovine enamel sections	In vitro static	Human saliva (samples from 10 volunteers); 12 h	2.5 ml trypticase soy broth+ 100 $\mu$ l of microbial suspension (36 h)	
van der Mei et al. (2007) [49]	<i>A. naeslundii</i> T14V-J1 and <i>S. oralis</i> J22	Quartz	In vitro dynamic	Human saliva (samples from 20 volunteers, good oral health) dissolved at 1.5 mg/ml in adhesion buffer (16 h)	Adhesion buffer (10 min) (a) <i>A. naeslundii</i> (in adhesion buffer or in adhesion buffer+ 1.5 g/l lyophilized human saliva, $1 \times 10^8$ bacteria/ml) until surface coverage of $1 \times 10^6$ bacteria/cm <sup>2</sup> Adhesion buffer (10 min) <i>S. oralis</i> (in adhesion buffer+ 1.5 g/l lyophilized human saliva, $3 \times 10^8$ bacteria/ml; 2 h) (b) <i>S. oralis</i> (in adhesion buffer+ 1.5 g/l lyophilized human saliva, $3 \times 10^8$ bacteria/ml) until surface coverage of $1 \times 10^6$ bacteria/cm <sup>2</sup> Adhesion buffer (10 min)	Parallel-plate flow chamber

Table 1 (continued)

First author (year of publication)	Bacteria	Substratum	Biofilm model characteristics	Pellicle formation (medium, duration)	Incubation of bacteria (medium, duration)	Specifics
Busscher et al. (2010) [25]	<i>A. naeslundii</i> T14V-J1 and <i>S. oralis</i> J22	Glass	In vitro dynamic	Human saliva (samples from 10 volunteers, good oral health) dissolved at 1.5 mg/ml in adhesion buffer (16 h)	<p><i>A. naeslundii</i> (in adhesion buffer or in adhesion buffer+1.5 g/1 lyophilized human saliva, <math>1 \times 10^8</math> bacteria/ml; 2 h)</p> <p>2 % THB-supplemented adhesion buffer (15 min)</p> <p><i>A. naeslundii</i> (in 2 % THB-supplemented adhesion buffer, <math>1 \times 10^8</math> bacteria/ml) until surface coverage of <math>1 \times 10^6</math> bacteria/cm<sup>2</sup></p> <p>Adhesion buffer (15 min)</p> <p><i>S. oralis</i> (in 2 % THB-supplemented adhesion buffer+1.5 g/1 lyophilized human saliva, <math>3 \times 10^8</math> bacteria/ml; 2 h)</p> <p>Adhesion buffer (15 min)</p> <p>100 % THB (16 h (growth period))</p> <p>Adhesion buffer (30 min)</p>	Parallel-plate flow chamber
Verkaik et al. (2010) [50]	(a) <i>S. mutans</i> NS (b) <i>S. oralis</i> J22  (c) <i>A. naeslundii</i> T14V-J1 (d) <i>A. naeslundii</i> T14V-J1 and <i>S. oralis</i> J22  (e) Bacteria of human saliva pool	Glass	(a, b, c, and d) in vitro dynamic (e) Ex vivo dynamic	Human saliva (samples from 20 volunteers, good oral health) dissolved at 1.5 mg/ml in adhesion buffer (16 h)	<p>(a) <i>S. mutans</i> NS (in 2 % THB-supplemented adhesion buffer+1.5 mg/ml lyophilized human saliva, <math>3 \times 10^8</math> bacteria/ml; 2 h)</p> <p>100 % THB (16 h (growth period))</p> <p>(b) <i>S. oralis</i> J22 (in 2 % THB-supplemented adhesion buffer+1.5 mg/ml lyophilized human saliva, <math>3 \times 10^8</math> bacteria/ml; 2 h)</p> <p>100 % THB (16 h (growth period))</p> <p>(c) <i>A. naeslundii</i> (in 2 % SB-supplemented adhesion buffer, <math>1 \times 10^8</math> bacteria/ml; 2 h)</p> <p>100 % SB (16 h (growth period))</p> <p>(d) <i>A. naeslundii</i> (in 2 % SB-supplemented adhesion buffer, <math>1 \times 10^8</math> bacteria/ml) until surface coverage of <math>1 \times 10^6</math> bacteria/cm<sup>2</sup></p> <p>Adhesion buffer (30 min)</p> <p><i>S. oralis</i> (in 2 % THB-supplemented adhesion buffer+</p>	Parallel-plate flow chamber

**Table 1** (continued)

First author (year of publication)	Bacteria	Substratum	Biofilm model characteristics	Pellicle formation (medium, duration)	Incubation of bacteria (medium, duration)	Specifics
Roberts et al. (2010) [28]	<i>S. mutans</i> (clinical isolate from the University of Washington)	HA	In vitro static	Sterile 2.5 % bovine mucin (30 min)	1.5 mg/ml lyophilized human saliva, $3 \times 10^8$ bacteria/ml; 2 h 100 % THB (16 h (growth period)) (e) Fresh human saliva (samples from 2 volunteers, good oral health) in adhesion buffer (1:1, $2 \times 10^8$ bacteria/ml; 2 h) 10 % fresh human saliva (same volunteers; 16 h (growth period)) Trypticase soy broth+yeast+20 % sucrose+ <i>S. mutans</i> (48 h)	

HA hydroxyapatite, THB Todd Hewitt broth, SB Schaedlers broth

The formation of multispecies biofilms was performed by means of dynamic systems; the apparatus selected varied from study to study. These included the parallel-plate flow chamber [25, 46, 49], the drip-flow system [27, 44], and the constant-depth film fermenter [40–43, 47]. These devices allow the continuous flow of a bacterial suspension and a nutrient medium. The duration of biofilm formation varied from 3 h to 8 days.

*Brushing protocols*

*Toothbrushes* Wu-Yuan et al. were the first to analyze the influence of a toothbrush with side-to-side action on biofilm reduction by noncontact brushing [45]. Side-to-side toothbrushes were either solely tested [39, 42, 44–46, 48] or compared with powered toothbrushes presenting other modes of action. Comparison with multidimensional toothbrushes was predominant [25, 27, 28, 40, 41, 43, 47, 49, 50]. Stanford et al. investigated a counter oscillation and a side-to-side toothbrush [37]. The most extensive study of powered toothbrushes in terms of different modes was performed by Buscher et al. [25]. The efficacy of a toothbrush with ultrasonic action in comparison to a side-to-side and a multidimensional toothbrush was also studied [28] (Table 2).

*Toothbrush apparatus* The angle of the toothbrush bristles toward the biofilm was 90° in most studies. The bristles were not in contact with any solid surface [25, 28, 37, 39, 44, 46, 48, 49]. Apart from the orientation of the bristles, there was additional movement of the toothbrush in some studies [27, 46, 49].

In addition, an interproximal model was developed to simulate the exposure of interproximal biofilm to powered brushing from the buccal surface [27, 40–43, 47]. The bristles were in direct contact with an artificial tooth surface. Hope et al. quantified the vertical and horizontal load of the bristles to be  $62 \pm 5$  g for the side-to-side toothbrushes in accordance to the manufacturers’ recommendations. The horizontal load of the multidimensional toothbrushes amounted to  $150 \pm 10$  g, while the vertical load was minimal [40–43]. The angle of the toothbrush head to teeth was 45° and 40° for side-to-side toothbrushes and 90° for multidimensional toothbrushes, respectively.

*Medium* The analysis of biofilm removal by hydrodynamic phenomena requires a liquid in which to immerse the toothbrush bristles and to imitate the mix of saliva, water, and toothpaste in the oral cavity.

For this purpose, phosphate-buffered saline solution [37, 40–43], phosphate-buffered saline solution supplemented with artificial saliva (i.e., hog gastric mucin) [37, 40–43], adhesion buffer [25, 46, 49], sterile Ringer solution [27],

Table 2 Brushing protocols

First author (year of publication)	Toothbrush	Distance (mm)	Time (s)	Control	n/condition	Toothbrush apparatus	Medium	Analysis method	Outcome variables	Results
Wu-Yuan et al. (1994) [45]	Sonicare <sup>a</sup>	(a) 0–2–4	(a) 15	Untreated substrate	(a) 3	90° (bristles to substrate)	PBS	(a) SEM	(a) Remaining bacteria/mm <sup>2</sup> (mean±SD)→ calculation of % removal	Significant more biofilm removal for all conditions compared with control (except for <i>Porphyromonas gingivalis</i> at 4 mm and 15 s and <i>Actinomyces naeslundii</i> at 4 mm and 5 s)* Decrease of biofilm removal with increasing distance Decrease of biofilm removal with shorter exposure time (major removal within 5 s)
		(b) 1–2–3–4	(b) 5–15–30		(b) 8	Manual movement (±3 mm, perpendicular to bristle movement, 2×/5 s) No contact	Short bristles 2 mm immersed	(b) Fluorescence microscope	(b) Reduction in fluoresce→calculation of % removal (mean±SD)	Removal of <i>Streptococcus mutans</i> —61 % (4 mm, 15 s) to 100 % (0 mm, 15 s) Removal of <i>P. gingivalis</i> —24 % (4 mm, 15 s) to 100 % (0 mm, 15 s) Removal of <i>A. naeslundii</i> : 24 % (4 mm, 15 s) to 87 % (1 mm, 15 s) Biofilm removal at distances >2 mm appears to vary with characteristics of bacteria
Stanford et al. (1997) [37]	Sonicare <sup>a</sup>	0–2–3 (side-to-side toothbrush)	5–10–15 (side-to-side toothbrush)	Untreated substrate	12 (side-to-side toothbrush)	90° (bristles to substrate)	PBS	SEM	Remaining log <sub>10</sub> of CFU/mm <sup>2</sup> (mean±SD)→calculation of % removal	Side-to-side toothbrush
	Interplak <sup>b</sup>	3 (counter oscillation toothbrush)	10 (counter oscillation toothbrush)		29 (counter oscillation toothbrush)	No movement	15 ml	Cultivation		Impact of distance—decrease of biofilm removal with increasing distance (significant at 5 s* and nonsignificant at 10 and 15 s)



**Table 2** (continued)

First author (year of publication)	Toothbrush	Distance (mm)	Time (s)	Control	n/condition	Toothbrush apparatus	Medium	Analysis method	Outcome variables	Results
Stanford et al. (2000) [39]	Sonicare <sup>a</sup>	0–2–3	5–15	Untreated substrate	10	90° (bristles to substrate)	Saliva+tap water	Cultivation	Remaining CFU/mm <sup>2</sup> → calculation of % removal (mean±SD)	Removal—58 (3 mm, 5 s) to 95 % (0 mm, 5 s) and 76 (3 mm, 15 s) to 95 % (0 mm, 15 s)
										No contact
Adams et al. (2002) [27]	Sonicare Elite <sup>d</sup>	7.5 (mean)	1.5	Untreated substrate	9	Brush head to tooth —45° (side-to-side toothbrush)	Ringer buffer	Live/dead staining	Biofilm thickness (mean±SD) →	Decrease of biofilm removal with shorter exposure time at 3 mm*
										Counter oscillation toothbrush—no biofilm removal
Adams et al. (2002) [27]	Manual Oral-B toothbrush <sup>c</sup>					Manual movement (10 or 30 mesial–distal strokes)				Decrease of biofilm removal with shorter exposure time at 3 mm by “on-modus” side-to-side toothbrush
										Differentiation in activated, e.g., “on-modus”, and inactivated, e.g., “off-modus”
Adams et al. (2002) [27]						Bristles in contact (load not defined)				Removal—65 (3 mm, 5 s) to 92 % (0 mm, 5 s) by “on-modus” side-to-side toothbrush and 5 (3 mm, 5 s) to 21 % (0 mm, 5 s) by “off-modus” side-to-side toothbrush
										Significant more biofilm removal at 0–1.5 mm by side-to-side

Table 2 (continued)

First author (year of publication)	Toothbrush	Distance (mm)	Time (s)	Control	n/condition	Toothbrush apparatus	Medium	Analysis method	Outcome variables	Results
	Oral-B 3D Excel <sup>c</sup>					and 90° (multidimensional toothbrush) Interproximal model	30 ml	CLSM	calculation of % removal	toothbrush compared with control*
						No movement	Bristles partially	CCD camera	Cell viability	No significant differences between biofilm thickness of areas exposed to multidimensional toothbrush and control
						Bristles in contact (load not defined)	Immersed		Flow visualization	Significant more biofilm removal by side-to-side toothbrush compared with multidimensional toothbrush* Decrease of biofilm removal with increasing distance by side-to-side toothbrush
										Removal—57 (0–5 mm), 53 (5–10 mm), and 43 % (10–15 mm) by side-to-side toothbrush and 16 (0–5 mm), 13 (5–10 mm), and 19 % (10–15 mm) by multidimensional toothbrush
										No influence on bacterial viability with either toothbrush
										Air bubbles projected through interproximal space
										Velocity of air bubbles and shear stress by side-to-side toothbrush higher than by multidimensional toothbrush
Hope et al. (2002) [40]	Sonicare Elite <sup>d</sup>	1.6 (nearest)	5	–	6	Brush head to tooth—40° (side-to-side toothbrush) and 90°	PBS+0.8 g/l hog gastric mucin	Cultivation	Remaining CFU/mm <sup>2</sup> →calculation of % removal (mean ±95 % CI)	Significant more biofilm removal by “on-modus” toothbrushes compared with “off-modus”*

**Table 2** (continued)

First author (year of publication)	Toothbrush	Distance (mm)	Time (s)	Control	n/condition	Toothbrush apparatus	Medium	Analysis method	Outcome variables	Results
	Oral-B 3D <sup>e</sup>					(multidimensional toothbrush) Interproximal model Differentiation in activated, e.g., “on-modus”, and inactivated, e.g., “off-modus” Movement (0.62 Hz, 9.5 mm) Bristles in contact —62±5 g horizontal and vertical load (side-to-side toothbrush) and 150±10 g horizontal and minimal vertical load (multidimensional toothbrush)	7 ml 7 ml			Significant more biofilm removal by “on-modus” side-to-side toothbrush (32.23 %) compared with “on-modus” multidimensional toothbrush (9.48 %)*
Hope et al. (2003) [41]	Sonicare Plus <sup>d</sup>	2.65 (mean) (side-to-side toothbrush)	5	—	6	Brush head to tooth—40° (side-to-side toothbrush), 90° (multidimensional toothbrush)	PBS+0.8 g/1 hog gastric mucin	Cultivation	Removed CFU/mm <sup>2</sup> —calculation of % removal (mean ±IR)	Significant more biofilm removal by “on-modus” toothbrushes compared with “off-modus” toothbrushes*
	Oral-B D15 <sup>e</sup>	1.62 mm (mean) (multidimensional toothbrush)				Interproximal model Differentiation in activated, e.g., “on-modus”, and inactivated, e.g., “off-modus” Movement (0.62 Hz, 9.5 mm) Bristles in contact: 62±5 g horizontal and vertical load	7 ml			Significant more biofilm removal by “on-modus” side-to-side toothbrush (48.45 %) compared with “on-modus” multidimensional toothbrush (15.86 %)*

Table 2 (continued)

First author (year of publication)	Toothbrush	Distance (mm)	Time (s)	Control	n/condition	Toothbrush apparatus	Medium	Analysis method	Outcome variables	Results
Hope et al. (2003) [42]	Sonicare Plus <sup>d</sup>	2.65 (mean)	15	Untreated substrate	10	(side-to-side toothbrush), 150 ±10 g horizontal and minimal vertical load (multidimensional toothbrush)	PBS+0.8 g/ l hog gastric mucin 7 ml	Cultivation	Removed CFU/mm <sup>2</sup> (mean±95% CI)→calculation of % removal (mean ±95% CI)	Significant more biofilm removal by “on-modus” toothbrushes (73.70%) compared with “off-modus” toothbrushes (3.66%)*
Heersink et al. (2003) [44]	Sonicare <sup>d</sup>	0–0.5–1–1.5	15	Untreated substrate	4	Interproximal model Differentiation in activated, e.g., “on-modus”, and inactivated, e.g., “off-modus” Movement (0.62 Hz, 9.5 mm) Bristles in contact (load of 62±5 g) 90° (bristles to substrate)	Nanopure water	Live/dead staining	Biofilm thickness (mean±SD)→calculation of % removal	Significant more biofilm removal for all distances compared with control* Decrease of biofilm removal with increasing distance
Busscher et al. (2003) [46]	Sonicare <sup>d</sup>	0–2–4–6	20	Handling of flow	3	No contact No contact	Adhesion buffer 7	Digital time-lapse microscope	Cell viability Flow visualization	Removal=20 (1.5 mm) to >99% (0 mm) No influence on bacterial viability after brushing compared with control Localized turbulence with air bubbles and fluid, opaque layer of remaining biofilm after exposure Decrease of biofilm removal with

**Table 2** (continued)

First author (year of publication)	Toothbrush	Distance (mm)	Time (s)	Control	n/condition	Toothbrush apparatus	Medium	Analysis method	Outcome variables	Results
Yuen et al. (2004) [47]	IntelliClean System (Sonicare) <sup>d</sup> Oral-B Professional Care 7000	1–2 mm	15	Untreated substrate Interproximal model Movement Bristles in contact (load not defined)	36	chamber without brushing  Movement (20 single strokes back and forth) No contact (at 0 mm load of <40 g)	7 ml diluted saliva substitute (33 % (v/v) Ringer's)	mm above biofilm or wetted brush (immersed in water once)+ film-wetted biofilm  Phase contrast microscope  CFU/mm <sup>2</sup> in adhering aggregates	cm <sup>2</sup> →calculation of % removal (mean ±SD)	increasing distance of >2 mm More biofilm removal for all conditions compared with control No influence of wetted and immersed states on biofilm removal Removal of <i>A. naeslundii</i> and <i>Streptococcus oralis</i> —33 (6 mm) to 94 % (0 mm), wetted and 14 (6 mm) to 99 % (0 mm), immersed Removal of <i>A. naeslundii</i> and <i>S. sanguis</i> —22 (6 mm) to 92 % (0 mm), wetted and 45 (6 mm) to 99 % (0 mm), immersed Significant more biofilm removal by side-to-side toothbrush (3.7 times) compared with multidimensional toothbrush*
Hope et al. (2005) [43]	Sonicare Plus  Oral-B 3D D17 <sup>c</sup>	2 mm (nearest)	5	—	7 (side-to-side toothbrush)  5 (multidimensional toothbrush)	Brush head to tooth—40° (side-to-side toothbrush), 90° (multidimensional toothbrush) Interproximal model Movement (0.62 Hz, 9.5 mm) and	PBS+0.8 g/l hog gastric mucin  7 ml	Cultivation	Removed CFU/ml (mean±SD) and log (CFU/ml; mean±SD)	Significant more biofilm removal by side-to-side toothbrush (32.00 % compared with multidimensional toothbrush (15.31 %))* Comparison to Hope et al. [42]—significant more biofilm removal without supplemented

Table 2 (continued)

First author (year of publication)	Toothbrush	Distance (mm)	Time (s)	Control	n/condition	Toothbrush apparatus	Medium	Analysis method	Outcome variables	Results
Brambilla et al. (2006) [48]	Sonicare Advance <sup>d</sup>	7	5–15–30	Untreated substrate	30	minimal vertical load (multidimensional toothbrush) 90° (bristles to substrate)	PBS	Viable biomass assay (MTT assay)	Optical density (mean±SD)→calculation of % removal	<p>sucrose (48.45 %) compared with supplemented sucrose (32.00 %) by side-to-side toothbrush</p> <p>No influence of sucrose supplementation on biofilm removal by multidimensional toothbrush</p> <p>Significant more biofilm removal of <i>S. mutans</i> and <i>S. sanguis</i> biofilms compared with control* and more biofilm removal with increasing exposure time</p> <p>Reduced removal of <i>L. acidophilus</i> and <i>V. alcalescens</i> biofilms</p> <p>No influence of wetted and immersed states on biofilm removal</p> <p>Significantly more biofilm removal of <i>A. naeslundii</i> and <i>S. oralis</i> sequence* compared with <i>S. oralis</i> and <i>A. naeslundii</i> sequence*</p> <p>Significantly more biofilm removal by contact-brushing compared with noncontact brushing*</p> <p>Significant more biofilm removal by side-to-side toothbrush compared with multidimensional toothbrush under all conditions*</p>
van der Mei et al. (2007) [49]	Sonicare Advance <sup>d</sup> Oral-B 3D Professional Care 7000 <sup>e</sup>	0–2–4	20	Handling of flow chamber without brushing	3	No movement No contact 90° (bristles to substrate) Movement (20 single strokes back and forth)	1 ml Adhesion buffer 7 mm above biofilm or wetted brush (immersed in water once)+ film-wetted biofilm	Spectrophotometer CCD camera Phase contrast microscope	Remaining bacteria/cm <sup>2</sup> →calculation of % removal (mean±SD) % of remaining bacteria in aggregates of ≥10	<p>Reduced removal of <i>L. acidophilus</i> and <i>V. alcalescens</i> biofilms</p> <p>No influence of wetted and immersed states on biofilm removal</p> <p>Significantly more biofilm removal of <i>A. naeslundii</i> and <i>S. oralis</i> sequence compared with <i>S. oralis</i> and <i>A. naeslundii</i> sequence*</p> <p>Significantly more biofilm removal by contact-brushing compared with noncontact brushing*</p> <p>Significant more biofilm removal by side-to-side toothbrush compared with multidimensional toothbrush under all conditions*</p>

**Table 2** (continued)

First author (year of publication)	Toothbrush	Distance (mm)	Time (s)	Control	n/condition	Toothbrush apparatus	Medium	Analysis method	Outcome variables	Results
Busscher et al. (2010) [25]	Oral-B S18 Sonic Complete  Sonicare Elite (Standard Elite brush head)  Sonicare Flexcare (Standard Flexcare brush head) Panasonic EW 1045 (SR18)	1–2–4–6	20	Handling of flow chamber without brushing Movement (20 single strokes back and forth) No contact	3	90° (bristles to substrate)	Adhesion buffer	Live/dead staining  CLSM	Biofilm volumes per unit area→calculation of % removal (mean ±SD)	Decrease of biofilm removal with increasing distances by both toothbrushes Sequence “streptococci in saliva followed by actinomyces in saliva” appeared more difficult to remove and left more large coaggregates than sequence “actinomyces in saliva followed by streptococci in saliva” regardless of toothbrush mode* Multidimensional toothbrush tended to leave more large aggregates than side-to-side toothbrush Decrease of biofilm removal with increasing distance Removal—60 to 78 % (1 mm) by all toothbrushes
									Percentage of live/dead bacteria in the volume→calculation of % viability of biofilms (mean)	31 to 40 % (6 mm) by several side-to-side and multidimensional toothbrushes Expansion, –29 to –86 % (4–6 mm) by several side-to-side and multidimensional toothbrushes No influence on bacterial viability after

Table 2 (continued)

First author (year of publication)	Toothbrush	Distance (mm)	Time (s)	Control	n/condition	Toothbrush apparatus	Medium	Analysis method	Outcome variables	Results
Verkaik et al. (2010) [50]	Care (EB17 brush head) D18 Professional Care (EB18 Pro White brush head)	0–2	20	Handling of flow chamber without brushing	3	90° (bristles to substrate)	No information	Phase contrast microscope with a ultra-long working distance objective	Fractional surface coverage by adhering bacteria→	No differences of biofilm removal between toothbrushes at 0 mm
	Oral-B Sonic Complete <sup>e</sup>					Movement (20 single strokes back and forth)	Immersed state	MatLab-based counting program	calculation of % removal (mean±SD)	More biofilm removal at 2 mm by side-to-side than by multidimensional toothbrush, lowest biofilm removal by manual toothbrush at 2 mm
Roberts et al. (2010) [28]	Manual Oral-B soft indicator Regular 40 <sup>c</sup>					No contact, at 0 mm—90 g load (side-to-side toothbrush), 150 g load (multidimensional toothbrush), and 220 g load (manual toothbrush)				Reduced biofilm removal of <i>A. naeslundii</i> and of dual species biofilms compared with <i>S. oralis</i> and multispecies biofilms at 0 mm
	Ultreo <sup>f</sup>	3	5	Untreated substrate	10	90° (bristles to substrate)	Dentifrice slurry (17 % toothpaste in distilled water)	Photographs with digital dental camera	Normalized pixel value (mean±SD)→calculation of % removal (mean)	Reduced biofilm removal of <i>A. naeslundii</i> biofilms (0–1 %) compared with remaining biofilms at 2 mm
	Modified Ultreo					No movement	Bristles immersed	Standard (4.8 mm) circular region of interest		Biofilm removal without bristle contact by all toothbrushes
										Significant more biofilm removal by ultrasonic toothbrush (64 %) compared with



**Table 2** (continued)

First author (year of publication)	Toothbrush	Distance (mm)	Time (s)	Control	n/condition	Toothbrush apparatus	Medium	Analysis method	Outcome variables	Results
	Sonicare Elite <sup>d</sup>					No contact				remaining toothbrushes* Modified ultrasonic, side-to-side, and multidimensional toothbrush treatments nonsignificantly different from each other
	Oral-B Triumph <sup>e</sup>									Ultrasonic, side-to-side, and multidimensional toothbrush treatments significantly different from positive control

*PBS* phosphate-buffered saline, *SEM* scanning electron microscope, *SD* standard deviation, *CFU* colony-forming unit, *CLSM* confocal laser scanning microscope, *CCD* charge-coupled device, *CI* confidence interval, *IR* interquartile range

<sup>a</sup> Bellevue, USA

<sup>b</sup> Tucker, USA

<sup>c</sup> Belmont, USA

<sup>d</sup> Snoqualmie, USA

<sup>e</sup> Kronberg, Germany

<sup>f</sup> Redmond, USA

\**p*<0.05

nanopure water [44], diluted saliva substitute [47], and a dentifrice slurry consisting of 17 % toothpaste in distilled water [28] were employed.

Despite the different liquid volumes, the toothbrush bristles were always partially immersed. In addition, biofilm removal was studied using a wetted substratum and a wetted toothbrush [46, 49].

**Exposure time and distance** To evaluate the influence of varying times and distances on biofilm removal by noncontact brushing, exposure time and distance were analyzed. Alteration of the distance along with a constant exposure time elucidated the influence of different distances on biofilm removal. The distances varied from 0 to 6 mm with an exposure time of 15 or 20 s [25, 44, 46, 49].

A few studies analyzed the alteration of both parameters to assess the impact of exposure time as well as of distance [37, 39]. Some studies, however, did not analyze either exposure time or distance [27, 28, 40–43, 47]. The distance between bristles and biofilm was not uniform because of the interproximal model [27, 40–43, 47]. The mean separation between bristles and biofilm was 2.65 mm. The exposure time of 5 s was arrived at by considering the average time of brushing in relation to the number of teeth [27, 28, 40–43]. Furthermore, an exposure time of 15 s was used [27, 42, 47].

## Results

**Toothbrush efficacy: biofilm removal** Biofilm removal was quantified by microscopic analyses, including the use of a confocal laser scanning microscope combined with live/dead staining [25, 27, 44], a scanning electron microscope [45], a phase-contrast microscope connected to a charge-coupled device (CCD) camera [46, 49], and a fluorescence microscope [45]. The quantification of biofilm removal was further determined by viable count procedures [37, 40–43, 47], by means of a viable biomass assay combined with the measurement of optical densities [48], and by normalized pixel values on photographs [28] (Table 2).

The included studies consistently reported biofilm reduction by noncontact brushing of tested side-to-side, multidimensional and ultrasonic toothbrushes [25, 27, 28, 37, 39–50]. Side-to-side toothbrushes yielded significantly more biofilm removal than multidimensional brushes [27, 40, 41, 43, 47, 49, 50]. Biofilms were not reduced by counter oscillation toothbrushing [37].

Considering that a distance of 2 mm and an exposure time of 15 to 20 s were the most commonly parameters for examining side-to-side toothbrushes, there was a wide range, i.e., from 38 to 99 %, in the percentage of biofilm

removed. Most studies, however, found >50 % biofilm removal by side-to-side toothbrushes [25, 46, 49, 50]. In contrast, biofilm reduction by multidimensional toothbrushes was frequently <50 % [49, 50]. The percentage of biofilm removal at a 2-mm distance and after 5 s of exposure was reported as 74 [45] and 65 % [37] by side-to-side action. Regarding the efficacy of side-to-side brushes in interproximal models with a mean distance of approximately 2 mm and an exposure time of 5 s, there was biofilm removal of 32 [40, 43], 48 [41], and 74 % [42]. In contrast, multidimensional toothbrushes achieved a biofilm reduction of 9 [40], 15 [43], and 16 % [41] using the same interproximal model.

Some studies analyzed the influence of different exposure times on biofilm reduction by side-to-side toothbrushes [37, 39, 48]. At distances of 0, 1, and 2 mm, there was no significant influence of exposure time (ranging from 5 to 30 s). The majority of bacteria were removed within the first 5 s of exposure. At a distance of  $\geq 3$  mm, however, exposures longer than 5 s had significantly greater effects [37, 39]. Brambilla et al. confirmed reduced biofilm removal at 7 mm after 5 s compared with exposures of 15 and 30 s [48].

Concerning the distance between toothbrush bristles and the biofilm, increasing distances almost invariably resulted in decreased biofilm removal by side-to-side and multidimensional toothbrushes [25, 27, 37, 44, 46, 49]. Nevertheless, biofilm removal at a 4-mm distance and after 15 to 20 s fluctuated around 60 % by side-to-side action in most studies [27, 46, 49]. Biofilm removal was also found at a distance of 6 mm [27, 46]. In contrast, Busscher et al. observed expansion of the biofilm after noncontact brushing for the first time [25]. At 4 mm, biofilm expansion amounted to approximately 86 % using a side-to-side toothbrush and to 29 % by a multidimensional toothbrush. At 6 mm, biofilms were expanded by 43 % using another side-to-side toothbrush. Other tested powered toothbrushes yielded biofilm removal between 30 and 40 % at a 6-mm distance [25].

The reduction of biofilm by noncontact brushing seems to be dependent on the types of bacteria constituting the biofilm [48, 49]. Removal of *S. mutans* and *S. salivarius* monospecies biofilms amounted to 41 and 30 %, respectively. In contrast, monospecies biofilms of *L. acidophilus* and *V. alcalescens* were not significantly reduced [48]. Moreover, biofilm removal differed among the sequences of inoculated bacteria [49]. Verkaik et al. compared the effect of noncontact brushing on monospecies, dual-species, and multispecies biofilms [50]. At a distance of 2 mm and after 20 s of exposure, monospecies biofilms of *A. naeslundii* (T14V) showed the least removal investigating both side-to-side and multidimensional toothbrushes. In contrast, monospecies biofilms of *S. mutans* were completely removed by handling of the flow chamber. The greatest differences

between side-to-side and multidimensional toothbrushes were observed for multispecies biofilms with side-to-side action showing more biofilm removal [50].

The state of immersion of the substratum and the bristles did not affect the percentage of biofilm removal [46, 49]. Biofilms with a thin liquid film were removed by a wetted toothbrush comparable with an immersed toothbrush. A completely dry powered toothbrush, however, removed significantly less bacteria.

*Toothbrush efficacy: viability of biofilm-associated bacteria* Studies using live/dead staining of biofilms reported no differences in the distribution of live and dead cells in tests and controls [27, 44]. The percentage viability of biofilms was 84 % before and 82 % after brushing [25].

*Hydrodynamic phenomena* A few studies examined the presence of hydrodynamic phenomena to explain biofilm removal by noncontact brushing. Adams et al. visualized the movement of fluid and air bubbles by means of a CCD camera [27]. They observed multiple air bubbles traveling through the interproximal area and calculated the velocity of air bubbles and the corresponding shear forces. The velocity of bubbles and the extent of shear forces did not differ significantly between side-to-side and multidimensional toothbrushes despite different biofilm removal efficacies. Using a digital time-lapse microscope, Heersink et al. also described fluid turbulences in which air bubbles were seen [44].

The importance of fluid, either in terms of fluid or even just as droplets, was emphasized by Busscher et al. when comparing the efficacy of completely dry, wetted and immersed toothbrushes [46]. Busscher et al. further analyzed the energy transfer by acoustic pressure waves and correlated it to powered toothbrushes [25].

## Discussion

The aim of the present review was to analyze the efficacy of powered toothbrushes in noncontact brushing. A total of 16 publications were included from the electronic search of the MEDLINE database and from a further manual search. Data were exclusively collected from in vitro studies.

Included studies were analyzed with regard to the technical and microbial aspects of the applied methods as well as in terms of quantitative biofilm removal, the viability of biofilm-associated bacteria and hydrodynamic phenomena.

The mode of action of powered toothbrushes was defined in two recent systematic reviews [17, 18]. A consensus exists that toothbrushes with side-to-side, multidimensional, and ultrasonic action enable biofilm removal by noncontact brushing although biofilms were not completely eliminated from surfaces [25, 27, 28, 37, 39–50]. Biofilm reduction

was more pronounced by side-to-side toothbrushes than by multidimensional action [27, 40, 41, 43, 47, 49, 50]. However, an extrapolation of these findings to powered toothbrushes not analyzed in this review should be undertaken with care. A shift in the distribution of live and dead bacteria after noncontact brushing was not observed. The interplay of hydrodynamic action, passing air bubbles and acoustic energy transfer was associated with biofilm removal.

Therapy of periodontal diseases and the establishment of periodontal health in the long term require well-performed dental plaque removal by the patient on a daily basis. Noncompliance with oral hygiene measures, in particular with difficult to access areas, such as the interdental area, however, is observed frequently [10, 14]. If powered toothbrushes are able to remove biofilms efficiently without bristle contact, they would probably simplify and consequently improve self-performed oral hygiene. However, up to now there is no proof that this will work in a clinical situation, as well.

When comparing the in vitro data from the included studies, differences in brushing protocols and biofilm formation need to be considered:

1. An important parameter is the strength of the in vitro biofilm. The adhesiveness of biofilms depends on the physical properties of the surface, such as roughness [51]. Irregularities of the tooth structure in terms of fissures and pits increase bacterial colonization and offer protection from shear forces. When using artificial substrates, such as glass and quartz [27, 46], the experimental results may be biased. The adhesion of salivary pellicle constituents and subsequently of adherent bacteria is presumably less than on tooth structures. Irrespective of physical and chemical differences with respect to enamel, titanium sections imitate implant surfaces [45]. The adhesiveness and thus the strength of biofilms is further dependent on the acquired salivary pellicle providing receptors for bacterial binding [52]. It is suggested that the strength of biofilms is less severe in methods evaluated without saliva for pellicle formation.
2. Bacteria have been identified adhering as early colonizers to tooth surfaces and preceding the attachment of late colonizers [53]. Both *Actinomyces* and *Streptococcus* species are considered to be early colonizers [54]. Using these bacteria in in vitro biofilms, adhesion to the salivary pellicle can be expected [25, 50]. Gram-negative bacteria, such as *P. gingivalis*, become more prominent in the biofilm at a later stage. They prefer receptors on other oral bacteria forming coaggregates to those of the salivary pellicle. This finding needs to be considered when using these bacteria in in vitro monospecies biofilms [48].
3. In addition to adhesiveness, which depends on the substratum, the acquired pellicle and early microbial colonizers,

the strength of oral biofilms depends also on cohesiveness [55]. Several factors may affect cohesiveness: bacteria involved in biofilm formation, the presence of exopolysaccharides and environmental factors such as hydrodynamics and nutrient concentration. Bacteria increase the strength of a biofilm by coaggregation to a defined set of partners. Using *A. naeslundii* and *S. oralis* in dual-species biofilms, coaggregation of these bacteria is expected [56]. Coaggregation of bacteria is also assumed in ex vivo biofilms derived from human whole saliva [40–43, 47] as well as in in vivo biofilms [37, 39].

4. The presence of exopolysaccharides strengthens the biofilm [55, 57]. The amount of exopolysaccharides rises with increasing time and nutrient concentration. Accordingly, the adhesion and growth curves of bacteria in biofilm model systems need to be considered.
5. Hydrodynamic conditions during growth influence biofilm strength. Increased hydrodynamic shear decreased the biofilm strength and changed the architecture from uniform carpet like to more fluffy with increased thickness [57]. Some model biofilm systems considered the impact of hydrodynamics. The parallel-plate flow chamber allows for the circulation of bacteria and nutrients by means of hydrodynamic pressure at a wall shear rate corresponding to physiological conditions [25, 46, 49]. Whereas adhesion of bacteria in a drip-flow reactor system occurs statically, nutrients subsequently flow during a 48-h growth period [27, 44]. In contrast, the strength of biofilms grown in static systems without shear differ significantly from that observed in natural intraoral biofilms [50, 58].
6. Brushing protocols as well as toothbrush apparatuses differed among the in vitro studies included. Basically, toothbrushes can be mounted either without any bristle contact to a solid surface or in an interproximal model with bristle contact with artificial teeth. Evidence exists that bristle load influences the deformation of bristles as well as the efficacy of brushing [32, 59]. Moreover, in interproximal models, the fluid is impelled into the interproximal space, generating high shear forces [25]. Without this narrow set-up, fluid and energy transfer would spread across a larger area and in several directions. It is suggested that these arrangements affect biofilm removal in interdental spaces [25].
7. Most liquids used to transfer hydrodynamic phenomena, such as phosphate-buffered saline, show lower viscosities and lower colloid content than the mix of saliva, toothpaste and water in vivo. The addition of artificial or human saliva, however, reflects the salivary component [28, 40–43, 47], whereas supplementation with toothpaste imitates the dentifrice slurry [28, 40–43]. It is suggested that different liquid viscosities and volumes influence the bristle velocity, the fluid flow, the formation of air bubbles and the shear forces [59]. Otherwise, the use of toothpaste in brushing protocols would probably distort toothbrush efficacy by affecting the chemical antibacterial efficacy [60].
8. The time spent for an oral hygiene session is on average 2 to 3 min [61, 62]. This means that the time available for cleaning a tooth surface interproximal space amounts to 4 to 6 s (2 min=120 s, 120 s/28 teeth=4.28 s; 3 min=180 s, 180 s/28 teeth=6.43 s). Therefore, exposure times of 5 s seem to be realistic and comparable to in vivo brushing times [40–43]. Exposure of the biofilm during a period of 20 s, however, would imply an overall brushing time far from clinical reality that may be of less clinical relevance.

To what extent the mechanisms of hydrodynamic action, passing air–liquid interfaces, and acoustic energy transfer contribute to the noncontact biofilm removal by powered toothbrushes was not separately investigated.

Hydrodynamic fluid shear forces were estimated by Adams and coworkers to amount approximately 0.9 Pa for the side-to-side toothbrush and 0.5 Pa for the multidimensional toothbrush [27]. In an aqueous solution, these values would correspond to fluid shear rates of about 0.9 and 0.5 s<sup>-1</sup>. For bacterial detachment from surfaces, however, shear rates of at least more than 10,000 s<sup>-1</sup> due to increased fluid flow were suggested to be essential. The efficacy of the hydrodynamic forces in bacterial reduction depends on the cell surface hydrophobicity of bacteria [19]. The transition of a laminar in a turbulent fluid flow generally results in an increase of shear forces [20]. Bristle motion may cause turbulences as described by Heersink and coworkers and thus shear rates are possibly high enough to stimulate microbial detachment [44].

The amount of entrapped air bubbles increases proportional to the fluid flow [19]. To remove bacteria from a surface the formation of a three-phase boundary between air bubbles, fluid and bacteria is required. Thus, surface tension forces become effective exceeding hydrodynamic shear forces by several orders of magnitude. The efficacy of bacterial detachment is influenced by the amount of gas in the fluid, the velocity and the diameter of the bubbles [22–24]. The visualization of air bubbles in the included studies supports the concept that powered toothbrushes involve passing air–liquid interfaces in biofilm removal although the proportion in detachment forces was not evaluated [27, 44].

In addition, the vibration of toothbrush bristles may enable acoustic energy transfer. The energy transfer to the biofilm is thought to depend on the frequency and amplitude applied [25]. The formation of oscillatory fluid motions and pressure waves may generate additional shear forces on the bacteria. Furthermore, the oscillation of entrapped air

bubbles is suggested to enhance shear forces by microstreaming and cavitation effects [26]. The extent to which the acoustic energy created by powered toothbrushes contributes to biofilm reduction, however, is unclear.

There may be a more complex interaction if there is intermittent tooth contact resulting in contact and noncontact biofilm removal. For clarity, the present review referred exclusively to noncontact biofilm removal.

#### Directions for further research

The data from this review are promising and suggest the possibility of noncontact biofilm removal by the toothbrushes analyzed. However, for future research the following parameter may be considered.

The use of

1. Multispecies biofilm models
2. A flow chamber model
3. Saliva for pellicle formation on a suitable substratum
4. An adjustable toothbrush apparatus, in terms of bristle angulation, distance, and load
5. Clinically relevant brushing protocols, in terms of brushing time, brushing distance, and liquid brushing environment.

Noncontact brushing may have the potential to improve oral hygiene in difficult to access areas, as up to now the clinical effectiveness of different types of powered toothbrushes does not correlate with the results of their respective noncontact biofilm removal capacities in vitro [18]. This conflict needs to be addressed in an appropriate clinical setting. Recently, a review provided a methodological guidance for randomized clinical trials comparing different toothbrushing modes [63]. The authors concluded that future clinical studies may require a longer follow-up, a standardization of indices and clinical relevant thresholds for differences in plaque and gingival health.

#### Conclusions

In conclusion, a great heterogeneity in microbial parameter, biofilm formation, and/or brushing protocols exist among the studies included in the review. However, noncontact biofilm removal by powered toothbrushes was a frequent finding. The results of this review can only be applied to the toothbrushes actually tested. Detachment forces, including shear forces, air bubbles interactions, and acoustic energy transfer are suggested to cause noncontact biofilm removal in vitro. Moreover, the clinical gain of noncontact biofilm removal is still unknown. There is no clinical study available, evaluating the extent of noncontact brushing to the observed superiority of powered over manual toothbrushing

in terms of plaque reduction. In vitro data should not be translated to the clinical situation.

**Conflict of interest** The authors declare that there is no conflict of interest. An in vitro analysis of the efficacy of powered toothbrushes is currently under preparation. The latter project is supported in part by an unrestricted grant by the Swiss Society of Dentistry (SSO) with the project number 264-12 (18.06.2012).

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