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Declaration of interests

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Abstract

In vitro and in situ studies modelling the wear of dental hard tissues due to erosion and abrasion exhibit a high variation in study designs and experimental parameters. Based on a summary of the existing protocols, the present review aimed to characterize and discuss the parameters which must be carefully considered in erosion-abrasion research, especially when it is intended to simulate clinical conditions. Experimental characteristics and parameters were extracted from a total of 42 in vitro and 20 in situ studies. The key experimental characteristics included parameters of erosion (duration, pH) and abrasion (duration, toothbrush, toothpaste, brushing force, time point) as well as co-factors (e.g. dental hard tissue). The majority of studies used models with alternating erosion/abrasion treatments intent to simulate clinical conditions, while other studies exaggerated clinical conditions intentionally, often using only a single erosion/abrasion treatment. Both in vitro and in situ models exhibited a high level of standardization but several studies showed a trend to severe erosion (e.g. >5 min/cycle) or extensive brushing (e.g. > 100 brushing strokes/cycle) at a high frequency and repetition rate. Thus, they often tend to produce a higher amount of wear than in the clinical situation, especially as modifying biological factors (e.g. dilution of the erosive solution by saliva, protective effect of the pellicle) cannot be simulated adequately. With a view to the existing models, it seems advisable to diminish duration and frequency of erosion and abrasion to clinically more realistic conditions when the everyday situation is to be simulated. Experimental parameters must be chosen with care to ensure that the problem is investigated in an appropriate mode at standardized conditions and with adequate measuring systems to allow prediction of clinical outcomes.

Introduction

In recent years, extensive research has been performed to gain better insight into, and understanding of, the processes involved in the development of erosive tooth wear. Erosion, abrasion and attrition rarely act alone but interact with each other, and abrasion of erosively altered dental hard tissues is considered as the most important interaction. Thus, numerous studies have investigated either the effects of toothbrushing on eroded dental hard tissues per se or the efficacy of different preventive agents under combined erosive/abrasive conditions. In these models the specimens are treated either in the oral cavity (in situ) or in the laboratory (in vitro). In some experiments a combination of an in vitro and in situ approach has been used. For instance, samples are carried in the oral cavity in special appliances, but are demineralised and abraded by brushing under standardized in vitro conditions. The intention, in using these setups, is to apply standardized procedures, which should simulate the intra-oral-conditions during demineralisation and abrasion as closely as possible. In other studies, the parameters are chosen to intentionally exaggerate clinical conditions. This is often done in order to create an amount of tissue loss, which might be measurable with the chosen method of determination. Other reasons for an exaggeration of the conditions are that a worst-case-scenario should be tested, that the limits of protective methods should become discernible or that special parameters (e.g. impact of different brushing forces) should be examined. These exaggerated conditions are used for the above mentioned reasons and will not be critically questioned in the following. Rather, the conditions chosen to mimic the real every-day-situations will be scrutinized in this critical review. Thus, the authors want to discuss how far the parameters chosen are able to simulate intra-oral clinical conditions in laboratory or in situ models as closely as possible.

General design

A total of 62 in vitro and in situ studies dealing with abrasion of eroded dental hard tissue were extracted from the database PubMed using the search term: abrasion AND (acid OR erosion OR softening) AND (enamel OR dentine OR dentin) AND (in vitro OR in situ OR

laboratory OR fluoride OR casein OR toothpaste OR dentifrice or toothbrushing OR brushing). Only full papers in English were taken into consideration. Several parameters used in the experimental designs in these studies were tabulated. This voluminous material is available online as Tables S1 and S2. ((Here, please insert URL for Wiegand Attin supplementary material)) Our recommendations are summarised in the body of the paper as Table 1.

A total of 42 studies with an in vitro design could be identified (Table S1). Most of these studies used cyclic erosion/abrasion models rather than single brushing treatment of eroded dental hard tissues. The number of erosive/abrasive challenges showed a wide range from 3 [Betke et al., 2003; Wiegand et al., 2004; Vieira et al., 2006b] to 720 [Bartlett et al., 1994] cycling treatments. Remarkably, a trend to severe erosion (e.g. > 5 min/ cycle) and/or extensive brushing (e.g. > 200 brushing strokes/ cycle) conditions could be observed in studies with very few cycles [Betke et al., 2003; Wiegand et al., 2004; Vieira et al., 2006b], while models using mild erosion and abrasion conditions are often performed at a higher frequency and repetition rate (e.g. 84 cycles, with each 30 s erosion and 15 s abrasion [Lagerweij et al., 2006]). Some reasons for the exaggeration of the conditions have already been mentioned above, but it has to be pointed out that these extreme models are hardly able to simulate tooth wear adequately, particularly under in vitro conditions.

Twenty in situ studies were found (Table S2). This means that the specimens were intra-orally carried in individual appliances allowing their removal from the oral cavity for further treatments, such as demineralisation and brushing.

Most of these studies applied cycling models with alternating de- and re-mineralisation phases. Alternating erosion/abrasion treatment also showed a great variety from 3 days with two cycles per day [Hara et al., 2003; Turssi et al., 2004] up to 21 days with three cycles per day [Vieira et al., 2007]. Erosion and abrasion were overwhelmingly performed extra-orally, and only in a few studies was either demineralisation [Hooper et al., 2003] or brushing [Jaeggi and Lussi, 1999; Lussi et al., 2004] done with the appliances in situ. Although both intra-oral erosion and brushing of the samples are more likely to better simulate real

conditions, it might be assumed that usually a higher standardization could be achieved with samples eroded and brushed extra-orally under standardized conditions. Moreover, extra-oral handling minimizes the risk of long-term acid exposure of the natural dentition. Statistical aspects of laboratory experiments (e.g. sample size) and in situ studies (e.g. subject and sample size, inter- and intra-individual salivary parameters) are not addressed in this review. However, to avoid the random impact of conditions on a single day condition of a volunteer, the cycles should run for a representative period of time such as 5-7 successive days. If this is not applicable, the treatments should be repeated on different days to rule out the random impact of a single day condition.

Extra-oral administration of the erosive and abrasive challenges allows a high level of standardization of the variables in both erosion (duration, pH) and abrasion (duration and number of brushing strokes; kind of toothbrush and toothpaste; brushing force). At the same time, however, this approach tends to produce a higher amount of wear than in the clinical situation, because modifying biological factors which are absent in vitro, such as dilution of the erosive solution by saliva, or erosion protection by native pellicle, cannot be simulated adequately. As a consequence, erosion and abrasion parameters, such as duration of erosion or amount of brushing strokes, must be carefully considered and probably diminished when performing erosion-abrasion research in laboratory experiments or in situ studies, with the intention to simulate the clinical situation.

Dental hard tissue

Erosion/abrasion experiments use enamel and/or dentin samples from human (usually wisdom teeth) or bovine (incisors) origin. Under the same cyclic erosion-abrasion conditions, dentin mostly revealed a higher susceptibility to wear in both bovine and human teeth [Hooper et al., 2003; Turssi et al., 2004; Attin et al., 2007; Wegehaupt et al., 2008; Wiegand et al., 2008a, 2010; Ranjitkar et al., 2009].

As almost half of the studies used bovine rather than human samples, it has to be asked whether bovine samples are an appropriate substitute for human teeth. In cyclic

erosion/abrasion models, bovine enamel showed a slightly higher susceptibility to wear than human, both in vitro [Attin et al., 2007] and in situ [Rios et al., 2006b], while human and bovine dentin specimens did not perform differently under the same in vitro conditions [Wegehaupt et al., 2008]. Bovine samples have the advantage that up to 4-5 specimens can mostly be gained out of a single bovine incisor. This allows for allocation of samples from the same tooth to different experimental groups, thus increasing comparability between those groups. Although it seems undoubtedly better to use human dental hard tissue to evaluate the impact of any kind of treatment, it seems to be acceptable to use bovine specimens instead of human ones, especially when relative tissue loss compared with controls, or the relative effect of different agents, is required rather than absolute tissue loss.

In situ models require that the samples are intra-orally fixed in appliances, in which they are usually placed either buccally in the lower jaw or palatally in the upper jaw. Both locations have advantages and shortcomings. In both locations, care has to be taken that no additional unintended abrasion of the samples surfaces through the buccal mucosa (lower jaw) or the tongue (upper jaw) interferes with the intended abrasion by toothbrushing. Additionally, at the palatal site, contact with saliva might be less intensive than in other regions of the oral cavity, where the major salivary glands empty into the oral cavity. However, if the acid solution is applied by drinking, the palatal area is more exposed than the buccal one. Owing to these considerations there seems to be no overall advantage of any of the two sites, and either could be chosen, in relation to the aim and set-up of the study.

Erosion

Duration of erosion varied between 15 s and 40 min (in vitro) and 40 s and 20 min (in situ) per cycle, while mostly an immersion time between 1 to 5 min/cycle was chosen. In only a single in situ study was the erosion performed intra-orally by a 10 min exposition to acidic drinks four times per day during 10 days [Hooper et al., 2003]. The investigators checked the tissue loss intermittently to ensure that harm to the natural dentition was minimal and took out participants in which tissue loss exceeded a critical limit. Nevertheless, the design of the

study led to 40 erosive challenges with a total of 400 min, which might be an ethical problem when using healthy volunteers for the experiment, since damage to the natural dentition could not be avoided completely.

Depending on the pH of the erosive agent, short erosion times of up to 3 min might result in a softened enamel layer, prone to brushing wear, of approximately 0.5 µm thickness [Wiegand et al., 2007b; Voronets and Lussi, 2010]. Considering that a high number of brushing strokes (approximately 500-1000) was necessary to remove this softened enamel layer almost completely [Wiegand et al., 2007b; Voronets and Lussi, 2010], it does not seem reasonable to increase the immersion time above this value. Moreover, it should be noted that the intra-oral challenge to the natural dentition, for instance when drinking an acidic beverage, is less pronounced because of dilution by, and interaction with, saliva and because of uneven distribution of the solution in the cavity. This results in only a short period of about 2 min during which the pH at a tooth surface is below the critical pH [Millward et al., 1997]. Thus, when performing the erosion extra-orally, exposure to the acidic environment without salivary interaction should not exceed a period of 2 min/cycle.

Less information regarding the abrasion-prone layer of erosion-affected dentin is currently available. Long-time erosion for 2 h led to a surface softening of 2 to 4 µm thickness which could be removed by ultrasonication [Vanuspong et al., 2002]. It can only be speculated how much of this softened dentin surface could be removed by brushing and whether shorter erosion times would produce a distinctly smaller softened zone. However, to allow comparison between enamel and dentin samples and to ensure realistic duration, short erosion periods should be preferred to long ones.

Regarding the erosion medium, many studies used commercial beverages, while others used citric or hydrochloric acid at a specific pH. Although it makes sense to use soft drinks in order to simulate the everyday situation, it seems also appropriate to use specific acidic solutions to test special properties and parameters of erosive media.

Interim storage (in vitro) and intraoral exposure (in situ) prior to brushing

In almost half of the studies the samples were not brushed immediately after erosion, but with a time delay of up to 4 h (mostly 30-60 min) to increase the abrasion resistance of eroded enamel and dentin. The efficacy of waiting periods to increase abrasion resistance is still questioned, since data are conflicting. Some studies show that abrasion resistance is significantly increased by storage in artificial saliva [Attin et al., 2000] or by intra-oral exposure [Jaeggi and Lussi, 1999; Attin et al., 2001b, 2004; Rios et al., 2006b], while others found no effect [Attin et al., 2001a; Hara et al., 2003; Ganss et al., 2007b; Sales-Peres et al., 2007; Kato et al., 2009]. However, a time delay before brushing might better represent conditions in daily life, as it seems rather unlikely that people brush their teeth immediately after each erosive challenge.

In laboratory experiments, samples were usually stored in artificial saliva for 1-60 min, while only three studies used human saliva as the storage medium [Kelly and Smith, 1988; Attin et al., 2001a; Hara et al., 2008]. Currently, it is not proven whether human or artificial saliva is more suitable as the immersion medium in vitro, as this was only checked in one study so far [Hara et al., 2008]. Although current in vitro models use a wide range of different saliva substitutes, artificial saliva provides the advantage that it can be prepared in sufficient amounts and with a consistent composition, which provide a high degree of standardization. Human saliva can be either collected from one donor [Kelly and Smith, 1988] or collected from several donors and pooled [Attin et al., 2001a; Hara et al., 2008]. Besides the problems that the composition of human saliva might show a high intra- and inter-sample variability and that large volumes of saliva are usually necessary in laboratory models, components of human saliva might be rapidly altered or degraded under in vitro conditions. Hence, it seems questionable whether the use of human saliva provides a distinct benefit in an in vitro set-up compared to saliva substitutes and hence whether it mirrors the clinical situation more closely. Thus, from current knowledge, the use of saliva substitutes as immersion medium prior to brushing seems to be appropriate if a delayed abrasion treatment is planned.

Generally, to simulate the everyday situation, brushing of eroded dental hard tissues should be delayed, by storage in artificial saliva in vitro or by intraoral exposure in situ. To omit the

intra-oral exposure before brushing only makes sense when an experiment is designed to evaluate immediate brushing of eroded surfaces.

Brushing abrasion

From Tables S1 and S2 it is evident that in abrasion experiments there is considerable variation in several brushing parameters, namely the frequency and duration of brushing, the kind of toothbrush and toothpaste and the brushing force.

Most cyclic models used an alternating treatment with equal numbers of erosive and abrasive challenges, which means that each erosive challenge is followed by brushing, irrespective of the waiting period applied. Only a few in vitro studies used a different setup, in which the samples were subjected to 6 erosive challenges but only 2 brushing cycles/day [Lagerweij et al., 2006; Ganss et al., 2007b, 2009a]. In addition, three in situ studies modified the alternating treatments in such a way that fewer abrasive than erosive challenges were performed each day [Vieira et al., 2007; Magalhaes et al., 2009; Wiegand et al., 2010]. This approach might reflect the clinical situation better, as most people brush their teeth twice daily [Ganss et al., 2009b] rather than after each contact with erosive foods or beverages.

In the majority of studies the samples were brushed with a manual toothbrush (25-5000 strokes/cycle), while fewer studies used electric toothbrushes (5 s to 1 min per cycle). Generally, laboratory experiments used a more severe abrasion treatment (duration or number of brushing strokes) than in situ studies. However, clinical surveys show that the overall brushing time for the whole dentition amounts to 30-90 s, which is equivalent to 300-400 brushing strokes [Macgregor and Rugg-Gunn, 1979, 1985; Ganss et al., 2009b]. Thus, it becomes evident that the regimes applied in most in vitro and in situ models exceed clinical conditions distinctly. Considering that, in vivo, each tooth might receive 10-15 brushing strokes with a manual toothbrush or less than 5 s brushing with an electrical toothbrush under clinical conditions, only 20% of the in vitro studies [Ponduri et al., 2005; Hemingway et al., 2006; Ganss et al., 2007b, 2009a; Wiegand et al., 2008b, 2009; Moretto et al., 2010] and 60% of the in situ studies [Attin et al., 2001b, 2004; Hara et al., 2003; Turssi et al., 2004;

Rios et al., 2006a,b, 2008a; Sales-Peres et al., 2007; Vieira et al., 2007; Wiegand et al., 2008a; Magalhaes et al., 2009] almost fulfilled this condition.

In vitro, usually both manual and electrical toothbrushes are fixed in automatic brushing machines or toothbrush holders which ensure a standardized movement of the brush over the sample surface as well as a constant brushing force [Parry et al., 2008]. Brushing forces vary between 0.2 and 4.5 N, but most of the studies used a brushing force of 2-3 N. These values match the clinical values of the mean load applied during brushing with a manual toothbrush [Ganss et al., 2009b]. It might be reasonable to reduce the brushing force of powered brushes to 1.5 or 2 N [Vieira et al., 2006a,b; Ganss et al., 2007b], not least as powered toothbrushes have shown a higher potential to damage eroded dental hard tissues than manual brushes at the same force [Wiegand et al., 2006a,b].

In most in situ studies, brushing was conducted extra-orally, with the exception of two studies [Jaeggi and Lussi, 1999; Lussi et al., 2004] in which the samples were brushed intra-orally, while the appliances with the specimens were still in the oral cavity. In all studies a high variability of tissue loss was observed, irrespective of whether samples were brushed intra- or extra-orally. This might be partly explained by differences in brushing forces used by the volunteers, even though they might be carefully trained and instructed. One possibility to achieve a higher level of standardization might be the use of brushing machines or holders [Wiegand et al., 2008a, 2010], the adjustment of a constant brushing force by scales [Ganss et al., 2007b] or brushing by the same person (investigator) [Hooper et al., 2003; Vieira et al., 2007]. Thus, there seems to be no distinct benefit from performing intra-oral brushing of the samples fixed in the appliances.

Various manual and powered toothbrushes are used in vitro. However, although the abrasion potential of toothbrushes is influenced by the brushing force [Wiegand et al., 2007a; Ganss et al., 2009a], the type of brush and the filament stiffness [Wiegand et al., 2008b; Wiegand et al., 2009], the impact of the toothbrush per se is considered to be significantly lower than the impact of the toothpaste [Wiegand et al., 2008b, 2009; Hara et al., 2009].

The abrasivity and the fluoride content are considered as relevant toothpaste parameters in erosion/abrasion experiments. Unfortunately, most of the published studies do not refer to the abrasivity (REA or RDA value) of the toothpaste, even though several studies indicate that abrasion of eroded enamel and dentin depends strongly on this [Hooper et al., 2003; Wiegand et al., 2008b, 2009; Hughes et al., 2008; Hara et al., 2009]. In contrast, authors usually specified whether they were using fluoridated or non-fluoridated toothpastes. Fluoridated toothpastes produced less wear on eroded enamel and dentin than non-fluoridated toothpastes both in vitro [Bartlett et al., 1994; Lagerweij et al., 2006; Hara et al., 2009] and in situ [Ganss et al., 2007b; Magalhaes et al., 2007, 2008]. Depending on the objective of the study, the use of either fluoridated or non-fluoridated toothpaste might be suitable, but it has to be considered that most toothpastes available on the international market contain fluoride. Thus, whenever possible, fluoride-containing toothpastes should be used to simulate clinical conditions.

Conclusion and recommendations

This review aimed to characterize and critically discuss the different designs of published in vitro and in situ models and to describe some important factors which influence the outcome of the respective studies and thus need to be considered carefully. Based on this review the authors try to give some recommendations (Table 1) for the variables in erosion/abrasion studies, with the aim of simulating as closely as possible the intra-oral daily life situation of patients suffering from erosion. These recommendations should be understood as guidance rather than as a standard schedule, although it has to be considered that a higher level of standardization within different experiments would allow better comparison among the study outcomes.

In vitro studies are important to clarify and estimate the relative effects of brushing of eroded dental hard tissue and the role of new anti-erosive methods or compounds, although in situ models have the potential to study fundamental aspects of the development of the

erosion/abrasion processes as closely as possible to the clinical situation without affecting the natural dentition.

The challenge of both laboratory and in situ studies is to strike a balance between the intention to simulate clinical conditions and the need to conduct the experiments in a rational, practicable and time-lapsed fashion and to gain measurable results. The variables suggested in Table 1 are applied in a very short experiment (either in situ or in vitro), so the amount of tissue loss might be below the detection limit of the assessment techniques, such as profilometry or microradiography, that are usually applied in erosion research [Attin, 2006]. This conversely emphasizes the need for further development in this field.

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	Dental hard tissue		Erosion				Interim storage/intra-oral exposure prior to brushing	Abrasion					
	Origin	Location of samples	Days, cycles/day	Procedure	Duration/ Cycle	Medium		Procedure	Cycles	Duration/ Cycle	Tooth-brush	Tooth-paste	Brushing force
In vitro	Human or bovine	-	Depends on the objective	-	≤ 2 min	Commercial beverage or specific erosive solution	Recommended, storage in artificial saliva	-	2/day	manual: 10-15 strokes powered: 5 s	powered or manual	F	1-2 N†
In situ	Human or bovine	Lower jaw buccal or upper jaw palatal	≥ 5 days with at least 2 erosions/day	extra-oral	≤ 2 min	Commercial beverage or specific erosive solution	Recommended, intraoral exposure	Intra- or extra-oral	2/day	manual: 10-15 strokes powered: 5 s	powered or manual	F	1-2 N†
				intra-oral	> 2 min possible*								

Table 1

Recommendations for adjustment of variables to simulate intra-oral real-life conditions as closely as possible in in vitro and in situ models testing erosion/abrasion of dental hard tissue. * if ethically justifiable, † standardization recommended.

Table S1
Summary and characteristic set-up parameters of the In vitro studies
AS: Artificial saliva, HS: Human saliva, BS: Brushing strokes, F: fluoridated toothpaste, NF: non-fluoridated toothpaste, n.a.: not available

Study	Dental hard tissue		Alternating erosion/ abrasion treatment		Erosion		Interim storage		Abrasion				
	Origin	Substrate	Days, cycles/day	total	Duration/ cycle	Medium	Duration	Medium	Cycles	Brushing/ cycle	Tooth-brush	Tooth-paste	Brushing force
Attin et al. [1997]	bovine	enamel	single treatment		1, 5, 15 min	Sprite light, pH 2.91	none		-	8000 BS	manual	NF	2.75
Attin et al. [1998]	bovine	dentin	-	5	5 min	Sprite light	1 min	AS	5	2000 BS	manual	NF	2.75 N
Attin et al. [1999]	bovine	enamel	-	4	5 min	Sprite light	1 min	AS	4	2000 BS	manual	F	2.75 N
Attin et al. [2000]	bovine	enamel	-	10	1 min	Sprite light	0-4h	AS	10	100 BS	manual	F	4 N
Attin et al. [2001a]	bovine	dentin	-	10	1 min	Sprite light	0-2h	HS	10	100 BS	manual	F	4 N
Attin et al. [2007]	bovine human	enamel	-	20	1 min	Citric acid	15 min	AS	20	100 BS	manual	F	3 N
Bartlett et al. [1994]	human	enamel	-	720	5 min	Citric acid, pH 3.5	none		720	200 BS	manual	F/ NF	0.2 N
Betke et al. [2003]	human	enamel	-	3	5 min	Citric acid	1 min	AS	3	2000 BS	manual	F	2.75 N
Davis and Winter [1980]	human	enamel dentin	single treatment		45 s 3 min	grapefruit/saliva mixture, pH 3.5	none		-	20, 50, 5000 BS 50, 1000 BS	manual	n.a.	2 N
De Menezes et al. [2004]	bovine	dentin	-	5	5 min	Sprite	1 min	AS	5	5000 BS	manual	F	3 N
Eisenburger et al. [2003]	human	enamel	-	4	10 min	Citric acid, pH 3.2	none		4	12, 500 BS	manual	NF	2 N
Ganss et al. [2007b]	human	dentin	9 d, each 6	54	2 min	Hydrochloric acid	none		9 x 2	15 s	powered	NF	2 N
Ganss et al. [2009a]	human	dentin	9 d, each 6	54	2 min	Hydrochloric acid	none		9 x 2	15 s	powered	NF	2, 3, 4 N
Hara et al. [2008]	human	enamel dentin	3 d, each 3	9	5 min	Citric acid, pH 3.75	30 min	HS, AS	3 x 3	500 BS 150 BS	manual	F	2 N
Hara et al. [2009]	human	enamel dentin	3 d, each 3	9	2 min	Citric acid, pH 3.75	60 min	AS	3 x 3	500 BS 150 BS	manual	F/ NF	2 N
Hemingway et al. [2006]	human	enamel	-	6	10 min	Juices	none		6	25 BS	manual	NF	2 N
Hughes et al. [2008]	human	dentin	3 d, each 3	9	2 min	Citric acid, pH 3.75	60 min	AS	3 x3	150 BS	manual	F	2 N
Kelly and Smith [1988]	human	enamel	-	60	5 min	Lemon juice	2 min	HS	60	200 BS	manual	NF	n.a.
Lagerweij et al. [2006]	bovine	enamel	14 d, each 6	84	30 s	Citric acid, pH 2.3	Few minutes	AS	14 x 2	15 s (60 BS)	manual	F/ NF	2.5 N
LIppert et al. [2004]	human	enamel	single treatment		1-3 min	Citric acid, pH 3.25	4 h	AS	single	30 s	powered	without toothpaste	Gentle force
Moretto et al. [2010]	bovine	enamel	7 d, each 4	28	5 min	Sprite, pH 2.8	none		28	15 s	powered	F	0.3 N

Ponduri et al. [2005]	human	dentin	-	5	40 min	Orange juice, Coca Cola	none		5	10 s (~8 BS)	manual	F	2 N
Ranjitkar et al. [2009]	human	enamel dentin	-	10	10 min	Citric acid, pH 3.2	none		10	200 BS	manual	NF	2 N
Schweizer-Hirt et al. [1978]	human	enamel	single treatment		5 min	Orange juice	none		-	5 min	manual	F	n.a.
Sobral et al. [2009]	bovine human	enamel	single treatment		30 min	Citric acid, pH 2.6	none		-	5000 BS	manual	F	2 N
Sulieman et al. [2004]	human	dentin	single treatment		30 min	Orange juice	none			1 min (50 BS)	manual	F	2 N
Sundaram et al. [2007]	human	dentin	-	50	5 min	Citric acid, pH 3.2	5 min	AS	50	100 BS	manual	Saline	0.2 N
Turssi et al. [2005]	bovine	enamel	-	5	5 min	Sprite diet, pH 2.7	1 min	AS	5	5000 BS	manual	F	3 N
Turssi et al. [2008]	bovine	dentin	5 d, each 5	25	5 min	Sprite diet	1 min	AS	5 x 5	40 BS	manual	F	3 N
Vieira et al. [2006b]	bovine	enamel	-	3	10 min	Demin.Solution, pH 3.0	none		3	1 min (200 BS)	powered	F	1.5 N
Vieira et al. [2006a]	bovine	enamel	-	3	10 min	Demin.Solution, pH 3.0	none		3	1 min (200 BS)	powered	F	1.5 N
Voronets et al. [2008]	human	enamel	single treatment		3 min	Citric acid, pH 4	none		single	15 s	manual	F	1.5 N
Voronets and Lussi [2010]	human	enamel	single treatment		3 min	Orange juice, pH 3.6	none		single	up to 590 BS	manual	F	1.5 N
Wegehaupt et al. [2008]	bovine human	dentin	-	20	1 min	Citric acid	15 min	AS	20	100 BS	manual	F	3 N
Wiegand et al. [2004]	human	dentin	-	3	5 min	Citric acid	1 min	AS	3	2000 BS	manual	F	2.7 N
Wiegand et al. [2006a]	bovine	enamel	-	10	5 min	Citric acid	15 min	AS	10	20, 80, 100 BS 20, 80 BS	manual powered	F	2.5 N
Wiegand et al. [2006b]	bovine	dentin	-	20	1 min	Citric acid	30 min	AS	20	20, 80, 100 BS 20, 80 BS	manual powered	F	2.5 N
Wiegand et al. [2007a]	human	enamel	single treatment		1 min	Hydrochloric acid, pH 2.0	none		-	up to 1000 BS	manual	F	1.5 , 2.5, 3.5, 4.5 N
Wiegand et al. [2007b]	bovine	enamel	single treatment		1 min	Hydrochloric acid	none		-	up to 500 BS	manual	F	2.5
Wiegand et al. [2008b]	bovine	enamel	15 d, each 4	60	15 s	Hydrochloric acid	none		15 x 4	40 BS	manual	NF	2.5 N
Wiegand et al. [2009]	bovine	dentin	15 d, each 4	60	15 s	Hydrochloric acid	none		15 x 4	40 BS	manual	F	2.5 N
Yu et al. [2009]	human	enamel	10 d, each 6	60	1 min	Citric acid	30 min	AS	10 x 6	1 min (100 BS)	manual	F	2.5 N

Table S2

Summary and characteristic set-up parameters of the In situ studies

BS: brushing strokes, F: fluoridated toothpaste, NF: non-fluoridated toothpaste, V: Brushing done by volunteer, no detailed information about brushing force, I: Brushing done by investigator, no detailed information about brushing force, * Standardized brushing force by means of a scale [Ganss et al., 2007a] or external brushing machine [Wiegand et al., 2008a; Wiegand et al., 2010].

Study	Dental hard tissue			Alternating erosion/ abrasion treatment		Erosion			Intraoral exposure before brushing	Abrasion					
	Origin	Substrate	Location of samples	Days, cycles/ day	total	Procedure	Duration/ Cycle	Medium		Procedure	Cycles	Duration / Cycle	Tooth-brush	Tooth-paste	Brushing force
Attin et al. [2001b]	human	enamel	Lower jaw buccal	21d, each 2	42	extra-oral	90 s	Sprite light	0 – 60 min	extra-oral	21 x 2	15 s	powered	F	V
Attin et al. [2004]	human	dentin	Lower jaw buccal	21d, each 2	42	extra-oral	90 s	Sprite light	0 – 60 min	extra-oral	21 x 2	15 s	powered	F	V
Ganss et al. [2007a]	human	enamel	Lower jaw buccal	5 d, each 2	10	extra-oral	20 min	Citric acid	0 h, 2 h	extra-oral	5 x 2	30 s	powered	F/ NF	2 N *
Hara et al. [2003]	bovine	dentin	Upper jaw palatal	3 d, each 2	6	extra-oral	90 s	Sprite light	0 – 60 min	extra-oral	3 x 2	40 BS	manual	F	V
Hooper et al. [2003]	human	enamel dentin	Upper jaw palatal	10 d, each 4	40	intra-oral	10 min	Orange juice, Coca Cola	none	extra-oral	10 x 4	1 min	n.a.	F	I
Jaeggi and Lussi [1999]	human	enamel	Lower jaw buccal	Single treatment		extra-oral	3 min	Citric acid	0 – 60 min	intra-oral?	-	30 s	manual	F	V
Kato et al. [2009]	bovine	dentin	Upper jaw palatal	5 d, each 4	20	extra-oral	5 min	Coca Cola	0 min, 30 min	extra-oral	5 x 4	30 s	powered	NF	V
Lussi et al. [2004]	human	enamel	Lower jaw buccal	Single treatment		extra-oral	3 min	Citric acid, pH 3.5	1 h	Intra-oral?	-	30 s	manual	F	V
Magalhaes et al. [2007]	human	enamel	Upper jaw palatal	7 d, each 4	28	extra-oral	5 min	Coca Cola	none	extra-oral	7 x 4	30 s	manual	F/NF	V
Magalhaes et al. [2008]	bovine	dentin	Upper jaw palatal	7 d, each 4	28	extra-oral	1 min	Coca Cola, pH 2.6	none	extra-oral	7 x 4	30 s	powered	F/NF	V
Magalhaes et al. [2009]	bovine	dentin	Upper jaw palatal	5 d, each 4	20	extra-oral	5 min	Coca Cola	none	extra-oral	5 x 2	15 s	powered	NF	V
Rios et al. [2006a]	bovine	enamel	Upper jaw palatal	5 d, each 4	20	extra-oral	10 min	Coca Cola	none	extra-oral	5 x 4	30 BS	manual	NF	V
Rios et al. [2006b]	bovine human	enamel	Upper jaw palatal	7 d, each 4	28	extra-oral	5 min	Coca Cola	0 – 30 min	extra-oral	7 x 4	30 BS	manual	F	V
Rios et al. [2008b]	bovine	enamel	Upper jaw palatal	7 d, each 4	28	extra-oral	1 min	Coca Cola, pH 2.6	none	extra-oral	7 x 4	30 s	powered	F	V
Rios et al. [2008a]	bovine human	enamel	Upper jaw palatal	7 d, each 4	28	extra-oral	1 min	Coca Cola	none	extra-oral	7 x 4	30 BS	powered	F/NF	V
Sales-Peres et al. [2007]	human	enamel dentin	Upper jaw palatal	5 d, each 4	20	extra-oral	5 min	Coca Cola	1 min, 30 min	extra-oral	5 x 4	10 BS	manual	F	V
Turssi et al. [2004]	bovine	enamel dentin	Upper jaw palatal	3d, each 2	6	extra-oral	90 s	Sprite light	none	extra-oral	3 x 2	40 BS	manual	F	V

Vieira et al. [2007]	human	enamel	Upper jaw palatal	21d, each 3	63	extra-oral	5 min	Sprite	1 h	extra-oral	21 x 1	5 s	powered	F	I
Wiegand et al. [2008a]	bovine	enamel dentin	Lower jaw buccal	14 d, each 3	42	extra-oral	40 s	Coca Cola	5 min prior or 5 after erosion	extra-oral	14 x 3	20 s (16 BS)	powered	F	2 N *
Wiegand et al. [2010]	bovine	enamel dentin	Upper jaw buccal	3 d, each 4	12	extra-oral	90 s	Sprite light	90 min	extra-oral	3 x 2	30 s	powered	F	1.2 N *