Experimental intra-oral caries models in fluoride research

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The use of experimental intra-oral caries models has increased in fluoride research. This paper focuses on the pre-clinical intra-oral models, the in situ and in vivo models, the various types, their benefits and disadvantages. Both preparation and sterilization of the hard tissue substrates can affect the substrates and therefore the results. Care needs to be taken that dentine samples are not exposed to drying and consequently shrinking during preparation and evaluation. Sterilization by γ-radiation is at present the least tissue-damaging method. The most realistic experimental model is the in vivo model, followed by the in situ model using specimens with natural surfaces. The most accurate and direct evaluation technique for demineralization and remineralization studies is quantitative transversal microradiography, whereas confocal laser scanning microscopy (CLSM) is the most sensitive qualitative evaluation technique. Other evaluation techniques discussed are microhardness testing and the iodine permeability test. In light of the present skewed caries situation in western countries we suggest that fluoride research focuses on experimental caries models that can mimic severe cariogenic challenge. Testing of fluoride combinations and dosages that can prevent lesion development rather than promote remineralization would then be a practical consequence. □ *Demineralization; fluoride; in situ; in vivo; remineralization*

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There are several reasons for choosing intra-oral models designed for clinical and mechanistic purposes instead of performing clinical trials. In most western countries a marked reduction in dental caries in children and adolescents has taken place (1). The general agreement is that fluoride and, in particular, the fluoride toothpastes are responsible for the change in disease prevalence and disease pattern (1–4). These dramatic changes represent obstacles in the planning and performance of clinical trials often resulting in large, time-consuming, and costly studies involving several hundreds of study subjects. Studies performed with experimental caries models in study subjects can be performed with fewer subjects in weeks or months (5–7) rather than years and are therefore much less expensive. Other reasons for choosing experimental caries models rather than clinical trials are the better control with the study subjects and better compliance because such studies take less time.

Lastly, most of the new and accurate examination techniques demand removal of the carious sites. Quantitative methods that have been used for evaluation of experimental caries are microhardness testing (5) and microradiography (8), whereas laser fluorescence quantification (9) may be described as a semi-quantitative method. Of these, only the laser fluorescence method can be used intra-orally without any preparation of the tooth samples. Qualitative methods that have been used for evaluation of experimental caries are polarized light microscopy, microscopy (10–12), iodine permeability (13), and confocal laser scanning microscopy (7). Iodine permeability and confocal laser scanning microscopy also require single tooth or enamel samples that are examined extra-orally.

The aim of this paper is to present some of the available experimental intra-oral models used in fluoride research and to give a brief presentation and discussion of some of the various implementations as well as the most used evaluation methods.

Experimental intra-oral models

Several experimental models have been designed for demineralization or remineralization studies. In the first experimental intra-oral models, small gold cups (14) or gold plates (10) were used when studying demineralization on vital teeth. In the first in situ model, foreign hard tissue material was used mounted in posterior flanges of lower dental acrylic prosthesis that was carried 24 h a day (15) (for more complete references see Table 1). Many of the later developed in situ appliances lean on Koulourides's model (16). In situ appliances can be of the Hawley type with palatal positioning of the test tissue (8, 13). Appliances fitted for the upper jaw with buccal positioning of the tissues (7, 17) have also been used. In situ models aim to mimic the prevention or development of lesions in the natural state as closely as possible. In the orthodontic banding model, introduced by Hals and Simonsen in 1972 (12), vital teeth are used, and so this model may be considered as even closer to reality than other intra-oral models. The orthodontic banding model of Øgaard et al. (18) used premolars to be extracted for orthodontic

Table 1. Historical intra-oral caries models.

Studies (refs)	Appliance site	Type of tissue	Tissue pretreatment	Plaque retention	Fluoride remin /prevention of demineralization	Evaluation technique
Bunting et al. 1926 (14)	bucc LJ	HE in vivo	N	Clasp with gold cup	No F	LM
Nygaard Østbye et al. 1957 (81)	bucc	HE in vivo	N	Gold cap	No F	Polarized LM
Nygaard Østbye et al. 1957 (10)	bucc LJ/UJ	HE in vivo	N	Gold plate	NaF ZnF	Polarized LM
von der Fehr et al. 1970 (11)	bucc	HE in vivo	Pumiced	nat	NaF	M (16×)
Hals et al. 1972 (12)	bucc	HE in vivo	N	OB	No F	Polarized LM
Koulourides et al. 1974 (15)	bucc LJ	BE	Ground	G	No F/1 ppm F in water—DEM/REM	MH

Appliance site: bucc: buccal, LJ: lower jaw, UJ: upper jaw.
Type of tissue: BE: bovine enamel, HE: human enamel.
Tissue pretreatment: N: natural, none.
Plaque retention: G: gauze, nat.: natural, OB: orthodontic band.
Fluoride—remin./prevention of demin: DEM: demineralization, NaF: sodium fluoride, no F: no fluoride, REM: remineralization, ZnF: zinc fluoride.
Evaluation technique: LM: light microscopy, M: microscopy, MH: microhardness.

reasons. The orthodontic bands were cemented around the teeth in such a way that plaque accumulated in a tiny "nice". The microflora behind the bands is similar to that associated with natural caries (19). The model is initially a demineralization type, but has also been used in the study of in vivo remineralization in the presence of fluoride (20). The model is independent of the degree of patient cooperation and is one of the few involving children. One advantage of this model is that the lesions develop in vital teeth (6). However, there may be variation in lesion development within the dentition of the same subject.

Sterilization

In the experimental in situ model, dental hard tissue of various origin is subjected to the study individual's oral micro-flora, cariogenic nutrition, and saliva. As a result, initial carious lesions may develop. Dental hard tissues harbor various microorganisms originating from the host. Therefore sterilization of the material is necessary prior to mounting in the appliances. In several of the papers sited in Table 2 no sterilization is mentioned (5, 9, 13, 17, 21–23). Since the mid-1970s, ethylene oxide has been used as a sterilizing medium in a number of studies (15, 16, 24–26). Because of high toxicity the use of ethylene oxide in sterilization has been markedly reduced during the last two decades. Chandler (27) assumed that this technique would be abandoned and suggested that dental workers should find other means of sterilization.

Gamma-irradiation is often used to sterilize heat-sensitive hospital items. According to EU regulations, the minimum dosage for sterilization by γ-irradiation is 25 kGy. In Norway a dosage of 32 kGy is used (28). In vitro studies have suggested both a decreased (29) and an increased (30) acid solubility of enamel after sterilization by γ-irradiation. The sterilization method mostly used in dentistry is autoclaving. Chandler (27) recommends both autoclaving and γ-irradiation as suitable methods for sterilizing enamel specimens. The often featured drying cycle as well as the high temperature used in autoclaving may damage the enamel-dentine specimens. Variations in expansion coefficients, due to variations in chemical composition between enamel and dentin (31), may result in enamel cracks or even in the separation of enamel and dentin. Air-drying of bulk dentine samples has been shown to increase remineralization of dentine considerably (32). Sensitivity to air-drying must also be considered when evaluating lesions in dentine samples. During the first 10 min of air-drying the lesion shrinks about 23% (33).

In our opinion, two facts support sterilization of dental hard tissue by γ-irradiation as being the method of choice. The tissue pieces can be kept wet during the procedure and they are not subjected to high temperatures as in autoclaving. In the in situ studies of Leach et al. (34) and Sønju Clasen et al. (7) γ-irradiation was used to sterilize the enamel pieces.

Substrates for experimental intra-oral caries models

Human enamel vs bovine

In the majority of more recent models designed for intra-oral testing, acrylic devices or prosthesis with test samples have been used mounted in various devices. The test substrates that have been used are human deciduous and permanent enamel blocks (5, 7, 17, 18), slabs of bovine enamel (15, 24), pieces of human root (18), of dentine (35), and even shark enamel (8) (for more complete references see Table 2). The only comparative in situ study known showed a significantly greater lesion development in primary than in permanent enamel, whereas no difference was obtained when using a sodium fluoride mouth-rinse once daily (7). Differences in chemical composition (36) as

Table 2. Intra-oral aries models.

Studies (refs)	Appliance site	Type of tissue	Tissue pretreatment	Plaque retention	Fluoride-remin /prevention of demineralization	Evaluation technique
Featherstone et al. 1982 (5)	bucc LJ	HE	Brushed	N	MFP REM	MH
Pearce 1982 (24)	bucc LJ	BE	Ground	GR	No F/SMFP and NaF DEM/prev. of DEM	MH qualitative MR
Creanor et al. 1986 (43)	ling LJ	HE sections	Pumiced DEM in vitro	N in trough	No F/MFP DEM/REM	MR
Mellberg et al. 1986 (25)	bucc LJ	HE thin sections	Ground and polished DEM in vitro	N	SMFP—remin	MR
Slater et al. 1986 (22)	bucc	HE	Etched and polished	N	No F DEM/REM	MR
Wefel et al. 1987 (21)	bucc/appr (mes and dist) LJ	HE	None ("cleaned")	N	NaF REM	Foto-MR
Øgaard et al. 1988 (18)	bucc (in vivo) pal (in situ)	HE and root	Pumiced natural surface	OB	NaF prev. of DEM	TMR
Øgaard et al. 1988 (8)	pal	HE and shark E	Pumiced natural surface	OB	No F DEM	TMR
Leach et al. 1989 (34)	bucc LJ	HE	None/natural	G	REM without F	Polarized LM
Schäfer et al. 1989 (16)	bucc/ling UJ/LJ	Human	Ground and polished	N	SMFP	MR
Meyerowitz et al. 1991 (17)	bucc UJ	HE	Brushed nat. surface DEM in vitro	R	NaF DEM/REM	MH
Zero et al. 1992 (13)	pal	BE	Ground	R with test plaque	No F DEM 45 min	Surface MH and IP
Dijkman et al. 1992 (23)	bucc LJ	HE	Ground	R	No F/F—releasing composites DEM/ prev. of DEM	TMR
Corpron et al. 1992 (26)	ling LJ	HE	Ground and polished	R	F-releasing device—demin and prev. of demin	SIMS and quantitative MR
Al-Khateeb et al. 1997 (9)	bucc UJ	HE	DEM in vitro	N	NaF F in lozengers/ chewing gum REM	QLF and TMR
Sønju Clasen et al. 1997 (7)	bucc UJ	HE perm. and dec.	Pumiced nat. surface	OB	NaF DEM/prev. of DEM	CLSM and TMR
Kashani et al. 1998	Approx. UJ	HE HD	Polished DEM in vitro	N	NaF-impregnated toothpicks	SIMS and TMR

Appliance site: Approx: approximal, bucc: buccal, dist: distal, ling: lingual, LJ: lower jaw, mes: mesial, UJ: upper jaw, pal: palatal.
Type of tissue: BE: bovine enamel, dec: deciduous, HD: human dentine, HE: human enamel, perm: permanent, shark E: shark enamel.
Tissue pretreatment: DEM: demineralized, nat: natural.
Plaque retention: G: gauze, N: none/natural, OB: orthodontic band, R: recess.
Fluoride—remin/prevention of demin.: DEM: demineralization, MFP: monofluorophosphate, NaF: sodium fluoride, noF: no fluoride, prev. of DEM: prevention of demineralization, REM: remineralization, SMFP: sodium monoflurophosphate.
Evaluation technique: CLSM: confocal laser scanning microscopy, LM: light microscopy, IP: iodine permeability, MH: microhardness, MR: microradiography, SIMS: secondary ion mass spectrometry, TMR: transversal microradiography, QLF: quantitative laser fluorescence.

well as the greater porosity (37) may be factors that contribute to variations in lesion development in primary and permanent human enamel.

Variations in enamels of different origin may not be of importance in studies comparing various caries-preventive agents on the same enamel type, but they may have to be considered when applying results to the clinical situation. Bovine enamel is considered to be a substrate with good reproducibility, especially when the outer surface of approximately 200 μm is ground off (38). The chemical composition of bovine enamel varies less than that of human enamel and is lower in fluoride concentration (39). Because of a greater porosity in bovine enamel, lesion development is faster than in human enamel (40, 41).

Choice of enamel may also be of importance in studies with mechanistic purposes. As indicated by the study of Sønju Clasen et al. (7) even the two types of human enamel may vary in lesion development and fluoride response.

In a few in situ studies, dentine (35) and root pieces (18) have been used to study lesion development, and also in the prevention of lesion development. Initial lesion development in root surfaces with cementum is very fast (18), despite the fluoride-rich cementum layer (42). Further lesion progression in root dentine takes place at a slower rate, probably because of an increasing content of organic matter (18). These in situ studies are of importance since they allow interactions to take place in the oral environment while enabling the use of sensitive laboratory techniques. These techniques are not applicable on dentine or root surfaces in their natural state.

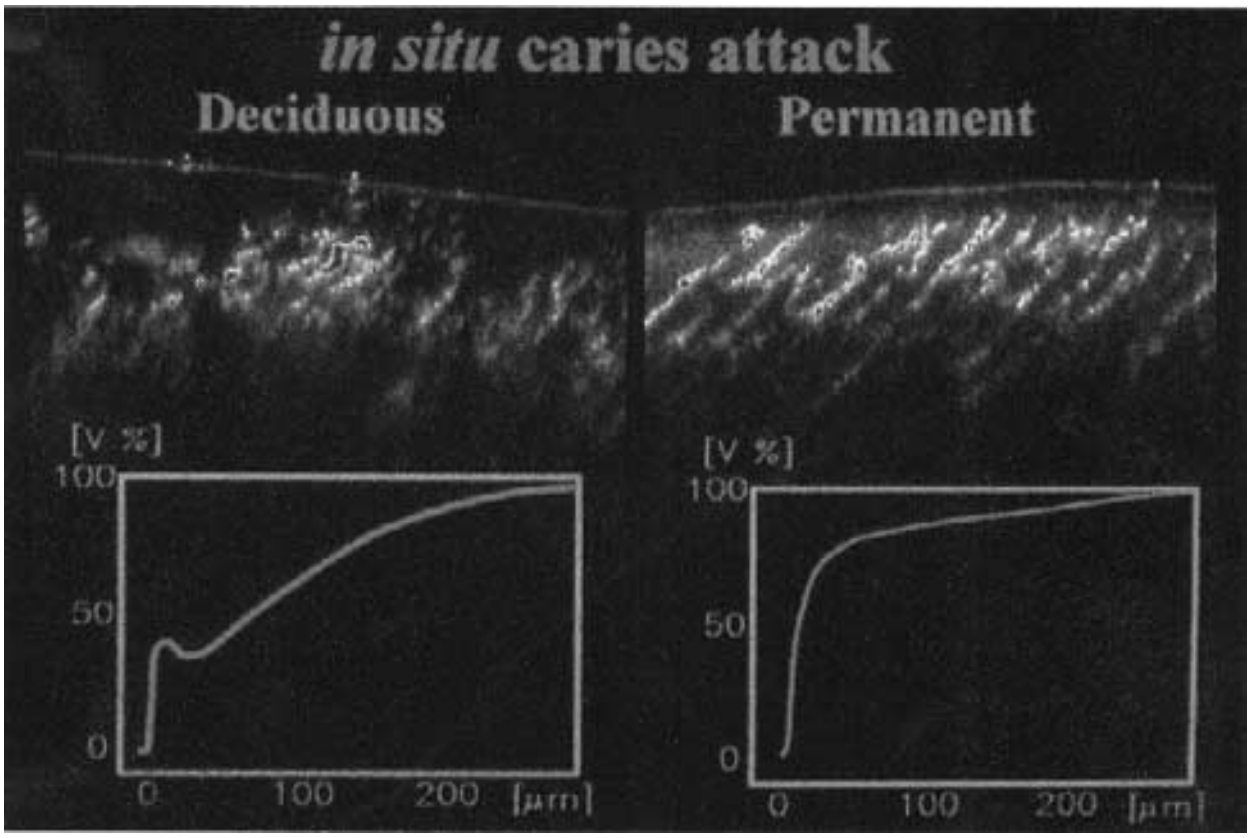

Fig. 1. (Top) CLSM images of deciduous and permanent enamel exposed to cariogenic challenge in situ in the absence of fluoride (xz plane). (Below) TMR scans of lesions in non-fluoridated deciduous and permanent enamel. With both evaluation techniques a more severe destruction of the deciduous tissue is apparent. Further description of the in situ study in Sønju Clasen et al. (7).

Bulk samples vs sections

A variation of the in situ model is the in situ enamel section model, in which thin sections of enamel (100–200 µm) are cut and mounted in the appliance (20, 43). A major advantage of the single-section technique is the exclusion of biological variation between samples during the study period, since pre-, post-, and repeated measurements can be performed on the single sections (44). A second advantage is the possibility of short periods between the measurements (45). A variation of the section model is the sandwich model (25), where the enamel is sandwiched between protective sheets of plastic and allows several layers of enamel sandwiches.

Ten Cate and Exterkate (45) compared the demineralization in bulk enamel and in thin sections by means of quantitative micro-radiography. They concluded that sectioning of the enamel increases its acid susceptibility. It was observed that 25% more mineral was lost from single sections than from the larger enamel samples. This has also been observed in other studies (25) and has been explained by damage at the prismatic level through sectioning and possibly leakage or diffusion from the sides of the sections (45). The authors therefore recommend the inclusion of bulk enamel specimens to verify the conclusions.

Pretreatment of the dental hard tissue samples may also result in a more pronounced demineralization. Pumicing, polishing, and grinding of the enamel or root samples reduces or removes the high-fluoride surface layer (25, 42) and therefore makes the tissue more susceptible to demineralization. Few demineralization studies have used natural tooth surfaces (7, 18), although this resembles the natural situation more closely. Only the orthodontic banding model provides demineralization on vital teeth (18) and is therefore not only an experimental in vivo model but also a model actually equivalent to the clinical situation during orthodontic treatment.

Intra-oral test sites

The majority of the models have used enamel samples placed in buccal sites in the lower jaw (5, 15, 21, 22, 24, 25, 34). Some have enamel samples placed in lingual sites in the lower jaw (43), in buccal sites in the upper jaw (7), in palatal sites (13, 18), or proximal (21, 34). For demineral-

alization to occur, plaque formation on the enamel samples is essential. The formation of plaque is initiated by the adsorption of salivary proteins to the enamel surfaces (46, 47). Recently, differences in the enamel pellicles formed on palatal and buccal enamel surfaces have been detected (48). Differences in pellicles may play a role in the formation of the plaque (49) and could therefore be of importance in the in situ demineralization or remineralization of the enamel samples.

Remineralization vs prevention of demineralization

When the mineral demineralization and remineralization equilibrium is disturbed, the result is lesion formation or lesion repair. In clinical trials the effect of fluoride is seen as prevention of the formation of carious lesions (50, 51). Several laboratory studies have contributed to the knowledge of the fluoride modes of action (52–56) and may have shifted the focus to the remineralization effect of fluoride rather than the prevention of demineralization. The in situ remineralization of in vitro prefabricated lesions has been the subject of several studies (5, 9, 21, 25). The in vitro lesions prefabricated for demineralization studies are known to develop differently from lesions formed in vivo (57). Caries development in vivo is an intermittent process (58). The remineralization itself has been shown to be dependent on the size of the initial lesion (38, 59). Lesions developed in vivo under experimental conditions are therefore more suitable for remineralization studies also since this is closer to reality than in vitro developed lesions. The severity of the cariogenic challenge may influence the reactivity of the hard tissue and hence lesion development and progression (60). The remineralization models mostly test anti-caries agents under moderate to low cariogenic challenge.

However, caries is an increasing problem in high-risk groups with severe cariogenic challenge. It may therefore be more realistic to test the anti-caries effect in experimental models by testing the prevention of caries by the test agent on tissue slabs heavily covered with plaque. Øgaard et al. (61) and Sønju Clasen et al. (7) tested the caries preventive effect of neutral sodium fluoride solutions on human dental tissues covered by plaque that was accumulated under orthodontic bands. Buyukyilmaz et al. (62) tested the cariostatic effect of TiF_4 on root surfaces in situ. In that study the in situ model of Øgaard et al. (18) was used. In 2 studies, anti-caries agents were tested under severe cariogenic challenge in the in vivo model of Øgaard et al. (18). Ullsfoss et al. (63) tested the effect of a combined chlorhexidine and NaF mouth rinse and Øgaard et al. (20) tested the effect of an acidulated NaF solution on the prevention of lesion development in vivo. All these studies showed that an improved cariostatic effect of fluoride during severe challenges can be achieved in various ways, e.g. by adding anti-microbials, modifying pH of the fluoride solution or introducing uncommon fluoride agents. Intra-oral model studies can thus increase our knowledge of the cariostatic mechanism of action of fluoride as well as suggest new procedures and agents for clinical testing.

Evaluation techniques

In clinical trials, relatively large caries lesions are generally registered by the DMFT/S index using mirror, probe, and sometimes bitewing X-rays. In intra-oral model studies, only initial lesions are studied, so changes in mineral content require advanced analytical techniques. In the earliest experimental models the examination techniques used qualitatively assessed the changes in the tissue. Mostly polarized light microscopy and light microscopy have been used (10, 14) (for more complete references see Table 1). In the last two decades two quantitative methods have frequently been used for evaluation of the demineralization or remineralization of tissues; microradiography and microhardness measurements (see Table 2). Microhardness and microradiography are known to be very sensitive methods. Of the two methods, microradiography is regarded as providing the most soundly based measurement of mineral content (38). Transverse microradiography is also regarded as the most practical technique for direct and quantitative measurement of mineral content, mineral changes, and mineral distributions (64, 65).

More recently, another type of microradiography has been developed, namely longitudinal microradiography (LMR) (66). Contrary to TMR, this technique is non-destructive and allows repeated mineral determinations of the tissue (67) on planoparallel or natural tooth surfaces (68).

Microhardness testing can be performed perpendicularly to the polished tissue surface (surface microhardness testing) or parallel to the tissue surface (cross-sectional microhardness testing) (64, 69). Although sensitive, microhardness testing has disadvantages in that the method does not allow direct measurement of the mineral content. Experiments using microhardness evaluation have been performed mainly on enamel. Used on dentine, the method is problematic because of relaxation of the indentation with time and drying-shrinkage effects of the material (70). The need of a flat polished surface to perform accurate microhardness testing results in the disadvantage of having to perform demineralization or remineralization studies on specimens with unnatural surfaces (15). All study results should ideally give information relevant to a clinical situation where natural tooth surfaces are predominant.

The iodine permeability test is a sensitive indirect quantitative method based on increased permeability due to mineral loss (38). However, the method is extremely operator-sensitive and is limited to the measurement of changes occurring in a relatively large area of enamel, defined by a window area (13). The iodine permeability test is most likely also sensitive to blockage of the pores (64). The indications that this test can be used reliably to

assess demineralization and remineralization are therefore weak (13).

More than a decade ago a very sensitive qualitative examination technique, confocal laser scanning microscopy (CLSM), was introduced and used in demineralization studies (71). Early enamel caries that cannot be detected by TMR can be visualized by CLSM, as shown in the in situ study by Sønju Clasen et al. (7) (see Fig). In this study it was also possible to visualize the fluoride effect on the enamel specimen.

The most recent evaluation method is the Quantitative Laser Fluorescence (QLF) technique. The enamel surface is illuminated by laser light and a CCD micro video camera connected to a computer monitors the tissue fluorescence. The software calculates the data to percentage fluorescence loss in the incipient lesion compared to sound enamel fluorescence (72, 73). The benefit with this method is that it is possible to monitor mineral changes in incipient enamel lesions in vivo longitudinally.

Fluoride dose-response studies and others

It is difficult at present to provide evidence that any particular in situ model is superior to another. In some studies (5, 16) the effect of various fluoride agents is compared using experimental in situ models. The best method for evaluating the caries-preventive effect of fluorides is a clinical trial. However, duration and high cost have resulted in use of the easier experimental in situ models (16). It is important that the models used for this purpose have a high level of validity in the clinical situation. The validity of a model can be defined as the degree of success with which the model actually provides information about the phenomenon or process it is being used to study (74). According to the committee of the US Council on Dental Therapeutics (75), the anti-caries efficacy of a fluoride dentifrice should be measured against the "gold standard" dentifrices (1000 ppm MFP or 1100 ppm NaF) and be at least "as good as" this. In the case of intra-oral studies the committee proposed that two requirements have to be fulfilled before it can be securely concluded that the test product provides a mean response within 10% of the gold standard in the clinical environment (75). Proskin et al. (75) noted, however, that the issue of making such a determination from data has not been completely resolved. To our knowledge, the validity issue has still not been completely resolved.

It is generally claimed that intra-oral models should demonstrate a dose-response to fluoride. However, in the clinical situation a dose-response to fluoride is not always apparent (80). In the orthodontic in situ and in vivo intra-oral models, too, no dose-response of concentrated fluoride agents has been observed (6, 20). In studies with these models, enamel lesion inhibition was almost the same regardless of fluoride concentration (61, 76). There are strong indications that during unfavorable conditions, e.g. in individuals with bad oral hygiene and severe plaque accumulation, fluoride agents may have a limited cariostatic potential since the remineralizing effect of fluoride is hampered by the low pH in plaque fluid (6, 58, 77). These high-risk individuals comprise the population group with the majority of the caries. Prevention care delivered by the public services in several Scandinavian countries has been aimed at improving the situation of high-risk individuals and to reach a higher cost-effectiveness (78, 79). Fluoride research may therefore increasingly have to focus on lesion prevention under severe cariogenic challenges, and for this purpose both the in vivo model and the in situ model providing rich plaque accumulation may be of further use. Even fluorapatite has been shown to demineralize with these intra-oral models.

Conclusions

Information about the caries process, microflora, and lesion development can be gathered from studies in which in situ or in vivo models have been implemented. Experimental intra-oral models are useful tools in mechanistic studies in caries and fluoride research. The in situ models represent an intermediate stage between laboratory models and clinical trials. It is not possible to claim that one model is superior to another. Care should be taken when drawing conclusions and implementing results from fluoride agents tested in experimental in situ models to the clinical situation. However, various experimental in situ models are suitable for screening fluoride agents and mechanistic studies.

Further research is needed on the cariostatic activity of the various new fluoride agents under severe cariogenic conditions. Suitable experimental models are the in vivo model and in situ models with heavy plaque accumulation.

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