

Antimicrobial efficacy of amine fluoride based tooth gels compared to a toothpaste in a phase 2/step 2 in-vitro test model

Mikrobiozide Wirksamkeit Aminofluorid basierter Zahngеле im Vergleich zu einer Zahnpasta in einem Phase 2/Stufe 2 in-vitro Testmodell

Abstract

Introduction: The aim of the present study was to determine the antimicrobial effect of various gel formulations on plaque formation; different tooth gels were compared to a toothpaste containing comparable antimicrobial ingredients with regard to its microbiocidal activity. The study was conducted under the assumption, that a chief requirement for the prevention of plaque formation is the combination of mechanical removal and antimicrobial activity, and not the sole capability of mechanical plaque removal.

Methods: Ledermix® fluoride gel as commercially available with preservative, and without preservative and perfume oils, Elmex® gelée, and Meridol® toothpaste were tested in a standardized in-vitro test modification of the quantitative suspension test EN 1040. Instead of testing in a suspension, the respective product was directly placed on a standardized contaminated sterile stainless steel disk without adding any bio-burden. 50% egg yolk in Aqua dest. was used as a neutralizer.

Results: Within 1 min, Elmex® gelée showed a RF $>5 \log_{10}$ against *S. pyogenes* and *S. sanguinis*. Against *S. mutans*, a \log_{10} RF of ≥ 5 was achieved after 2 min, against *C. albicans* after 5 min, and against *P. aeruginosa* after 10 min. *S. aureus* was the most difficult organisms to be reduced. After an application time of 10 min, only a \log_{10} RF of 2.4 was achieved. Ledermix exceeded the antimicrobial efficacy of Elmex® gelée against *S. mutans* and *C. albicans* and was already effective against these organisms after 1 min, but did not show the same antimicrobial efficacy as Elmex® gelée against *P. aeruginosa*. Similar to Elmex® gelée, a required reduction of $>5 \log_{10}$ for antimicrobials under no organic challenge was not achieved against *S. aureus*. Ledermix® fluoride gel without preservatives and Ledermix® fluoride gel without preservatives and perfume oil did not show the antimicrobial efficacy of the standard Ledermix® fluoride gel formulation, indicating that the observed antimicrobial efficacy is chiefly based on the preservative, and possibly the perfume oil. Compared to the tested gels, Meridol® toothpaste was less effective and reached any antimicrobial effect $>5 \log_{10}$ only against *S. sanguinis* after 10 min.

Conclusion: All unmodified tested gels showed an antimicrobial effect. Because no relevant antimicrobial efficacy against plaque forming bacteria was achieved within 2 min, in practice, an anti-plaque forming effect based on the antimicrobial action of gels cannot be assumed when used in the oral cavity. However, the results of the present study indicate that the antimicrobial efficacy of gels is determined by their formulation and that for the prevention of plaque formation the combination of mechanical removal and antimicrobial activity is not the chief requirement only, but a sustained antimicrobial effect may be of greater importance.

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Keywords: fluoride gels, toothpaste, amino fluoride, sodium monofluorophosphate, EN 1040, antimicrobial efficacy

Zusammenfassung

Hintergrund: Das Ziel der vorliegenden Studie war die Überprüfung antimikrobieller Beeinflussung unterschiedlicher Zahngеле auf die Plaque-Bildung. Die der Studie zugrunde liegende Annahme war, dass ein Haupterfordernis für die Prävention der Plaque-Bildung die Kombination einer mechanischen und antimikrobiellen Komponente, und nicht nur die mechanische Komponente allein ist.

Methoden: Ledermix® Fluorid Gel mit als auch ohne Konservierungsmittel und ohne Parfümstoffe, Elmex® gelée und Meridol® Zahnpaste wurden in einem standardisierten in-vitro Model nach einem modifizierten quantitativen Suspensionstest nach EN 1040 untersucht. An Stelle in einer Testsuspension wurde das jeweilige Prüfprodukt auf ein standardisiert kontaminiertes Testplättchen ohne Proteinbelastung aufgetragen. Als Neutralisator wurde 50%iger Eidotter in Aqua dest. verwendet.

Ergebnisse: Bei 1 min Einwirkungszeit erreichte Elmex® gelée einen RF $>5 \log_{10}$ gegenüber *S. pyogenes* und *S. sanguinis*. Gegen *S. mutans* wurde ein \log_{10} RF von ≥ 5 nach 2 min erreicht, gegen *C. albicans* nach 5 min und gegen *P. aeruginosa* erst nach 10 min. *S. aureus* war der am schwierigsten abzutötende Erreger bei einem \log_{10} RF von 2,4 nach 10 min. Ledermix übertraf die antimikrobielle Wirksamkeit von Elmex® gelée gegen *S. mutans* und *C. albicans* und war bereits nach 1 min gegen diese Erreger effektiv, zeigte aber eine geringere Wirksamkeit als Elmex® gelée gegenüber *P. aeruginosa*. Ähnlich wie Elmex® gelée wurde eine erforderliche Reduktion $>5 \log_{10}$ ohne organischer Belastung für eine antiseptische Wirksamkeit nicht gegenüber *S. aureus* erreicht. Ledermix® Fluoride Gel ohne Konservierungsstoffe und Ledermix® Fluoride Gel ohne Konservierungs- und Parfümstoffe zeigten nicht die antimikrobielle Wirksamkeit des handelsüblichen Ledermix® Fluoride Gels. Dies lässt den Schluss zu, dass die beobachtete antimikrobielle Wirksamkeit hauptsächlich auf den Konservierungs- und möglicherweise Parfümstoffen beruht. Im Vergleich zu den Gelen war die Zahnpasta Meridol® weniger effektiv und erreichte einen RF $>5 \log_{10}$ nur gegen *S. sanguinis* nach 10 min.

Schlussfolgerung: Alle unmodifizierten Gele zeigten einen antimikrobiellen Effekt. Da allerdings keine relevante antimikrobielle Wirkung innerhalb von 2 min gegenüber Plaque-bildenden Bakterien feststellbar war, kann in Praxis nicht darauf geschlossen werden, dass ein anti-Plaque-Effekt durch Verwendung von Zahngelen erzielbar ist. Die Ergebnisse der vorliegenden Studie weisen jedoch darauf hin, dass der antimikrobielle Effekt von Zahngelen durch die Galenik und Wirkstoffzusammensetzung bestimmt ist und das ein anti-Plaque-Effekt nicht nur durch mechanische Einwirkung, sondern auch durch antimikrobielle Wirksamkeit bestimmt ist, welche einen remanenten Effekt hinsichtlich Plaque-Neubildung bewirken kann.

Schlüsselwörter: Fluorid Gele, Zahnpasta, Amin-Fluorid, Natrium monofluorophosphat, EN 1040, antimikrobieller Effekt

Introduction

In the common household, still mechanical-chemical dental plaque control plays an imminent role for prophylaxis of caries, gingivitis, and ultimately, periodontitis. For this, the mechanical cleaning effect of a toothbrush is significantly improved by combined usage of toothpaste

[1], and shorter brushing times are possible achieving the same or even better dental plaque removal [2], [3]. Additionally, the usage of toothpastes significantly increases the time of new formation of dental plaque, as compared to mechanical tooth brushing without use of any toothpaste [4]. Not only the structure and formulation influences the outcome, but also different compounds in

toothpastes and tooth gels, such as adding antimicrobial compounds, may achieve varying effects [5]. Toothpastes and tooth gels achieve their optimum effect only on a tooth surface free of biofilms, because the uptake of fluoride may be decreased by the glycocalyx structure of a biofilm. Therefore, not only for toothpastes, but in particular for tooth gels an additional antimicrobial effect may be decreases in those areas, where biofilms were not adequately removed through mechanical cleansing. The oral cavity provides a number of micro-ecologic niches for microorganisms. A complete denture provides approximately 130 bacterial habitats, which represent difficult to reach interdental areas [6]. In between these structures, microorganisms may accumulate and form biofilms. Ultimately, an imbalance between bacteria colonization and immunological defense mechanisms may lead towards a chronic pathogenic state. In adults, this phenomenon supporting caries formation and periodontitis is particularly found in predilection sites such as dental approximal spaces. Therefore, it is justified and recommended to use antimicrobial compounds in addition to mechanical cleansing of teeth [7], [8], [9].

Contrary to toothpaste, fluoride gels are highly concentrated fluoride preparations to increase the dental enamel's resistance against acid damage. Usually, fluoride gels contain 1% to 1.25% fluoride, which is approximately ten times higher than what is found in toothpaste. Therefore, fluoride gels are chiefly used in the prophylaxis of caries and must be used based on a clean indication following the instructions of a dentist [6]. Next to the chief compound amino fluoride and/ sodium monofluorophosphate [10] tooth gels may contain additional compounds, such as the antimicrobially active chlorhexidine in a concentration of 1%, but contrary to toothpaste, gels never contain abrasive compounds. They are used once a week or once every 14 days, and after a local application time of 2–3 min they are spat out. Gels are indicated for intensive prophylaxis of caries, for the supportive treatment of initial caries, as well as for the treatment of sensitive teeth necks. In dental practice, fluoride gels are additionally used for enamel decalcification below removable appliances, partial prosthesis, and orthodontic appliances, as well as for fluoridation of abraded or damaged enamel areas [11]. If amine fluoride gels are combined with antimicrobial compounds, they are also used for prophylaxis of gingivitis [12], for the treatment of stomatitis due to prosthesis wear, oral soor, and reduction of *Streptococcus mutans* [13].

The aim of the present study was to determine the antimicrobial effect of various gel formulations on plaque formation; four different tooth gels were compared with one toothpaste containing comparable antimicrobial ingredients with regard to its microbiocidal activity. The study was conducted under the assumption, that a chief requirement for the prevention of plaque formation is the combination of mechanical removal and antimicrobial activity, and not the sole capability of mechanical plaque removal.

Methods

The selection of the tested formulas aimed at comparing the effectiveness of Ledermix® fluoride gel (RIEMSER Arzneimittel AG, Greifswald, Germany), which antibacterial effect has not been studied yet, with the market leader Elmex® gelée (GABA GmbH, Lörrach, Germany), since both products are used for the identical indications. Additionally, a toothpaste with declared antimicrobial effect, Meridol® toothpaste (GABA GmbH, Lörrach, Germany), was selected to allow the comparison between gels and toothpaste, and hence, a possible added mechanical effect due to abrasive compounds in the toothpaste.

The following products and lots were used for this study: Elmex® gelée (lot no. 865705) – compounds in 100 g: Dectafluor 0.287 g, Olafluor 3.032 g, Sodium-fluoride 2.210 g (total fluoride concentration: 1.25%); Ledermix® Fluorid Gel (lot no. 609711) – compounds in 100 g: Dectafluor 2.74 g, Olafluor 3.036 g, 2.021 g Sodium-fluoride (total fluoride concentration: 1.25%); Ledermix® Fluorid Gel without preservatives (RIEMSER Arzneimittel AG, Greifswald, Germany, lot no. 150707) and Ledermix® Fluoride Gel without preservatives and without perfume oil (RIEMSER ArzneimittelAG, Greifswald, Germany lot no. 150707), and finally Meridol® toothpaste (lot no. 73331F) containing 0.14% fluoride, thereof 350 ppm amino fluoride and 1.050 ppm stannous-fluoride.

Test setting and experimental procedure

Because of the inhomogeneity of the test suspension no reproducible results could be obtained in a pre-testing phase, the quantitative suspension test according to EN 1040 [14] had to be modified. Therefore, instead of testing in a suspension, the respective product was directly placed on a standardized contaminated sterile stainless steel disk without adding any bio-burden, as described previously [15].

The disk carriers were contaminated with 0.05 mL of bacterial test suspension, which was allowed to dry under a laminar flow work bench for 1 hour. The following test organisms were used: *S. mutans* (ATCC 20175), *S. sanguinis* (DSM 20068), *S. pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), and *Candida albicans* (ATCC 10231). The test suspension contained each 10^8 – 10^9 cfu/mL.

The contaminated test disk carrier was completely plated with 0.35 g of the test product on a calibrated scale. After passing of the examined application time, the test carriers were placed with their upside downwards in a sterile polypropylene container (BEHAWE GmbH, Germany) together with 5 glass beads (diameter: 3–4 mm) and 10 mL of a neutralizing solution. Because the neutralizers proposed by the EN 1040 were not able to sufficiently neutralize all test products, sterile egg yolk suspended in 1:1 Aqua dest. (50% sterile egg yolk neutralizer) was used. The polypropylene containers were placed on a plane shaker and shake thoroughly for 2 minutes. After further 5 minutes, 0.1 mL of the solution was obtained, and

serial dilutions of 10^1 to 10^4 were prepared in NaCl-tryptone following standard microbiological methods. 0.1 mL of the respective dilution was spread in duplicate on casein-soy peptone-agar plates and incubated at $36 \pm 1^\circ$ for 48 h.

The calculation of the \log_{10} reduction factor (\log_{10} RF) was performed following EN 1040. Briefly, the respective \log_{10} RF = \log_{10} (cfu KO) – \log_{10} (cfu KD), whereby KO = number of cfu/mL of the control using sterile water standardized hardness instead of the test suspension, and KD = number of cfu/mL after effect of the test product. All tests were done in duplicate.

Results

Within 1 min, Elmex® gelée showed a RF $>5 \log_{10}$ against *S. pyogenes* and *S. sanguinis*. Against *S. mutans*, a \log_{10} RF of ≥ 5 was achieved after 2 min, against *C. albicans* after 5 min, and against *P. aeruginosa* after 10 min. *S. aureus* was the most difficult organisms to be reduced. After an application time of 10 min, only a \log_{10} RF of 2.4 was achieved (Table 1).

Ledermix exceeded the antimicrobial efficacy of Elmex® gelée against *S. mutans* and *C. albicans* and was already effective against these organisms after 1 min, but did not show the same antimicrobial efficacy as Elmex® gelée against *P. aeruginosa*. Similar to Elmex® gelée, a required reduction of $>5 \log_{10}$ for antimicrobials under no organic challenge was not achieved against *S. aureus*. Ledermix® fluoride gel without preservatives and Ledermix® fluoride gel without preservatives and perfume oil did not show the antimicrobial efficacy of the standard Ledermix® fluoride gel formulation, indicating that the observed antimicrobial efficacy is chiefly based on the preservative, and possibly the perfume oil.

Compared to the tested gels, Meridol® toothpaste was less effective and reached any antimicrobial effect $>5 \log_{10}$ only against *S. sanguinis* after 10 min (Table 1).

Discussion

While all tested tooth gels showed an antimicrobial efficacy against the relevant plaque forming bacteria *S. mutans* and *S. sanguinis* within 1 min, the tested toothpaste reached only against *S. sanguinis* and *C. albicans* a \log_{10} RF >5 after 10 min application time. *S. mutans* remained unaffected during this application time, while an effect on *S. pyogenes* was seen even though a \log_{10} RF of 3.5 was not reached. Because no relevant antimicrobial efficacy against plaque forming bacteria was achieved within 2 min, in practice, an anti-plaque forming effect based on the antimicrobial action of gels cannot be assumed when used in the oral cavity. An antimicrobial efficacy was expected for all tested products because of the contained concentration of amine fluoride [16]. The antimicrobial effect of amine fluoride is based on a decreased acidity tolerance of the

bacterial cell, as well as on the disruption of the enzyme-dependent glucose transport of the bacterial cell and its consecutively metabolism [17]. As a result, plaque formation is impaired [17], [18]. This effect is enhanced in combination with tin [19]. In contrast, sodium fluoride shows only a bacteriostatic effect at high concentrations [19], [20]. Arweiler et al. [21] showed that combining triclosan with amine and sodium fluoride was more effective than a chlorhexidine solution. With the exception of sodium monofluorophosphate all used fluoride compounds in dentistry exist in the form of ionic bound fluoride (e. g. NaF, SnF_2 , ZnF_2). Bivalent cations such as Zn^{2+} , Sn^{2+} or Cu^{2+} can inhibit enzymes of the bacterial metabolism and have a plaque-busting effect for durations of approx. 6 hours by binding with sulfhydryl groups. Furthermore, they are capable of binding to saliva and the tooth surfaces, which results in a prolonged retention time in the oral cavity [22]. Currently, stannous fluorides in combination with amine fluorides, such as used in Meridol® toothpaste, are recommended for daily prophylaxis [23]. Indeed, Hombach et al. [24] were able to show in vivo, that the application of either 0.12% chlorhexidine solution or amine stannous fluoride solution twice daily were comparable regarding their efficacy to inhibit plaque formation.

Although Meridol® toothpaste contains a combination of amine fluoride and stannous fluoride, and therefore a more effective fluoride combination as well as a potential additional antimicrobial active compound, the polyalcohol sorbitol, Elmex® gelée and Ledermix® fluoride gel showed a considerably higher antimicrobial efficacy. This may be due to the 10-fold higher concentration of amine fluoride/sodium fluoride in these gels and the combination of essential oils. This view is supported by the observation that the tested formulation of Ledermix® fluoride gel without preservatives and particularly without perfume oil was less antimicrobially effective. Indeed, Willershausen et al. [25] demonstrated an inhibiting effect of herbal extracts in toothpastes and mouthwashes on the inhibition of plaque, which was confirmed in subsequent studies [26], [27]. Apart from their antimicrobial effect [28], essential oils also affect the ion permeability of cell membranes [29], which explains their local anaesthetic and anti-inflammatory effects. Due to dilution effects and swallowing, the time of action in the oral cavity, however, is only short and compared to the antimicrobial efficacy and plaque-busting properties of chlorhexidine, essential oils and herbal extracts must be regarded equally or less effective [30]. Therefore, their use in gels may be considered to be more advantageous than in antimicrobial toothpastes.

Pertaining to effects due to preservatives, Lenz [31] demonstrated an inhibiting effect on dental plaque, which was doses dependant. Obviously, while present, the effect of preservatives in the tested gels was not high enough for a significant plaque inhibition. However, the primary purpose of a preservative is to prevent bacterial decay of an organic product, and not to serve as an agent for sustained inhibition of plaque formation. Fluorides as

Table 1: Log₁₀ reduction factors (RF) of tooth gels and one toothpaste against six micro-organisms tested under practical conditions

Test organism	1 min					2 min					5 min					10 min				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
S. aureus	0.03	<0.2	1.0	<0.2	<0.2	0.1	<0.1	1.3	<0.1	<0.1	2.1	<0.1	1.3	1.1	<0.1	2.4	0.8	2.4	2.3	<0.02
P. aeruginosa	3.2	2.1	1.9	0.7	0.9	5.0	2.1	2.1	1.0	0.8	7.7	2.4	2.1	1.7	1.5	7.7	3.2	1.8	2.1	1.8
C. albicans	3.9	5.2	3.5	3.6	2.9	4.1	5.1	3.5	3.5	3.2	5.1	5.1	3.5	5.1	3.8	5.2	5.2	5.2	5.2	5.2
S. pyogenes	5.0	6.0	4.2	1.9	1.0	6.2	6.2	6.2	6.2	1.3	6.3	6.3	6.2	6.3	2.1	6.0	6.1	6.0	6.0	3.7
S. mutans	2.7	6.0	6.0	2.1	0.1	6.0	6.0	6.0	4.2	0.1	6.3	6.3	6.0	6.3	0.5	6.2	6.2	6.2	6.2	0.7
S. sanguinis	5.3	5.2	1.4	3.5	0.6	5.4	5.4	3.3	5.4	1.2	5.5	5.5	3.3	5.5	2.9	5.4	5.4	5.4	5.4	5.4

A = Elmex® gelée; B = Ledermix® Fluorid Gel; C = Ledermix® Fluorid Gel without preservative; D = Ledermix® Fluorid Gel without preservative and without perfume oil; E = Meridol® toothpaste; red numbers: indicates antimicrobial efficacy $\geq 3.5 \log_{10}$ RF as required for antimicrobial compounds without organic challenge.

weak acids, however, may doses dependant also be able to inhibit lactate production of *S. mutans* [32], [33]. And finally, Leikanger et al. showed more than 20 years ago that benzoic and sorbic acid affect streptococci in a plaque- and acid-inhibiting modality at higher concentrations [34].

Our study has one important limitation, which is that the investigations were performed only in-vitro. The antimicrobial efficacy of the tested products was assessed under modified standardised conditions following the European Norm EN 1040. However, these results are the first allowing an assessment of possible antimicrobial effects. Furthermore, to our knowledge, this is the first study investigating the antimicrobial efficacy of various products under the same standard conditions. In vivo, the clinical efficacy of gels and toothpaste is severely influenced by the difficulty to permeate dental biofilms and the galenic form of the matrix the antimicrobial compounds are embedded in. To which extend the antimicrobial effect may be observed under real conditions, must be further investigated in in-vivo studies.

In conclusion, all unmodified tested gels showed an antimicrobial effect. Because no relevant antimicrobial efficacy against plaque forming bacteria was achieved within 2 min, in practice, an anti-plaque forming effect based on the antimicrobial action of gels cannot be assumed when used in the oral cavity. However, the results of the present study indicate that the antimicrobial efficacy of gels is determined by their pharmacological formulation and that for the prevention of plaque formation the combination of mechanical removal and antimicrobial activity is not the chief requirement only, but a sustained antimicrobial effect may be of greater importance.

Notes

Competing interests

The authors declare that they have no competing interests.

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