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Evaluation of antibacterial properties of fluoride-containing mouth rinses differing in their acidic compound using a *Streptococcus mutans* biofilm

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SUMMARY

This *in vitro* study assessed the antibacterial effect on *Streptococcus mutans* biofilms of mouth rinses with 700 ppm F- (derived from NaF) that differed only in their acid compounds (malic, citric, tartaric, fumaric, hydrochloric, phosphoric, and lactic acid) used to adjust pH.

S. mutans (ATCC™ 25175) was grown for 22 h at 37°C, harvested, resuspended in simulated body fluid and biofilm formation followed for 24 h at 37°C. Thereafter, biofilms were treated with experimental rinses for 30 s and placed in TAM48 isothermal microcalorimeter at 37°C for 72 h. Applying Gompertz growth model the parameters lag time and growth rate were determined from heat flow curves; additionally, reduction of active biofilms was calculated. Moreover, samples were live/dead-stained and analysed by confocal scanning microscopy.

All mouth rinses were showing statistically significant lag time and reduction of active biofilm ($p < 0.05$, A 19.1 ± 2.3 h and 58.5 ± 7.7%, B 15.5 ± 1.1 h and 41.9 ± 5.3%, C 17.6 ± 1.9 h and 53.1 ± 7.5%, D 18.4 ± 2.4 h and 55.8 ± 8.8%, E 20.2 ± 3.3 h and 61.5 ± 10.0%, F 20.2 ± 3.0 h and 61.6 ± 9.3%, and G 18.3 ± 2.5 h and 55.3 ± 8.9%). Interestingly, there were no differences found between the treated groups ($p > 0.05$, A 0.064 ± 0.004 1/h, B 0.063 ± 0.005 1/h, C 0.065 ± 0.004 1/h, D 0.067 ± 0.004 1/h, E 0.066 ± 0.006 1/h, F 0.067 ± 0.004 1/h, G 0.066 ± 0.006 1/h) for the maximum growth rate. Vitality staining supported these findings. The present investigation demonstrates that the type of acid compounds used to produce the rinses did not show any negative effect on the antimicrobial properties of the tested products as all of them exhibited a similar efficacy against *S. mutans* biofilms.

Introduction

Mouth rinses are used in many different clinical situations for prophylactic as well as therapeutic purposes to improve oral health by reducing plaque and gingivitis or prevent infections after tooth extraction or intraoral surgery (Osso & Kanani 2013; Solderer et al. 2019). The main advantage of mouth rinses in comparison to other oral healthcare products is the simplicity of utilisation due to chemical and not mechanical plaque control by using a floss, interdental brush, or toothbrush that require fine motor skills and coordination ability. Not only might elderly people experience difficulties over time in executing proper oral care, but also people with systemic diseases or special needs can profit from the use of a mouth rinse to improve oral health (Jaccarino 2009; Sanchez-Garcia et al. 2011, Subramaniam & Gupta 2013).

Chlorhexidine, a widely-used compound of mouth rinses, has shown positive effects against plaque and gingivitis (Roldan et al. 2004; Osso & Kanani 2013) and reduces bleeding index (Polizzi et al. 2019). However, it also has side effects (e.g., loss of taste, staining of tongue and mucosa, or resulting in a burning sensation) which make it unsuitable for long-term use (Brookes et al. 2020). Moreover, exposure of clinical isolates of *Klebsiella pneumoniae* to chlorhexidine *in vitro* has led not only to stable resistance toward chlorhexidine but also cross-resistance toward the last line antibiotic colistin (Wand et al. 2017). Furthermore, highly translucent materials or monolithic zirconia can lose aesthetic properties when exposed to chlorhexidine (Derafshi et al. 2017; Lee et al. 2020). Thus, other studies have tried to find antimicrobial products suitable for daily use combining essential oils (Pan et al. 2010; Serbiak et al. 2018), Peptides (Mickels et al. 2001), or quaternary ammonium compounds (Rao et al. 2011).

Another important factor in caries prevention and control is the bioavailability of fluoride. This depends on the solubility of the fluoride containing compound and its adhesion to the tooth surface. The type of fluoride in rinses can be either inorganic (e.g., NaF) or organic (AmF) (Ten Cate 1999). It has been shown that organic fluorides have a greater anticariogenic property for two reasons: firstly, the amine component has an antibiofilm effect by inhibiting bacterial adhesion, and secondly, a tensioactive property which allows accumulation of fluoride close to the tooth surface providing a sustained fluoride release over time (Sh et al. 2013).

The weak hydrofluoric acid is 98% dissociated at pH above 5.0, but at pH 4.0 already 12% of the undissociated form exists as Hydrofluoric acid (HF). Fluoride enters the cells as Hydrofluoric acid (HF) and dissociates when exposed to higher intracellular pH (Breaker 2012). Thus, fluoride is stored both intracellularly as well as extracellularly. Intracellularly it is predominantly bound to cytoplasm proteins (e.g., enolase, urease, pyrophosphatases, aminopepidades) and extracellularly as CaF_2 stabilised by organic phosphates and matrix proteins (Marquis et al. 2003).

Every rinse is adjusted to a certain pH to have maximal efficacy against oral pathogens, and for this acidic compounds are added to the products. However, little is known whether these components have an effect on the efficiency of rinses. The difference of two acids on salivary bacterial counts and plaque formation has been investigated: either malic acid or gluconic acid was added to a mouth rinse with $NaClO_2$, and both formulations were compared to a chlorhexidine containing mouth rinse. No significant differences in efficacy were found between the chlorhexidine mouth rinse and the two $NaClO_2$ formulations (Yates et al. 1997).

Thus, to investigate further the effect of acid compounds in rinses to their efficacy, this in vitro study has the aim to assess the antibacterial effect of seven identical mouth rinses with 700 ppm F- (derived from NaF) that differ only by their acid compounds (malic, citric, tartaric, fumaric, hydrochloric, phosphoric, and lactic acid) with the null hypothesis that the efficacy remains the same, regardless of the acid compound.

Materials and Methods

Preparation of rinses

In the first step of rinse preparation, concentrated solutions were prepared with the same fluoride concentration and similar amine base plus organic acid concentrations. In the second step, 5% dilutions of the original solutions were made with demineralised water. The pH of each of these 5% dilutions was measured before the samples were released for further use. The pH of these working solutions stayed within the range of 4.1 to 4.3.

Biofilm formation and treatment

10 µl of *S. mutans* (ATCC™ 25175) stock solution was spread on a Columbia blood agar plate (BBL Columbia Agar Base; BD, Allschwil, Switzerland) and incubated for 48 h at 37°C. Thereafter, one colony was picked and added to 25 ml of Todd Hewitt medium (Bacto Todd Hewitt broth; BD, Allschwil, Switzerland) supplemented with 0.5% sucrose (D(+) sucrose; Fluka, Buchs, Switzerland). The culture was incubated for 22 h at 37°C. Then, the bacteria were harvested by centrifugation (8500 rpm, 5 min, RT; Sigma 4-16KS, Kuhner, Basel, Switzerland) and resuspended in simulated body fluid (7.996 g NaCl, 0.35 g $NaHCO_3$, 0.224 g KCl, 0.228 g $K_2HPO_4 \times 3H_2O$, 0.305 g $MgCl_2 \times 6H_2O$, 0.278 g $CaCl_2$, 0.071 g Na_2SO_4, 6.057 g $(CH_2OH)3CNH_2$ dissolved in 1 l of ultra-pure water, pH adjusted to 7.25 with 1 mol/l HCl, all chemicals from Sigma Aldrich, Buchs, Switzerland; according to Cho et al. 1995) supplemented with 10% Todd Hewitt medium and 1% sucrose. Meanwhile, 5 mm glass disks (Biosystems Switzerland AG, Muttenz, Switzerland) were coated with sterile pooled saliva mixture for 15 min. Briefly, saliva was collected by paraffin stimulation from young healthy volunteers in accordance with the World Medical Association Declaration of Helsinki. Before pooling, anonymised samples were ultrasonicated for 30 s (30 W; Vibracell, Sonics & Materials, Newtown, Connecticut, USA), filtered through a 70 µm filter (Cell Strainer, Becton-Dickinson, Basel, Switzerland) and centrifuged at 22 000 × g for 40 min at 4°C. The supernatant was sterile-filtered through two connected filters (0.45 and 0.22 µm; Millex-HV and Millex-GV, respectively; Merck-Millipore, Schaffhausen, Switzerland). The coated disks placed in 24-well plates (Sarstedt AG, Sevelen, Switzerland) and 1 ml of bacterial suspension and 0.5 ml of Todd Hewitt medium were added to each well, and the disks were incubated for 24 h at 37°C.

After 24 h, the disks with biofilms were dipped three times in 0.9% NaCl (Merck, Zug, Switzerland) and then placed for 30 s in 50% v/v mouth rinse solutions (Tab. 1) and placed in 0.9% NaCl. Biofilms exposed to 0.9% NaCl for 30 s served as untreated growth controls. Each set consisted of five replicates and experiments were repeated three times altogether for each of the mouth rinses and for the untreated control (n = 15).

Tab. I Seven experimental mouth rinses used in this study based on their major amine base

Mouth rinse	F– (ppm)	PEG–3 tallow aminopropylamine (ppm)	Acid added to adjust pH to 4.1–4.3
A	700	similar*	Malic acid
B	700	similar*	Citric acid
C	700	similar*	Tartaric acid
D	700	similar*	Fumaric acid
E	700	similar*	Hydrochloric acid
F	700	similar*	Phosphoric acid
G	700	similar*	Lactic acid

* More details regarding the used concentrations cannot be disclosed as these are subject to intellectual property rights.

Isothermal microcalorimetry (IMC)

Columbia blood agars were prepared, and treated biofilms as well as untreated controls were placed on the agar with the biofilm facing the agar. The IMC ampoules were closed in aerobic conditions and placed in TAM 48 instrument (TA Instruments; New Castle, Delaware, United States), where the metabolic activity of the biofilms was recorded at 37 °C for up to 72 h.
The heat flow data obtained over time was analysed for growth rate (1/h) and lag time (h) by fitting the heat-over-time curve (i.e., resulting from the integration of the heat flow curve) with Gompertz's equation with the "grofit" package in R statistical software (R Foundation for Statistical Computing, Vienna, Austria) as described earlier (Astasov-Frauenhoffer et al. 2014). Considering an average biofilm growth rate and that the lag time increase (roughly a biofilm doubling time) corresponds to a 2× reduction in the bacterial population (Braissant et al. 2015), the reduction of the biofilm population was estimated by using the following approach (1):

$$Inhibition = 100 - \frac{100}{2^{\left(\frac{(lag\,time_{sample} - lag\,time_{control})}{\frac{\ln(2)}{growth\,rate_{controls}}}\right)}}$$

Vitality staining

Biofilms were prepared and treated as described for IMC. The biofilms were thereafter stained with a live/dead staining kit according to the manufacturer's instructions (LIVE/DEAD™ BacLight™ Bacterial Viability Kit, for microscopy, Invitrogen, Thermo Fisher Scientific, Allschwil, Switzerland). 20 µl of the prepared staining solution was added to each sample followed by 15 min incubation in the dark at room temperature before assessing the biofilms with a Leica SP8 microscope (Leica SP8, Heerbrugg, Switzerland) using 63× (1.4×) oil immersion objective with Z-stack step of 0.3 µm.

Statistical analysis

Normality test was applied to the samples and differences between mouth rinses and untreated controls were assessed by Student's t-test with significance set to $p < 0.05$, and differences between the different acids and amino bases were assessed by one-way ANOVA and Tukey post hoc test using GraphPad Prism (version 9.2.0 for Mac, GraphPad Software, La Jolla, California USA, www.graphpad.com).

Results

This study shows that all biofilms treated with mouth rinses, independently of the acid used to adjust pH, had a significantly prolonged lag time of the bacteria ($p < 0.05$, rinse A 19.1 ± 2.3 h, rinse B 15.5 ± 1.1 h, rinse C 17.6 ± 1.9 h, rinse D 18.4 ± 2.4 h, rinse E 20.2 ± 3.3 h, rinse F 20.2 ± 3.0 h, and rinse G 18.3 ± 2.5 h) (Fig. 1 A) which means that a larger number of bacteria was killed in the treated biofilms compared to the non-treated ones (8.8 ± 0.5 h). Mouth rinse B, however, had a significantly lower antimicrobial effect than all other mouth rinses demonstrating a significantly lower lag time ($p < 0.05$) (Fig. 1 A). As the only difference in the composition of mouth rinse B was citric acid, it is reasonable to assume that citric acid reduced the antimicrobial properties of the mouth rinse. Among the other mouth rinses, no significant difference in bacterial lag time was observed showing that all other acids tested had similar antibacterial properties ($p > 0.05$) (Fig. 1 A).

Similar results were obtained for the maximum growth rate (Fig. 1 B) as all mouth rinses significantly decreased this parameter for the biofilms ($p < 0.05$, A 0.064 ± 0.004 1/h, B 0.063 ± 0.005 1/h, C 0.065 ± 0.004 1/h, D 0.067 ± 0.004 1/h, E 0.066 ± 0.006 1/h, F 0.067 ± 0.004 1/h, G 0.066 ± 0.006 1/h) in comparison to the untreated control biofilms (0.087 ± 0.003 1/h). Interestingly, citric acid showed a similar effect to the other acids ($p > 0.05$) indicating that all mouth rinses tested in this study, independently of the acid added, had a similar bacteriostatic effect on the biofilms (Fig. 1 B).

The calculation of active biofilm is shown in equation 1 and indicates a reduction of the biofilm activity after mouth rinse treatment in comparison to the control of untreated biofilms in percentages (Fig. 1C). Here again, mouth rinse B containing citric acid shows significantly lower reduction of the activity (41.9 ± 5.3%) than the other mouth rinses ($p > 0.05$, rinse A 58.5 ± 7.7%, rinse C 53.1 ± 7.5%, rinse D 55.8 ± 8.8%, rinse E 61.5 ± 10.0%, rinse F 61.6 ± 9.3%, and rinse G 55.3 ± 8.9%) correlating with the lower antimicrobial properties already described. The other mouth rinses showed no significant differences between each other (Fig. 1C).

Vitality staining allows the assessment of efficacy based on cell membrane intactness. No differences in the pattern or

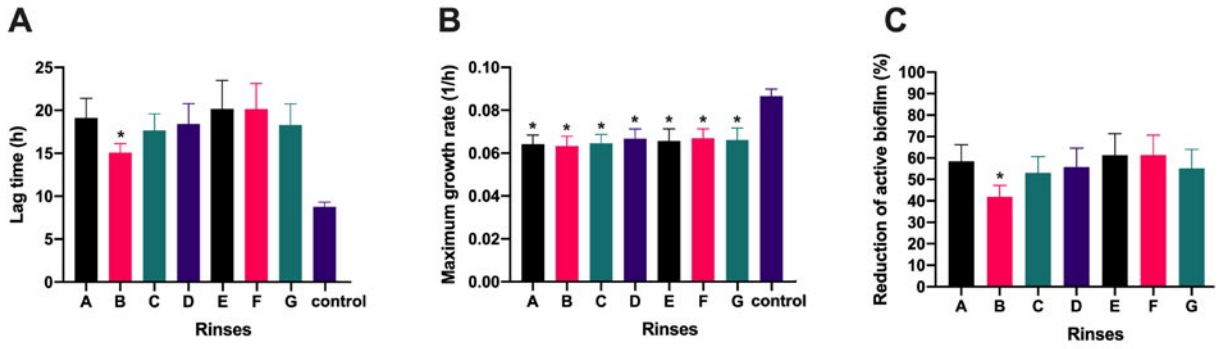

Fig. 1 Three different parameters were assessed from IMC data: (A) lag time in h, (B) maximum growth rate in 1/h, and (C) reduction of active biofilm in % compared to untreated control biofilm calculated using equation 1. Seven rinses were produced using different acids and examined for their antimicrobial properties: A (malic acid), B (citric acid), C (tartaric acid), D (fumaric acid), E (hydrochloric acid), F (phosphoric acid), and G (lactic acid); as well as the untreated control. * indicates statistical difference ($p < 0.05$) to the untreated control (in A and B), and to other groups (in C).

depth of penetration through the biofilm could be observed between the samples. These results demonstrate similar effect with all rinses as can be seen in Fig. 2. Thus, these qualitative images support the quantitative results obtained by IMC.

Discussion

Mouth rinses are easy-to-use oral health products, and their compounds can have diverse benefits on oral health such as antibacterial effects (Polizzi et al. 2019), the reduction of aphthous ulcerations (Sharquie et al. 2006), or the remineralisation of early carious lesions (Hegazy & Salama 2016). In this study, different acids in the composition of experimental rinse together with 700 ppm F- were evaluated to assess their efficacy against the growth of *S. mutans* biofilms. These acids were initially added primarily to adjust the pH. However, as no other component differed within the tested formulations, the effect of the acids on antimicrobial properties of the rinses could be directly evaluated.

With respect to the bactericidal or bacteriostatic effect of an antimicrobial, using IMC, it can be considered that a thermogram shifted in time (increase of lag duration) is indicative of a bactericidal effect (a fraction of the inoculum is killed, and the rest grows "normally"). On the contrary, a thermogram showing lower peaks and a heat-over-time curve with a clear difference in slopes (decrease in growth rate) are considered as indicative of a bacteriostatic effect. This is exemplified by the paper of von Ah and colleagues (2009) where curves for chloramphenicol (typically considered as bacteriostatic) and gentamycin (typically considered as bactericidal) can be seen (Von Ah et al. 2009).

The results show that all experimental rinses had a similar antibacterial effect independently of the acid used meaning that all rinses were able to inhibit bacterial growth. The bactericidal effect differed between the mouth rinse with citric acid added and the other acids tested: the addition of citric acid as pH adjuster showed a slightly lower lag time of *S. mutans* and a lower

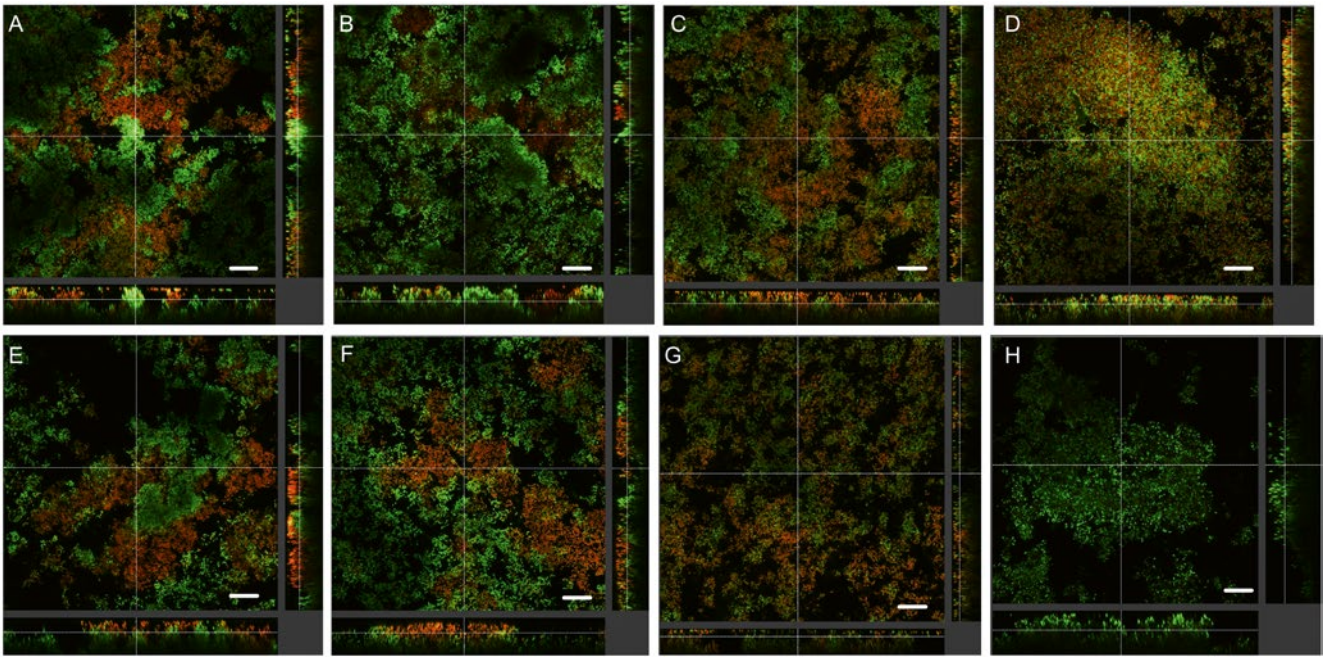

Fig. 2 Vitality staining of biofilms treated with seven rinses produced using different acids: A (malic acid), B (citric acid), C (tartaric acid), D (fumaric acid), E (hydrochloric acid), F (phosphoric acid), and G (lactic acid); as well as the untreated control (H). The bar indicates 10 µm. Green signal indicates alive cells while red signal corresponds to dead cells.

reduction of active biofilm than the other acids. This result was surprising as the different acids were only used to adjust the pH of the mouth rinses and not as an active ingredient. In dental medicine, citric acid has been used before as a final irrigant during endodontic treatment to remove residual bacteria from the root canal system (Yamaguchi et al. 1996; Siqueira et al. 1998). In this context, citric acid was shown to have a bigger inhibition potential on *S. mutans* than the 2% chlorhexidine solution (Siqueira et al. 1998). However, in this study, citric acid was part of a formulation, and its low concentration or the interaction with other compounds in the experimental rinse might have slightly altered its antibacterial properties leading to a reduction in original surviving inoculum detected by lag time. The overall bactericidal efficacy was, however, not affected. Growth rate measurements were comparable to other rinses.

All other acids had a statistically similar bactericidal potential on *S. mutans* growth. For this reason, other properties of the different acids might be considered. Lactic acid in a mouth rinse has been shown to be good against aphthous ulcers reducing signs and symptoms of the disease in comparison to a placebo (Sharquie et al. 2006) or to another mouth rinse (Ibrahim et al. 2020). Fumaric acid has also been studied for this purpose as it has been shown to modulate cytokine production in immune cells (Lehmann et al. 2007). A case report of a patient suffering from aphthous ulcers and treated with tablets of fumaric acid ester has shown a complete remission of the symptoms for about 17 months (Guenova & Hoetzenecker 2011). Thus, further clinical studies with patients suffering from recurrent aphthous stomatitis comparing the use of mouth rinses with fumaric or lactic acid as pH adjusters should be done to see if beneficial effects can be achieved.

Malic acid in lozenge has been shown to increase saliva production in people with Sjögren's syndrome resulting in xerostomia relief and an improved quality of life (Da Mata et al. 2020). Thus, it would be interesting to assess whether the addition of malic acid as a pH adjuster in a mouth rinse could have beneficial effects for patients with Sjögren's syndrome. This could also open up new perspectives for prosthetic restorations in patients with Sjögren's syndrome (D'orto et al. 2020).

Tartaric acid also appears in the literature as an antimicrobial ingredient. It has been used in effervescent mouth rinse tablets (Singh et al. 2020) or diluted in a solution to study the effect of different ingredients on *Neisseria gonorrhoeae* (Van Dijck et al. 2019). However, no study on the beneficial or adverse effect of tartaric acid on oral biofilms could be found, highlighting the fact that more studies are also needed here to evaluate potential clinical impact.

All rinses used in this study contained 700 ppm F– as rinsing with water directly after tooth brushing might remove residual fluoride (Pitts et al. 2012). However, higher levels of fluoride can inhibit demineralisation during acid challenge and enhance remineralisation when the pH level rises (Ten Cate 2013). For this reason, rinsing with a mouth rinse containing fluoride is beneficial and may compensate for this possible loss (Duckworth et al. 2009). A study on the interaction between sodium fluoride and a CPC-containing mouth rinse formulation showed that sodium fluoride did not affect the antibacterial and antibiofilm properties of the formulation, emphasising the benefit of adding fluoride (Latimer et al. 2015). Similar to toothpastes, fluoride in a mouth rinse can be added in different forms including amine fluoride, stannous fluoride, sodium monofluorophosphate and sodium fluoride. Stannous fluoride has been shown to be very effective in preventing erosive substance loss (Schlueter et al. 2009), in contrast to amine fluoride that has been shown to have no effect on erosion progression (Ganss et al. 2008). For the remineralisation of demineralised enamel area, an *in vitro* study has shown that sodium monofluorophosphate in association with calcium glycerophosphate was very effective (Puig-Silla et al. 2009). For caries prevention in adolescents, the daily or weekly use of sodium fluoride mouth rinse was shown to reduce the number of decayed/missing/filled surfaces up to an average of 39%. Additionally, for the prevention of root caries, the daily use of a 0.2% sodium fluoride mouth rinse is likely to be the most effective self-applied topical fluoride method (Zhang et al. 2020).

The main limitation of the present study was the use of a single-species biofilm to screen for the antimicrobial effect on cariogenic biofilms. *S. mutans* has been considered a keystone pathogen in the initiation and progression of caries (Tanzer et al. 2001), and its levels have been used as part of caries risk assessment (Guo & Shi 2013; Edelstein et al. 2016). However, also contradicting studies exist reporting no correlation between caries experience and *S. mutans* counts (Aas et al. 2008). A possible explanation for the differing in population could be the ecological plaque hypothesis (Marsh 1994), which proposes that *S. mutans* might not be the primary aetiological factor for caries. According to this hypothesis, caries results from a disrupted homeostasis of the resident microflora driven by changes in local environmental conditions (Wade 2013). Thus, a more complex biofilm would allow for broader conclusions regarding antimicrobial efficacy, covering claims over more species. However, *S. mutans* is still very often used as a model organism for caries for first screening of anticariogenic agents in vitro. Another limitation of the study is the in vitro design. However, the primary screening for the best performing formulation needs to be assessed in a lab environment before going into clinical studies. The low number of replicates and the rather high number of groups used in the statistical analysis is clearly a limitation of this study. To further investigate such limitation, a retrospective power analysis was performed. It showed that statistical analysis were sufficiently powered (power > 0.9) to detect differences between the controls and other groups, smaller differences between treatment groups should, however, be considered with care.

Conclusions

The present investigation demonstrates that the use of all seven rinses tested leads to a significant reduction of vitality in the *S. mutans* biofilms regardless of the acid compound used for pH adjustment. Furthermore, the acids have no negative effect on the antimicrobial properties of the formulations used in this study.

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Zusammenfassung

Einleitung

Mundspülungen werden in vielen verschiedenen klinischen Situationen sowohl zu prophylaktischen als auch zu therapeutischen Zwecken verwendet, um die Mundgesundheit durch die Reduzierung von Plaque und Gingivitis zu verbessern oder Infektionen nach Zahnextraktionen oder intraoralen Eingriffen zu verhindern. Jede Spülung wird auf einen bestimmten pH-Wert eingestellt, um eine maximale Wirksamkeit gegen orale Krankheitserreger zu erzielen; zu diesem Zweck werden den Produkten saure Verbindungen zugesetzt. Es ist jedoch wenig darüber bekannt, ob diese Bestandteile die Wirksamkeit der Spülungen beeinflussen.

In dieser In-vitro-Studie wurde die antibakterielle Wirkung auf *Streptococcus-mutans*-Biofilme von sieben Mundspülungen mit 700 ppm F- (abgeleitet von NaF) untersucht, die sich nur durch die zur Einstellung des pH-Werts verwendeten Säureverbindungen (Apfel-, Zitronen-, Wein-, Fumar-, Salz-, Phosphor- und Milchsäure) unterschieden.

Material und Methoden

S. mutans (ATCC™ 25175) wurde 22 Stunden lang bei 37 °C gezüchtet, in der stationären Wachstumsphase geerntet und in frischem nährstoffarmem Medium resuspendiert. Die Bakterien durften 24 Stunden lang bei 37 °C an proteinbeschichteten Scheiben haften. Danach wurden die Biofilme 30 Sekunden lang mit experimentellen Spülungen behandelt, und die Wärmekurven wurden 72 Stunden lang in einem isothermen TAM48-Mikrokalorimeter bei 37 °C gemessen. Das Gompertz-Wachstumsmodell wurde verwendet, um zwei Wachstumsparameter aus den ermittelten Wärmekurven zu bestimmen: Anlaufsphase und Wachstumsrate; ausserdem wurde die Reduktion der aktiven Biofilme berechnet. Darüber hinaus wurden alle Probengruppen nach einer Vitalitätsfärbung mittels konfokaler Rastermikroskopie analysiert.

Resultate

Diese Studie zeigt, dass alle mit Mundspülungen behandelten Biofilme, unabhängig von der zur Einstellung des pH-Werts verwendeten Säure, eine signifikant verlängerte Anlaufsphase der Bakterien aufwiesen ($p < 0,05$), was bedeutet, dass in den behandelten Biofilmen eine grössere Anzahl von Bakterien abgetötet wurde als in den unbehandelten. Somit hatten alle in dieser Studie verwendeten Mundspülungen eine bakterizide Wirkung. Ähnliche Ergebnisse wurden für die maximale Wachstumsrate erzielt, da alle Mundspülungen diesen Parameter für die Biofilme signifikant verringerten ($p < 0,05$). Im Vergleich zwischen den Mundspülungen hatte jedoch die Spülung B, deren pH-Wert mit Zitronensäure eingestellt wurde, eine signifikant geringere bakterizide Wirkung als alle anderen Mundspülungen und wies eine signifikant geringere Anlaufsphase auf ($p < 0,05$). Da der einzige Unterschied in der Zusammensetzung der Mundspülung B die Zitronensäure war, kann man davon ausgehen, dass die Zitronensäure die bakteriziden Eigenschaften der Mundspülung verringert. Die durch Vitalitätsfärbung gewonnenen qualitativen Bilder unterstützen die quantitativen Ergebnisse der Isothermal-Microcalorimetry(-IMC)-Experimente.

Diskussion

Es ist schon gezeigt geworden, wie sich zwei Säuren auf die Anzahl der Speichelbakterien und die Plaquebildung auswirken: Einer Mundspülung mit Natriumchlorit wurde entweder Apfelsäure oder Gluconsäure zugesetzt, und beide Formulierungen wurden mit einer chlorhexidinhaltigen Mundspülung verglichen. Da keine signifikanten Unterschiede in der Wirksamkeit zwischen der Chlorhexidinmundspülung und den beiden Natriumchloritformulierungen festgestellt wurden, kann die mit Apfelsäure oder Gluconsäure angesäuerte Natriumchloritspülung für den klinischen Einsatz empfohlen werden.

Alle Spülungen in dieser Studie zeigten sowohl bakteriostatische als auch bakterizide Wirkungen gegen den getesteten Biofilm von *S. mutans*. Die Ergebnisse der vorliegenden Untersuchung zeigen, dass die Art der zur Herstellung der Spüllösungen verwendeten Säureverbindungen keinen negativen Einfluss auf die antimikrobiellen Eigenschaften der getesteten Produkte hatte, da alle eine ähnliche Wirksamkeit gegen kariogene Biofilme aufwiesen.

Résumé

Introduction

Les bains de bouche sont utilisés de manière prophylactique et thérapeutiques dans de nombreuses situations cliniques. Ils permettent d'améliorer la santé bucco-dentaire en réduisant la plaque et la gingivite, ou en diminuant le risque d'infection après une extraction ou intervention chirurgicale. Pour une efficacité maximale, le pH de chaque formulation de bain de bouche est ajusté à l'aide de composés acides. Pourtant, peu d'informations relatives au choix du composé acide sur l'efficacité des formulations de bains de bouche sont disponibles.

Dans cette étude, nous avons analysé l'effet antibactérien de sept bains de bouche enrichis avec 700 ppm de fluor (NaF) sur un biofilm de *Streptococcus mutans*. La composition des sept bains de bouche était identique à l'exception de l'acide utilisé pour réguler le pH de la solution. Les acides suivants ont été testés : acide malique, acide citrique, acide tartrique, acide fumarique, acide chlorhydrique, acide phosphorique et acide lactique.

Matériel et méthodes

La souche ATCC™ 25175 de *S. mutans* a été cultivée durant 22 heures à une température de 37 °C, récoltée en phase stationnaire et resuspendue en milieux de culture pauvres en nutriments. Les bactéries ont ensuite été incubées à 37 °C sur des lamelles de verre avec revêtement de protéines permettant une croissance en adhésion et la formation d'un biofilm. L'effet des différentes solutions de bains de bouche a été testé sur les biofilms 30 secondes, puis, la courbe de production de chaleur des biofilms a été enregistrée durant 72 heures dans un microcalorimètre-TAM48 isotherme. Le modèle de croissance Gompertz a été utilisé pour déterminer deux paramètres de croissance : le temps de latence initial et taux de croissance. De plus, la réduction du biofilm actif a été calculée et des photographies ont été prises avec un microscope confocal à balayage laser après coloration des cellules viables.

Résultats

Les résultats de cette étude montrent une différence significative sur l'effet bactéricide ainsi que sur la phase de croissance initiale ($p<0,05$) de la solution B, dont le pH a été ajusté au moyen d'acide citrique, en comparaison avec les autres solutions de bains de bouche. L'acide utilisé pour la régulation du pH étant le seul composé variable entre les différentes solutions, on peut donc suggérer que l'acide citrique réduit l'effet bactéri-

cide de cette composition de bain de bouche. Les images effectuées au microscope confocal à balayage laser sont en accord avec les analyses quantitatives.

Néanmoins, tous les biofilms traités avec les solutions de bains de bouche testées (incluant la solution B et indépendamment de l'acide utilisé pour l'ajustement du pH) présentaient une phase de croissance initiale prolongée ($p<0,05$) indiquant une plus grande réduction du nombre de bactéries au contact des solutions de bains de bouche que les contrôles non traités. Toutes les solutions de bains de bouche présentent donc un effet bactéricide. De manière similaire, le taux de croissance affiche une réduction significative après traitement avec les différents bains de bouche ($p<0,05$).

Discussion

Des études antérieures ont montré que l'addition d'acide malique ou d'acide gluconique à des solutions de chlorite de sodium avait un effet comparable sur le nombre de bactéries salivaires ainsi que sur le développement de la plaque. En effet, une solution de bain de bouche a été enrichie en acide malique ou en acide gluconique et les deux formulations ont été comparées à une solution contenant de la chlorhexidine. Aucune différence significative n'a été observée entre la solution contenant de la chlorhexidine et les deux solutions de chlorite de sodium. Aussi bien l'addition d'acide malique que l'addition d'acide gluconique à une solution de chlorite de sodium peuvent donc être recommandées pour l'usage clinique.

Dans cette étude, des résultats similaires ont été obtenus. En effet, toutes les solutions de bains de bouche affichaient un effet bactériostatique ainsi que bactéricide sur un biofilm de *S. mutans*. Les résultats montrent donc qu'aucun des acides testés ne supprime les propriétés antimicrobiennes des solutions de bains de bouche car toutes les solutions affichaient un effet significatif sur un biofilm cariogène. D'autres propriétés des différents acides devraient donc être prises en compte pour choisir l'acide adapté aux différentes situations cliniques.

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