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Short communication

# The effect of delmopinol and fluoride on acid adaptation and acid production in dental plaque biofilms

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ABSTRACT

*Objective:* To investigate the effect of delmopinol and fluoride alone or in combination on acid adaptation and acid production in plaque biofilm bacteria *in vitro*.

*Design:* The effect of delmopinol and fluoride on acid adaptation was tested by exposing the biofilm bacteria, grown in a mini-flow cell system under static conditions, to pH 5.5 overnight in the presence of 0.16 mM delmopinol, 1 mF NaF or a combination of both. The following day, acid adaptation was evaluated by exposing the cells to an acid challenge for 2 h at a pH known to kill non-adapted cells (pH 2.5). The cells were stained using LIVE/DEAD® BacLight™ Viability stain and the number of viable (acid tolerant) cells was determined using confocal scanning laser microscopy. Control cells were treated in the same manner but without the exposure to delmopinol or fluoride. How delmopinol and fluoride affected acid production was assessed by measuring the pH-drop after glucose pulsing in the presence of delmopinol and/or different concentrations of fluoride.

*Results:* Fluoride alone or in combination with delmopinol affected the acid adaptation and significantly reduced the acid tolerance of the plaque biofilm. This effect was more pronounced when the two compounds were combined. Delmopinol alone did not affect acid adaptation. A combination of delmopinol and fluoride also reduced acid production at concentrations where neither of the compounds in isolation had an effect.

*Conclusion:* Fluoride and delmopinol can work synergistically to affect acid adaptation and acid production in plaque biofilm bacteria.



## 1. Introduction

Bacteria in the oral cavity are subjected to many environmental stresses, including recurrent cycles of low pH. This results from the production of organic acid by the bacteria themselves following exposure to dietary carbohydrates.[1] Frequent intake of easily fermentable carbohydrates will result in prolonged periods of low pH in plaque and this will favour the growth of bacteria that are acid-tolerant. While some bacteria such as lactobacilli are constitutively acid tolerant, others can, in response sub-lethal pH values, initiate an acid tolerance response (ATR) and thereby adapt to an acidic environment. The ATR is a phenotypic change of the bacteria and involves changes in protein expression, increased ATPase activity and shifts to lower pH optimum for glucose

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transport and glycolysis.[2–4] The ATR thereby allows the bacteria to survive and continue to produce acids at low pH values.[3,5,6] *Streptococcus mutans* biofilm grown cells have been shown to express a more acid tolerant phenotype. This characteristic disappears if the biofilms cells are dispersed indicating that the acid tolerance are of phenotypic and not genotypic nature.[7] An acid tolerant microflora can cause prolonged periods of low pH in plaque and this will cause demineralization of the enamel and the development of dental caries.[6,8]

Fluoride is well known for its ability to reduce the incidence of caries. Its primary anti-caries effect is thought to be the conversion of hydroxyapatite to fluorapatite, which resists acid dissolution to a greater extent than hydroxyapatite.[9,10] However, another important property of fluoride is its effect on bacterial physiology. Fluoride inhibits bacterial carbohydrate metabolism by affecting the enzyme enolase in the glycolytic pathway.[11,12] Fluoride has also been shown to affect a range of other enzymes such as catalase, urease and the F-ATPases.[13–15] The proton-translocating ATPases play a major role in the acid-base physiology of oral streptococci and lower ATPase activity results in a reduced bacterial acid tolerance.[16] A more recently discovered property of fluoride is that it can inhibit the induction of an ATR in *S. mutans* and it has been shown to reduce acid tolerance in plaque biofilms *in vivo*.[17,18]

Delmopinol is a surface-active agent used in dental products. In comparison with other products on the market, delmopinol has a low antimicrobial profile and promotes a microbial flora compatible with dental health.[19,20] The compound binds to hard and soft oral tissues as well as to bacterial surfaces and affects several of the steps in the formation and establishment of dental biofilms. These include displacement of components from the pellicle,[21] and interference with the build-up and cohesion of plaque by reducing glucan synthesis and glucan viscosity.[22–25] Delmopinol may also reduce cell to cell adhesion by changing the colloidal stability of bacterial suspensions[26] and by detaching or solubilising surface structures of oral bacteria,[27] and has been demonstrated to affect acid production in oral bacteria.[19] The purpose of the present study was therefore to investigate whether delmopinol and/or fluoride can inhibit acid adaptation and/or acid production in plaque biofilms *in vitro*, and if the combination of delmopinol and fluoride would enhance the effect.

## 2. Materials and methods

### 2.1. Media and test compounds

For the establishment of a plaque biofilm, a semi-defined growth medium Bacto™ Todd Hewitt Broth (Becton, Dickinson and Company, USA) was used. For the acid adaptation and acid killing experiment a fully defined minimal medium (MM4) was used. MM4 contains six amino acids: glutamate, serine, cysteine, valine, leucine, asparagine, 20 mM glucose and 40 mM phosphate/citrate buffer adjusted to pH 7.5, 5.5 or 2.5 and has been used previously in acid adaptation experiments.[28] Test compounds used were delmopinol hydrochloride, batch No. RB997 (Sinclair Pharma AB, Sweden) and sodium fluoride, (Sigma–Aldrich, USA).

### 2.2. Plaque biofilm formation

After refraining from brushing the teeth for 12 hours, plaque was sampled in the morning from the buccal and lingual tooth surfaces of a healthy individual and pooled. The same donor was used throughout the entire study. The plaque sample was suspended in 50 μl 0.1 M potassium phosphate buffer (PBS) followed by the addition of 1.5 ml Todd–Hewitt broth pH 7.0 and mixed by vortexing. To each channel of a mini flow-cell, μ-Slide VI for Live Cell Analysis (Ibidi®, Ibidi GmbH, Martinried, Germany) with a growth area of 0.6 $cm^2$, 120 μl of plaque suspension was added and incubated under static conditions for 24 h at 37 °C in air with 5% $CO_2$ in a humid chamber. These biofilms were then used for either acid adaptation or acid production experiments (Fig. 1). All experiments were run under static conditions.

### 2.3. Acid adaptation in plaque biofilms

To test if delmopinol and fluoride could affect acid adaptation in plaque biofilm cells, a modified version of a method developed previously for testing the effect of fluoride on acid adaptation was employed.[17] The 24-h plaque biofilms, prepared as described above, were exposed to MM4 pH 5.5 with the addition of 1 mM fluoride or 0.16 mM delmopinol alone or in

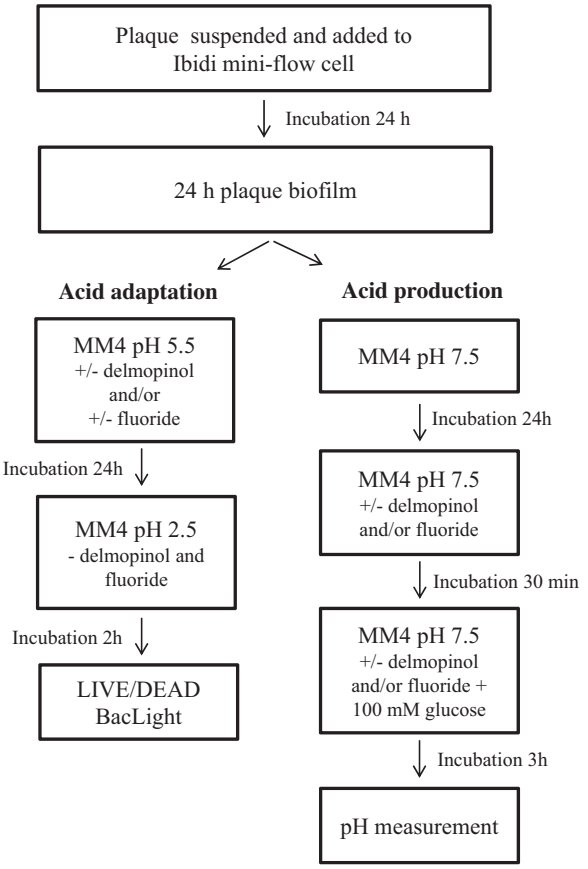


**Fig. 1 – Schematic overview plaque biofilm formation, acid adaptation and acid production experiments.**