Ratiometric monitoring of extracellular pH at the microscale in in situ-grown dental biofilms exposed to different saliva flow velocities and film thicknesses

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Background

Extracellular pH in *in situ*-grown dental biofilms has never been monitored at the microscale in the presence of **flow** with undiluted **whole saliva** as the flow medium and a **saliva film thickness** that matches intraoral estimates.

Aim

This study aimed to monitor extracellular pH inside *in situ*-grown biofilms from 9 **healthy participants** (48-h, n=27; 96-h, n=27) and determine which factors influence the pH at the microscale.

Materials and methods

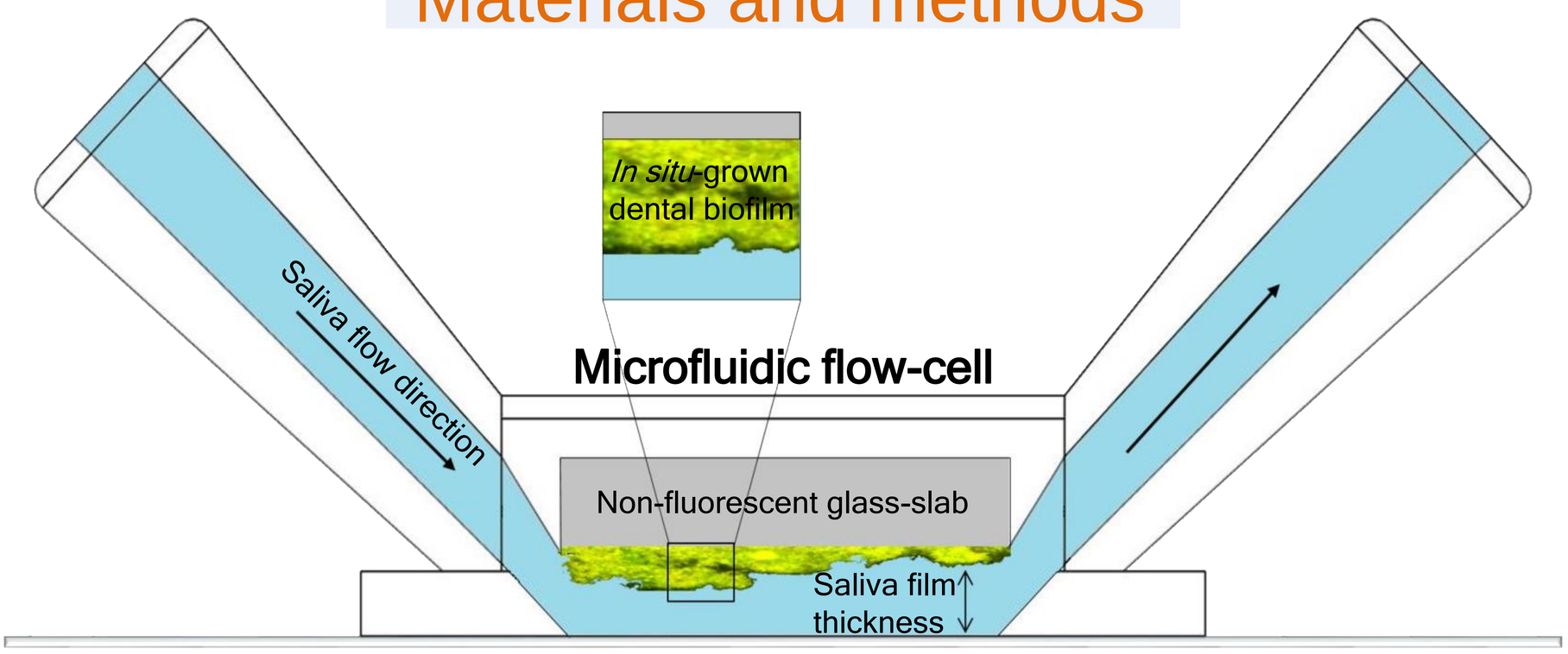

Figure 1. Schematic illustration of the experimental setup. Extracellular pH was monitored inside *in situ*-grown dental biofilms using confocal-microscopy-based pH ratiometry. In each biofilm, pH was measured under static and different flow conditions (0.8 mm/min; 8 mm/min; 30 mm/min) in 12 different locations at the biofilm top and base. pH results were correlated to the microbial composition of the biofilms, as determined by 16s rRNA sequencing.

Results

Determinants of extracellular pH in dental biofilms

	Biofilm thickness	Biofilm age	Horizontal location	Vertical location	Saliva film thickness	Flow velocity
High pH	Thin	48 h	Up-stream	Biofilm top	Thick	High
Low pH	Thick	96 h	Down-stream	Biofilm bottom	Thin	Low

Figure 2. Determinants of biofilm pH. Each of the identified factors had a significant effect on the extracellular pH in dental biofilms (P<0.05).

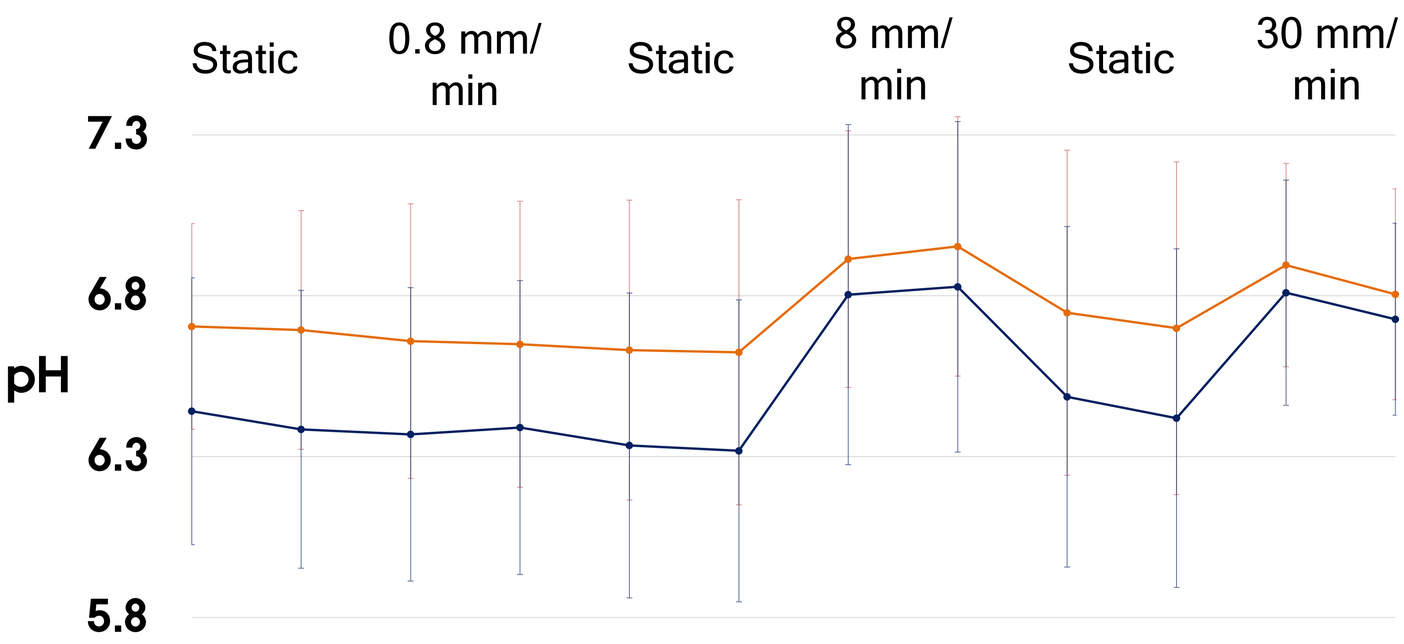

Figure 4. Average biofilm pH under static and flow conditions. Average pH of all 48-h biofilms (orange; n=27) and 96-h biofilms (**dark blue**; n=27). A high flow velocity (8 mm/min) increased the pH significantly (48-h, P=0.0038; 96-h, P= 0.001). Error bars = SD

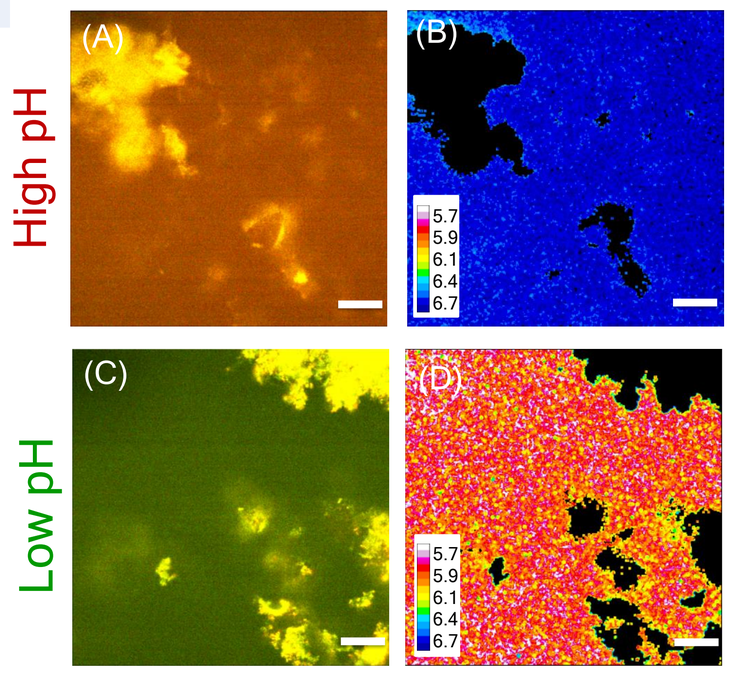

Figure 3. Representative images of extracellular pH in dental biofilms. (A+C) Raw images. (B+D) Corresponding processed images that illustrate extracellular pH in a location with high (B) and relatively low (D) pH. Scale bars = 20 µm.

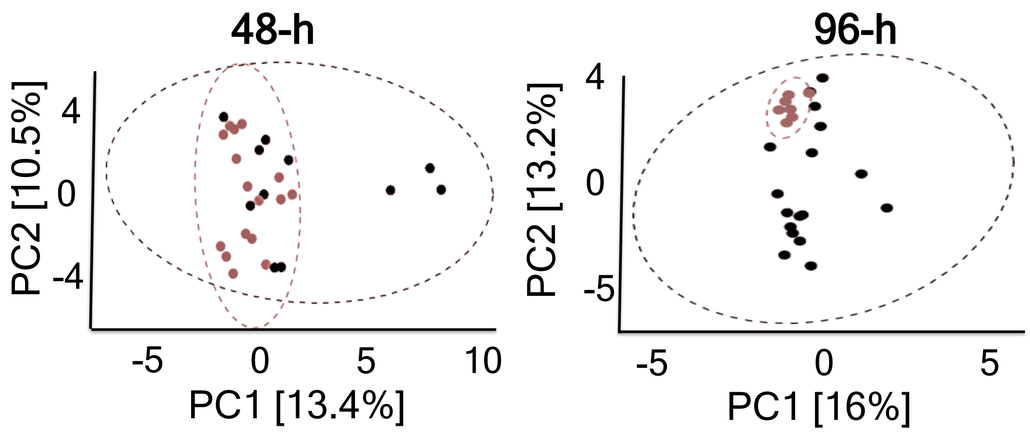

Figure 5. Principal component (PC) analysis of bacterial composition. The bacterial composition of the biofilms clustered according to pH (**weak** or **strong** pH response, defined as a pH drop of **≤0.5** or **>0.5** pH units after 5 min of sucrose challenge).

Conclusions

The pH response of *in situ*-grown dental biofilms to sucrose is influenced by a variety of factors, including biofilm age and thickness, the microbial composition of the biofilms, the saliva flow velocity and the thickness of the saliva film. Biofilms exhibit both horizontal and vertical pH gradients, with lower pH downstream and at the biofilm base.