Microfluidic “plaque-on-a-chip” with pH new imaging concept probes individual factors in tooth decay

Your teeth are covered in biofilm called plaque. This biomaterial consists of bacteria in a protective matrix of proteins, polysaccharides and other long organic molecules. When you eat or drink, bacterial respiration and other metabolic processes convert molecules like sugars into acid by-products, which become trapped against your teeth. Dentists know that a rapid decrease in pH (acidification) occurs after a meal, followed by a slow return to neutral conditions. The lower the pH value and the longer it stays below a critical value (around 5.5), the more risk there is for tooth decay. Not only are current methods for acquiring such data indirect, but the mechanisms responsible for the acidification and recovery are not well-studied. To help dental health professionals better understand how chemistry and fluid movement around teeth play a role in the oral acid cycle, a microfluidic platform was developed capable of mapping surface acidification of intact oral biofilms.

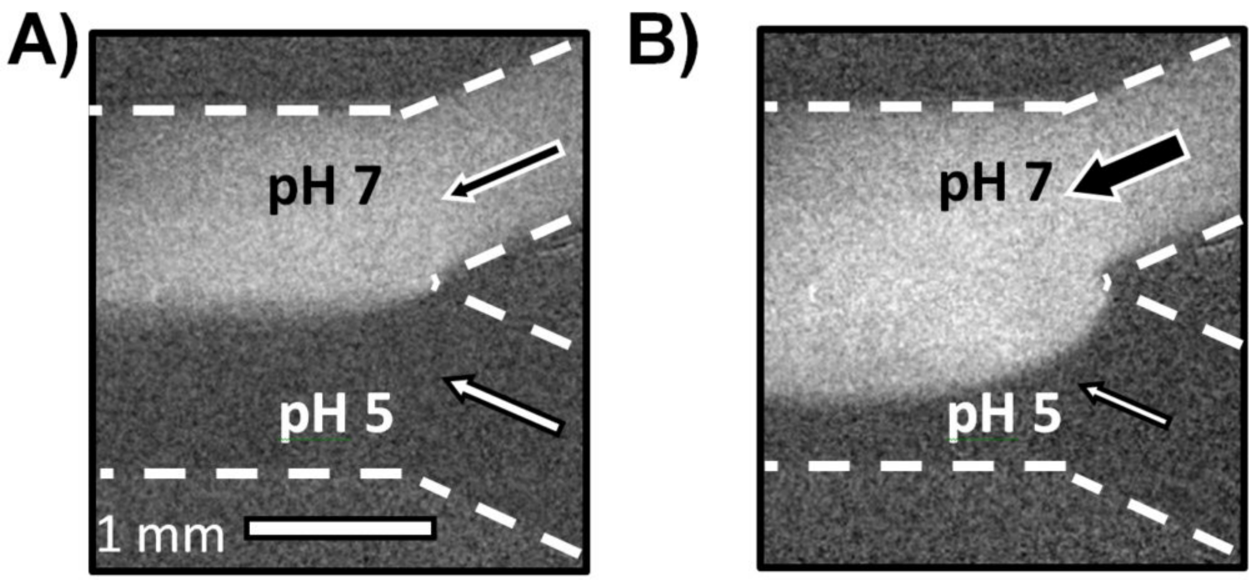

Fig. 1. Fluorescence microscopy images of a channel with co-flowing pH 7 and pH 5 water, before (A) and after (B) flow disturbance.

Mapping pH in aqueous systems is not easy. Acidic and basic solutions are indistinguishable under microscopes and spectrophotometers, but their chemical properties are very different. The pH of a liquid is measured by the logarithmic pH scale

$$\mathrm{pH} = -\log 10^{[\mathrm{H}^+]}$$

where $[H^+]$ is the concentration of protons. So the difference in proton concentration after a meal (say, pH 5) can be 100 times higher than normal (pH 7). We took advantage of the fact that the intensity of some fluorescent molecules varies with pH to measure the acidity of their environment. But, calibrating fluorescence intensity to pH is notoriously unreliable. We solved this problem by encapsulating fluorophores in nanoparticles, giving excellent brightness, stability and response time. This was demonstrated in a microfluidic device by co-flowing streams of pH 5 and pH 7 side-by-side and generating flow disturbances (Fig. 1). The chemically bound pH-sensitive nanoparticle layer at one of the microchannel walls enabled real-time pH imaging using a microscope (Fig. 1).

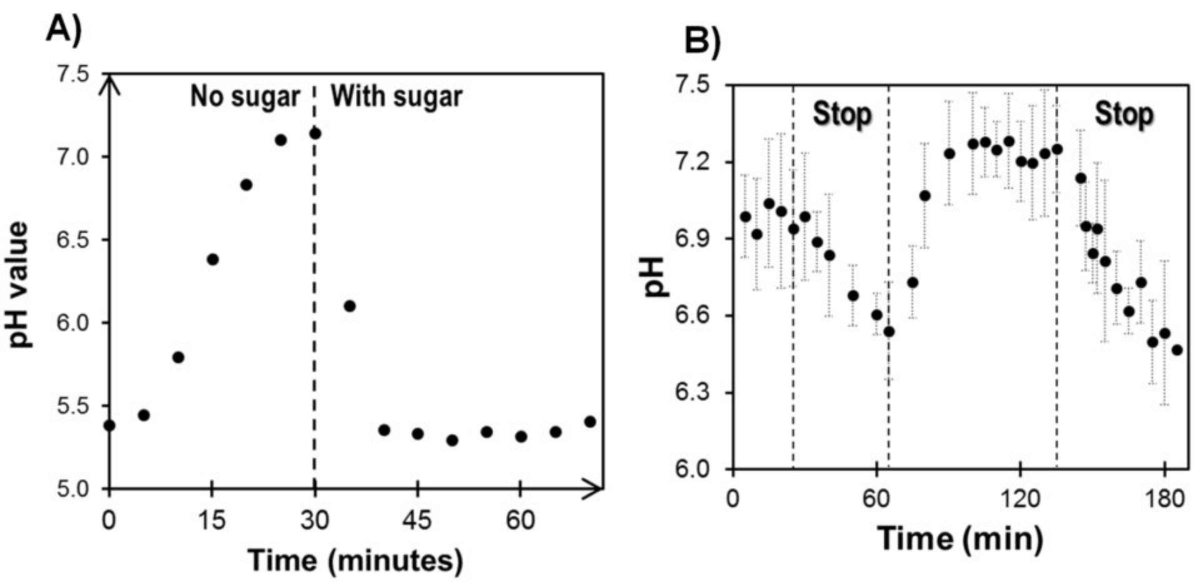

Fig. 2. (A) Typical pH response to glucose (sugar) exposure and removal. (B) Influence of liquid flow rates to pH. Increased acidity (reduced pH) when flow stops, and reduced acidity (higher pH) due to enhanced mass transfer of acid by-products under flow conditions.

Next we introduced the oral bacteria *Streptococcus salivarius* into the palm-sized working platform to produce a biofilm similar in composition to oral plaque. Thanks to the strong attachment of the nanoparticles, they stayed connected and continued operating normally while the biofilm grew on top of them. Also, since they were more than ten thousand times smaller than the bacteria, every pixel of a picture encoded a pH value exactly at the biofilm-attachment surface interface. This essentially gave us an image of the pH at the exterior tooth surface. Then, we simulated a meal by changing the concentration of glucose in the solution that washed through the channel and measured the resulting acidity at the surface of our “microfluidic tooth”. Figure 2a shows that after removing glucose, pH slowly returned to neutral conditions, but decreased rapidly after re-exposure. The recovery of pH to neutral pH values (7) is largely based on rinsing out acidic biofilm by-products under different flow rates of liquid, as seen in Figure 2b. Now, for the first time, chemical and hydrodynamic contributions to oral health can be probed rapidly and independently with high precision.

Jesse Greener, Professor
Jérémie Asselin, Ph.D. candidate

Publication

A microfluidic platform with pH imaging for chemical and hydrodynamic stimulation of intact oral biofilms.
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