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Research Paper

Streptococcus mutans biofilm transient viscoelastic fluid behaviour during high-velocity microsprays

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ABSTRACT

Using high-speed imaging we assessed *Streptococcus mutans* biofilm–fluid interactions during exposure to a 60-ms microspray burst with a maximum exit velocity of 51 m/s. *S. mutans* UA159 biofilms were grown for 72 h on 10 mm-length glass slides pre-conditioned with porcine gastric mucin. Biofilm stiffness was measured by performing uniaxial-compression tests. We developed an in-vitro interproximal model which allowed the parallel insertion of two biofilm-colonized slides separated by a distance of 1 mm and enabled high-speed imaging of the removal process at the surface. *S. mutans* biofilms were exposed to either a water microspray or an air-only microburst. High-speed videos provided further insight into the mechanical behaviour of biofilms as complex liquids and into high-shear fluid–biofilm interaction. We documented biofilms extremely transient fluid behaviour when exposed to the high-velocity microsprays. The presence of time-dependent recoil and residual deformation confirmed the pivotal role of viscoelasticity in biofilm removal. The air-only microburst was effective enough to remove some of the biofilm but created a smaller clearance zone underlying the importance of water and the air–water interface of drops moving over the solid surface in the removal process. Confocal and COMSTAT analysis showed the high-velocity water microspray caused up to a 99.9% reduction in biofilm thickness, biomass and area coverage, within the impact area.
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1. Introduction

Dental plaque biofilms are the heterogeneous bacterial communities attached to teeth and soft tissues and embedded in a matrix composed mainly of extracellular DNA, proteins, and polysaccharides (Marsh and Bradshaw, 1995). Oral biofilms are associated with the development of caries, gingivitis and periodontitis (Costerton et al., 1995; Donlan and Costerton, 2002). Dental caries occurs through the dissolution of the enamel by acidogenic bacteria such as *Streptococcus mutans*, *Streptococcus sobrinus*, and lactobacilli (Featherstone, 1999). Biofilm complex structure makes dental diseases difficult to control and to eradicate, thus becoming a worldwide public health problem (Selwitz et al., 2007). When biofilms are subjected to different flow conditions, they mechanically behave as viscoelastic fluids (Klapper et al., 2002; Peterson et al., 2015; Towler et al., 2003; Wilking et al., 2011). This means that at low-shear rates biofioms have a "solid-like" behaviour and are able to store energy, while at high-shear rates they become "fluid-like" and lose their ability to store elastic energy. Energy dissipation through viscoelasticity is an important characteristic because it allows biofilms to tolerate rapidly-changing shear stresses without detaching from the surface. In dentistry, fluid shear stresses generated via either non-contact toothbrushing or fluid flow play a major role in biofilm detachment (Hope et al., 2003; Hope and Wilson, 2003; Paramonova et al., 2009) since dental plaque mainly accumulates in particular areas inside the mouth (such as pits, fissures, interproximal (IP) spaces and subgingival areas) inaccessible for toothbrush bristles and dentifrices (Fried, 2012). Therefore, the understanding of biofilm mechanical properties under various hydrodynamic flows represents an important part for the design of more effective strategies to remove and to control dental plaque biofilms. Oral irrigators, which generate a continuous pulsating or steady water jet designed to remove interdental and subgingival plaque are widely used as a supplement to toothbrushing, or to replace traditional flossing (Barnes et al., 2005; Jahn, 2010). More recently, mechanical biofilm removal either using low volume, high-velocity water droplets (Cense et al., 2006) or by entrained air bubbles (Parini and Pitt, 2006; Sharma et al., 2005b) has shown positive results due to the droplets' impact pressure, hydrodynamic shear stresses and the surface tension effects of the passage of an air–water interface over a solid surface (Busscher et al., 2010b).

In previous studies we grew *S. mutans* biofilms on and between two central incisors of a periodontal model to recreate the realistic geometry of the IP space (Rmaile et al., 2012). Then we performed high-speed imaging to assess biofilm removal and viscoelastic behaviour during the exposure to high-velocity microbursts (Rmaile et al., 2014). We also performed Computational Fluid Dynamics (CFD) simulations to predict wall shear stresses generated over the tooth surface during the burst (Rmaile et al., 2015). However, due to the opaque nature of the surface we could not see the details of biofilm removal process at the surface. Here we developed an in vitro IP model allowing the parallel insertion of two biofilm-colonized glass slides which could be monitored through the side of the slide by a high-speed camera. Biofilms were exposed to high-velocity water microsprays or air-only microbursts to assess the effects of these different fluid flows on the biofilm–burst interactions and biofilm viscolastic mechanical behaviour with respect to the removal process.

2. Materials and methods

2.1. Bacteria and growth media

Biofilms were inoculated with a *S. mutans* UA159 (ATCC 700610) adjusted overnight culture (10^6 cfu/mL) grown in a 2% sucrose-supplemented brain-heart infusion (BHI+S medium) (Sigma-Aldrich). Type II porcine gastric mucin (Sigma-Aldrich) was added to the BHI+S medium (BHI+SM medium). Petri plates or microscope glass slides were conditioned with 10 mL of the BHI+SM medium for 24 h to allow mucins to cover the surface. Then, biofilms were grown in static conditions for 72 h at 37 °C and 5% CO_2 with BHI+SM medium replacement every 24 h. We also grew biofilms on non-mucin conditioned plates and in BHI+S medium (control *S. mutans* biofilms) to assess the influence of mucin on the mechanical properties.

2.2. Uniaxial compression tests

Uniaxial compression experiments were performed on control *S. mutans* biofilms and on *S. mutans* biofilms grown on mucin-conditioned petri plates and with mucin-supplemented medium using an Electroforce 3200 testing instrument (Bose). Since biofilms are known to be viscoelastic materials and their mechanical behaviour varies with the strain rate applied, we performed uniaxial compression experiments at a constant rate of 0.05 mm/s. An upper cylindrical plunger of a diameter (D) of 7.75 mm compressed the biofilm and a 5 N capacity load cell (Honeywell Sensotec, Columbus, OH, USA) recorded the resulted force. Biofilm stiffness under constant strain rate was measured calculating the Young's modulus (E) from the stress–strain curves as previously described (Rmaile et al., 2012). Six independent replicate experiments were performed ($n=6$). Statistical analysis was performed using unpaired two samples t-test for normally distributed data and difference considered significant where $p<0.05$.

2.3. In vitro IP model and high-velocity microsprays

To allow high-speed camera imaging at the surface we developed an in vitro IP model (Fig. 1). The model consisted of a rectangular clear plastic holder, in which two grooves were made for the parallel insertion of two *S. mutans* biofilm-colonized slides at a distance of 1 mm. Slides were cut at 10 mm (10 mm-length slice) as a representative length, in the outside-in direction, of the proximal surface of the human molars. Since most of the biofilm was rapidly cleared from the 10 mm length of the slide we also grew *S. mutans* biofilms on full-length slides (75 mm × 25 mm) in order to more clearly assess the fluid nature of the biofilm which was most evident at the interface between the spray and the biofilm. Prior to the insertion into the IP model, the initial thickness of the

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