CHAPTER 1

General Introduction

and Aim of the Thesis
ORAL BIOFILM

Oral biofilm, also known as dental plaque, is a complex community of microorganisms, surrounded by an extracellular matrix, consisting of extracellular polymeric substances (EPS). The oral biofilm has been identified to retain around 700-1000 bacterial species (Ten Cate, 2006; Frias-Lopez and Duran-Pinedo, 2012). Often, the pathogenicity of oral biofilm is a result of a shift in bacterial composition due to environmental changes, e.g. a pH change of the oral cavity or a dietary change of the host. In the oral cavity, a compositional imbalance of oral biofilm can cause gingivitis, periodontitis and caries. Bacteria, released from oral biofilm and transmitted by blood or other routes to remote areas of the human body, can cause for example atherosclerosis (Reyes et al., 2013), endocarditis (Nagata et al., 2005; Wilson et al., 2008), bronchopneumonia (Imsand et al., 2002), otitis media (Topcuoglu et al., 2012), rhinosinusitis, osteomyelitis (Rana and Monnis, 2011) and infections related to biomaterial implants or devices (Mombelli and Decaillet, 2011).

Effective prophylaxis is important in order to maintain a healthy microbial balance. Common methods of oral biofilm control include mechanical removal (e.g. brushing) and chemical control (e.g. antimicrobials). Preventive measures based on both brushing and antimicrobials are widely used. Unfortunately, neither provides a 100% control over oral biofilm in vivo (Deery et al., 2004; Rosema et al., 2008; Busscher et al., 2010).

BIOFILM RESISTANCE TO CHEMICAL CONTROL

Antimicrobials are intentionally added to toothpastes and mouthrinses to prevent oral diseases by killing or inactivating bacteria in the oral biofilm.
Generally, antimicrobials are used to retard biofilm formation or to suppress the growth of pathogenic bacteria and the production of toxins (Mohammadi and Abbott, 2009; Shen et al., 2009). It has been proven that planktonically grown bacteria are more susceptible to antimicrobials than bacteria grown in a biofilm (Nickel et al., 1985; Van der Mei et al., 2006). This resistance to antimicrobials is due to the protective role of EPS and insusceptible bacteria in a biofilm. EPS limits the diffusion of antimicrobials into a biofilm, which has been extensively studied in oral biofilms of different bacterial species and with different antimicrobials (Corbin et al., 2011). There are several mechanisms involved in this limited penetration of antimicrobials, such as molecule size of the antimicrobial (Thurnheer et al., 2003), reaction and interaction with biofilm components (Stewart, 2003), absorption (Otten et al., 2012), electrostatic interactions (Ganeshnarayan et al., 2009), hydrophobicity of the antimicrobial (Campanac et al., 2002; Sandt et al., 2007), and neutralization by enzymes (Stewart et al., 2000). However, the resistance provided by EPS may be discouraged by longer exposure time or higher concentration of antimicrobials, both are often impossible for oral applications. Biofilm resistance also depends on the intrinsic resistance provided by bacteria inside a biofilm (Drenkard, 2003). Several possible mechanisms have been proposed, e.g. that bacteria develop a resistant phenotype during adhesion and biofilm formation (Campanac et al., 2002), or that the metabolic activity of bacteria is different due to limited oxygen and nutrition availability.

**BIOFILM RESISTANCE TO MECHANICAL REMOVAL**

Clinically, most of the oral biofilm is removed mechanically by daily toothbrushing. Mechanical removal of biofilms depends on the strength of
the biofilm matrix, its Young’s modulus and biofilm structure (Bol et al., 2009). Studies have shown that powered toothbrushes provide better biofilm removal than manual toothbrushes (Rosema et al., 2008). Powered toothbrushes can also mechanically disrupt biofilm that is beyond the reach of bristles by bubble cavitation via aqueous media (Adams et al., 2002; Parini and Pitt, 2006). However, there is never 100% biofilm removal in the oral cavity (Busscher et al., 2010) and biofilm will always remain in interproximal spaces, fissures, gingival pockets and on orthodontic appliances.

**BIOFILM VISCOELASTICITY**

Biofilm is widely accepted as a viscoelastic material, which is mainly attributed to its extracellular matrix, containing polysaccharides, proteins and extracellular DNA (Rupp et al., 2005; Flemming and Wingender, 2010). The extracellular matrix provides the biofilm with a structural stability against both chemical and mechanical challenges from outside. Studies have shown that biofilm behaves as a viscoelastic polymeric fluid (Klapper et al., 2002), responding to a short term strain as an elastic solid and as a viscous fluid to long term strain (Shaw et al., 2004; Rupp et al., 2005). This bipolar feature of biofilms is a protective mechanism to survive external mechanical stress, for instance, toothbrushing. The elastic feature cushions the stress energy during deformation, and the viscous feature leads to irreversible deformation to resist transient stress. Both are the strategic compromise to external challenge through re-arrangement of biofilm structure.
Common modes of measuring the viscoelasticity of a biofilm are tension (Hohne et al., 2009), compression (Korstgens et al., 2001) and shear (Rupp et al., 2005). The low load compression test (LLCT) as used in this thesis applies a uniaxial force on the biofilm with a relative large plunger which gives an average measure of the biofilm ruling out influences of heterogeneities (De Beer et al., 1994; Stoodley et al., 1997).

The viscoelastic properties of a biofilm are barely influenced by the properties of the substratum, once a mature biofilm has formed (Whitehead and Verran, 2009). Instead, the viscoelasticity of a biofilm correlates with its structural and compositional features and is influenced by hydrodynamics during growth and the initial biofilm architecture (Paramonova et al., 2009). The limited penetration of antimicrobials into a biofilm is said to be due to differential diffusion, hampered by structural and compositional heterogeneity of the biofilm (Stewart, 2003; Corbin et al., 2011). Therefore, we hypothesise that the viscoelasticity of a biofilm will relate with antimicrobial penetration, since both depend on biofilm structure and composition.

**AIM OF THE THESIS**

To study the viscoelasticity of *in vitro* and *in vivo* oral biofilms and relate the viscoelastic response to mechanical deformation with antimicrobial penetration, in order to confirm or deny the above hypothesis. The aim can be divided into the following sub-aims:

- To compare different *in vitro* biofilm models with respect to their viscoelasticity and antimicrobial penetration.
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- To develop an *in vivo* model for biofilm collection and to compare the viscoelasticity and antimicrobial penetration of *in vivo* formed biofilm with the one of *in vitro* formed biofilms.

- To investigate the influence of non-contact, powered brushing on viscoelasticity and antimicrobial penetration into *in vitro* formed, oral biofilms.
REFERENCES


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