CHAPTER 6

GENERAL DISCUSSION
Biofilms play an important role in medically-related fields, as they are a major cause of many clinical infections, such as cystic fibrosis pneumonia, osteomyelitis and biomaterial-related infections (2). Mechanical properties of biofilms influence how biofilms deform due to external forces, cell detachment and dispersal from biofilms and mass transfer into biofilms. Previous studies have shown that biofilms possess viscoelastic behavior (14, 20) and their strength is influenced by increased extracellular polymeric substance (EPS) production (7), EPS chemistry (18) and presence of divalent cations (11, 15), growth medium (3), hydrodynamic conditions during growth (6, 17) and quorum sensing (4). However, the great majority of studies were conducted with environmental biofilms and the information for medically-relevant biofilms is scarce.

Medically-relevant biofilms are rather different from environmental biofilms. On one hand, their growth conditions are less diverse, since temperature, pH and nutrients available are usually within a range of parameters present in a human body. On the other hand, species diversity of medical biofilms is rather high. For example, oral biofilms contain more than 700 different species and are among the most complex microbial communities known (16). Additionally, medical biofilms can contain a mixture of bacterial and fungal species, such as biofilms isolated from a voice prosthesis (14). Mechanics of medical fungal biofilms especially received very little attention, despite the fact that they rank number four in frequency among species causing bloodstream infections (5), and oral biofilms were used in only few studies (2, 3, 21). Therefore, we chose to focus on oral biofilms and fungal biofilms of Candida species. We studied how morphological and species composition, biofilms
architecture, hydrodynamic conditions during growth, EPS composition and quorum sensing influence biofilm mechanical properties.

In this thesis, we showed that hyphal content was a determining parameter for the strength of fungal biofilms, which could be adversely affected by the presence of eDNA, but the effect was not as strong as in bacterial biofilms, previously reported in the literature (13). We determined that mechanical properties of oral biofilms were influenced by hydrodynamic conditions and species composition during growth. Biofilm compressive strength generally declined with increased shear during growth, along with a transformation in biofilm architecture. Multi-species oral biofilms were generally stronger than single species biofilms, however, when non-coaggregating species were grown together in a biofilm, biofilm strength declined.

Knowledge about mechanical properties of medically-related biofilms can be beneficial for modeling of biofilm development, for making predictions about effectiveness of treatments and for development of new medications. For instance, in fungal biofilms, hyphal production should be reduced or suppressed to weaken the biofilms. Hyphal production can be suppressed by adjusting growth conditions to non-favorable for germination, for example, by reducing the growth temperature, limiting amount of serum, changing pH (1). However, the conditions favorable for germination, such as neutral pH, temperatures around 37°C and presence of serum, are the condition present in a human body and may be difficult to change. The use of quorum sensing (QS) molecules, which work in a manner similar to farnesol and target hyphal production (5) appears to be a more attractive solution. On the other hand, treatments targeted to reduce slime production by biofilms may not be as effective for fungal biofilms as for bacterial biofilms, since the role of EPS in
maintaining structural integrity of fungal biofilms is not as crucial as in bacterial biofilms.

Knowledge about influences of hydrodynamics and species interaction on oral biofilm structure and strength and about the relationship between these parameters can help to create more realistic models and improve current treatment methods. Several coaggregation assays can be performed in laboratory conditions (2,4,6) to determine if the interactions between species are favorable or not. Coaggregation parameters can be incorporated into models to account for species interaction within biofilms. Additionally, models can be simplified to include only several species, as their biofilms possess mechanical properties and structure similar to those of full dental plaque, as we have shown in Chapter 4 of this thesis.

Suggestions for future research

Despite its usefulness for measurement of biofilm mechanical properties and, especially, for determination of biofilm thicknesses and factors influencing biofilm strength, compression testing has certain limitations. First of all, mechanical properties, in addition to compressive strength, also include shear and tensile strength not measured by our technique, which can actually be more important in respect to biofilm erosion and cell release from a biofilm. Secondly, flow induced shear is a more common load case for biofilms than compression. Ideally, a method incorporating measurements of compressive, shear and tensile strength would be a preferred way of evaluating biofilm mechanical properties. We attempted to design an instrument which could be used as an attachment to low load compression testing (LLCT) and would allow for measurement of shear and tensile strength of biofilms, in
addition to compressive strength. The main components of design are presented in Figs. 1 and 2 and testing procedure and preliminary results are described below.

**Figure 1.** Tensiometer assembly with the arrows pointing in the directions in which the main parts of the assembly are pulled apart.

Biofilms are grown in an assembly at a regulated shear rate, preferably the same shear rate used for growing biofilms for compression testing. The assembly is placed on a load cell of LLCT, fixed to it and the top is screwed into the top plate in place of a plunger. The assembly is pulled apart and the force required to separate two parts of the assembly is recorded by data acquisition system. This force is used to calculate tensile strength of biofilms by dividing the force over biofilm cross-section. Dimension of the cross-section are estimated from confocal laser scanning microscopy (CLSM) analysis for which inserts with biofilms, directly taken out of the sides of assembly, are used.
For shear testing, a part of the assembly is taken apart and each of two parts is placed on the load cell. A plunger is inserted into a top plate and carefully aligned to move inside the assembly without touching inner walls to avoid friction. The force required to remove a biofilm and position and speed of the plunger are recorded. The force is normalized per cross-sectional area of biofilm, known from CLSM analysis, to obtain shear strength.
Preliminary test results with 36 h old biofilms of *Enterococcus faecalis* BS385 showed that surface tension force of water between two parts of the assembly was higher than tensile strength of the biofilms and interfered with the measurement of strength. Therefore, adjustments should be made to the assembly to reduce surface tension force by placing a Teflon insert between the two parts or using a coating on the contacting parts which would increase their hydrophobicity, or using different materials for design.

During shear testing, no detectable friction force was measured during a “dry” run without a biofilm and a medium, showing that the contribution of friction force could be neglected. During shear tests with a biofilm of *E. faecalis* BS385, shear force measured increased with the distance as the plunger moved through the biofilm, potentially indicating buildup of removed biomass in front of the plunger (Fig. 3). More tests are needed to determine the accuracy and reproducibility of the technique.
Despite its shortcomings, the technique has a potential since it is the first known method that incorporates measurements of compressive, shear and tensile forces in one system. Additionally, the system has low costs and can be assembled in-house which makes it more accessible to groups with both high and low research budgets.

\[ y = 0.0001x - 0.0188 \]
\[ R^2 = 0.953 \]

**Figure 3.** The force measured in shear experiment with *E. faecalis* BS385 biofilm vs. distanced traveled by the plunger; deformation speed 1 µm s\(^{-1}\).
REFERENCES


