SUMMARY
The aim of this work was to study which biological and environmental factors influence the strength of medically-relevant biofilms. Out of many medically important biofilms, we focused 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. Biofilm mechanics were studied using a new method, low-load compression testing (LLCT), which directly measures the resistance of biofilms to compression and allows for accurate determination of biofilm thicknesses. Additionally, phase contrast and confocal laser scanning (CLSM) microscopy were used to evaluate biofilms structure and composition.

**Chapter 1** introduces the reader into the subject by giving a definition of biofilms, an overview of medically-relevant biofilms and a summary of recent studies of biofilm mechanical properties.

In **Chapter 2**, we present Low Load Compression Testing (LLCT), as a new method for measuring biofilm thickness. Biofilm thickness is an important parameter in biofilm characterization. Current methods of measuring biofilm thicknesses have several limitations, including application, availability, and costs. With LLCT, biofilm thicknesses are measured during compression by inducing small loads of up to 5 Pa corresponding to 0.1% deformation, making LLCT essentially a non-destructive technique. Comparison of the thicknesses of various bacterial and yeasts biofilms derived from LLCT and obtained using confocal laser scanning microscopy (CLSM), yields the conclusion that CLSM underestimates the biofilm thickness due to poor
penetration of different fluorescent dyes, especially through the thicker biofilms, whereas LLCT does not suffer with this thickness limitation.

The aim of the study presented in Chapter 3 was to identify the factors that determine the compression strength of Candida biofilms. Candida albicans is the most frequently isolated human fungal pathogen among species causing biofilm-related clinical infections. Mechanical properties of Candida biofilms have been given no attention hitherto, despite the fact that mechanical properties are important for selection of treatment or dispersal of biofilm organisms due to a bodily fluid flow. Biofilms of C. albicans wild-type parental strain Caf2-1, mutant strain Chk24, lacking Chk1p, known to be involved in a regulation of morphogenesis (yeast-to-hyphae transition) and gene-reconstructed strain Chk23 were evaluated for their resistance to compression along with biofilms of C. tropicalis GB9/9 and C. parapsilosis GB 2/8, derived from used voice prostheses biofilms. Additionally, cell morphologies within the biofilm, cell surface hydrophobicities, and EPS composition, were determined. Our results suggest that the hyphae-to-yeast ratio influences the compression strength of C. albicans biofilms. Biofilms with hyphal content over 50% possessed significantly higher compressive strength and were more difficult to destroy by vortexing and sonication compared to the biofilms with lower hyphal content. However, when the amount of extracellular DNA in biofilms of C. albicans Caf2-1 and Chk24 increased, biofilms strength declined, suggesting that eDNA may adversely influence biofilms integrity.

In Chapter 4, the influence of hydrodynamic conditions and species composition on oral biofilms strength were investigated. Single and multi-species biofilms of Streptococcus oralis J22, Actinomyces naeslundii TV14-J1 and full dental plaque were grown at shear rates ranging from 0.1 to 50 s⁻¹ and their compressive
strength was measured. Subsequently, biofilms architecture was evaluated using confocal laser scanning microscopy. Multi-species biofilms were stronger than single species biofilms, with strength values ranging from 6 to 51 Pa and from 5 to 17 Pa, respectively. In response to increased hydrodynamic shear, biofilms strength decreased and architecture changed from uniform carpet-like to more “fluffy” with a higher thickness. *S. oralis* biofilms grown under variable shear of 7 and 50 s⁻¹ possessed properties intermediate of the ones measured at the respective single shears.

The study presented in Chapter 5, aimed to determine if biofilms of coaggregating bacteria were stronger than of non-coaggregating and single species biofilms. Coaggregation, known as a specific cell-to-cell recognition that occurs between genetically distinct cell types, is one of the types of microbial interaction. Single species biofilms of *Streptococcus oralis* J22, *Streptococcus sanguis* PK1889 and *Actinomyces naeslundii* TV14-J1, biofilms of a coaggregating pair *Streptococcus oralis* J22 and *Actinomyces naeslundii* TV14-J1, and a non-coaggregating pair *Streptococcus sanguis* PK1889 and *Actinomyces naeslundii* TV14-J1 were grown in parallel plate flow chamber and subjected to compression forces in a uniaxial low load compression tester (LLCT). Biofilms architecture was evaluated using confocal laser scanning microscopy. Coaggregating species biofilms were 4 times stronger than biofilms of a non-coaggregating pair and 2 to 7 times stronger than single species biofilms of *S. oralis* and *A. naeslundii*. When *S. sanguis* were grown in combination with *A. naeslundii*, biofilm strength decreased 3 times compared to *S. sanguis* grown alone. A correlation was established between the percentage of voids and biofilm strength: the biofilms with the lowest percentage of
voids possessed the highest strength. Biofilm thicknesses, which were on average 20 
µm for all biofilms, did not influence their strength.

In Chapter 6, the general discussion, main conclusions of the studies are 
summarized, potential applications for acquired knowledge are offered and a new 
method for measuring mechanical properties of biofilms is presented.