Chapter 1

General introduction
Over the past decades, the parallel plate flow chamber has been widely used in dynamic studies of bacterial and cellular adhesion to substratum surfaces.\textsuperscript{1-3} A typical design of a parallel plate flow chamber consists of a Teflon spacer, stainless steel body (with inlet and outlet) and glass coverslips at both top and bottom for real time observation (see Figure 1).\textsuperscript{4} The velocity of the flow determines the shear rate on the bottom plate of the chamber, usually employed for study purposes rather than the top plate.\textsuperscript{5} The bottom plate can be made out of various materials to be studied and can be coated with a conditioning film, consisting of serum, plasma or salivary proteins, depending on the bacterial niche of interest.\textsuperscript{6-8}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{parallel_plate_flow_chamber.png}
\caption{Schematic overview of the parallel plate flow chamber system and detailed view of a parallel plate flow chamber as often used within the Department of Biomedical Engineering at UMCG, The Netherlands. The O-rings serve to prevent leakage of fluid from the chamber. An \textit{in situ} obtained image of bacteria adhering on a glass substratum is shown on the right-hand side.}
\end{figure}
From its early development, parallel plate flow chamber designs have been equipped with real-time, \textit{in situ} observation techniques, usually comprising a CCD camera and an ultra-long working distance objective to the microscope system enabling to focus through the depth of the channel and the thickness of the top- and bottom plates of the chamber (see also Figure 1). \textit{In situ} observation avoids the use of slight-rinsing or dipping to remove so-called loosely bound bacteria, which has been demonstrated to be a flawed expression as most papers applying slight rinsing or dipping to remove loosely bound bacteria do not define how strong “loosely” bound is.\textsuperscript{9-11} \textit{In situ} observation allows to state that one enumerates bacteria able to adhere under the well-defined prevailing shear conditions of the experiment.\textsuperscript{12} Real-time observation also allows the detailed study of bacterial adhesion as a function of time and to monitor individual adhering bacteria for their residence-time dependent behavior.\textsuperscript{13,14} Despite the extensive use of \textit{in situ} observation by various research groups over the world, a special trait shown by adhering bacteria has hitherto been completely ignored: adhering bacteria exhibit nanoscopic, random vibrations around their equilibrium positions.\textsuperscript{15} The lack of attention of this phenomenon is the more surprising, since previous studies have pointed out that also a-biotic polystyrene particles and red blood cells exhibit similar vibrations when adhering on a glass surface.\textsuperscript{16,17}

In earlier studies, bacterial adhesion was evaluated by various biochemical and physico-chemical approaches that mostly assumed silently that the bond between a bacterium and a substratum surface was a completely rigid one.\textsuperscript{18-20} The existence of nanoscopic vibrations exhibited by adhering bacteria already points out, that this is a wrong assumption and in fact suggests that the bond should be considered as a spring, whose spring constant can be derived from the vibration amplitudes, as done in the past for abiotic particles.\textsuperscript{16,17} Atomic force microscopy has also been applied extensively to determine the viscoelastic bond properties of adhering bacteria,\textsuperscript{21,22} but AFM involves the application of an external load during measurement while obtaining data over a statistically reliable number of individual bacteria is tedious. QCM-D (Quartz Crystal Microbalance with Dissipation monitoring) is another technique to determine viscoelastic bond properties,\textsuperscript{23,24} that does allow to measure the bond over a large number of adhering bacteria in one and the same experiment, but in QCM-D adhering bacteria are forced into high-frequency oscillation, again by an external force. Therewith, bacterial vibration spectroscopy is the only method to study bacterial bond properties under naturally occurring environmental conditions.

\textbf{Mechanisms of bacterial adhesion}

\textbf{Non-specific approach to bacterial adhesion}

In a non-specific approach to bacterial adhesion to surfaces, bacterial adhesion is
assumed to be mediated by an interplay of repulsive or attractive electrostatic double-layer energies and attractive Lifshitz-Van der Waals energies,\textsuperscript{19,20} that can be derived from measured zeta potentials and contact angles on the interacting surfaces, respectively. Zeta potentials are calculated from the measured bacterial mobility in an applied electric field under a defined salt concentration and pH.\textsuperscript{25,26} However, the magnitude of the charge varies from species to species and is probably influenced by culture conditions, age of the culture, ionic strength and pH. Under most natural conditions, bacterial cell and substratum surfaces are negatively charged and regardless of the ionic strength of the suspending fluid,\textsuperscript{27} adhering bacteria are held at a small distance from the substratum surface by a repulsive electrostatic energy barrier, which increases with decreasing ionic strength. Bacterial cell surface hydrophobicity is reflected by the water contact angles on bacterial lawns\textsuperscript{28,29} from which the Lifshitz-Van der Waals interactions can be estimated (see Chapter 3 for details). Residence in the so-called secondary energy minimum enables adhering bacteria to vibrate under the influence of Brownian motion forces while remaining in an adhering state.

\textit{Specific approach to bacterial adhesion}

The outermost bacterial cell surface is composed of a variety of different, mostly proteinaceous surface appendages and a matrix of extracellular polymeric substances (EPS) including eDNA, proteins and polysaccharides.\textsuperscript{30,31} EPS can either be tightly bound to the cell surface or excreted. Usually EPS incorporates large amounts of water and covers the bacterial cell surface in a contiguous layer. Surface appendages can come in various forms, like fibrils or flagella. Fibrils are cell wall-associated proteins projecting outwards from the cell surface and are usually less than 200 nm long. Individual fibrils are very thin but they often form a ‘hairy fuzz’ over the entire cell surface or aggregate into tufts.\textsuperscript{32} Flagella are whip-like structures (up to 20 µm long and 10-30 nm thick) protruding from the bacterial cell wall and are responsible for bacterial motility (i.e. movement).\textsuperscript{32} The flagella beat in a propeller-like motion to help a bacterium move toward nutrients and away from toxic chemicals. However, the density and length of the fibrils and flagella also influence the viscoelastic properties of the bond between adhering bacteria and substratum surfaces. In the non-specific approach these surface appendages are thought to be able to pierce the energy barrier between the secondary and primary minimum and cause irreversible binding.

Often however, these appendages are equipped with highly specific ligands that allow strong binding with receptors sites, mostly located on proteinaceous conditioning films on (bio)materials surfaces,\textsuperscript{33,34} such as albumin, fibronectin, fibrinogen, laminin or collagen.\textsuperscript{35-37} Whereas specific ligand-receptor bindings are sometimes called a “special type of interaction”, they represent the same basic
physico-chemical force types as mediating non-specific interactions. However, in ligand-receptor binding, these forces are organized in a highly directional fashion and spatially confined and consequently operative over relatively short distances (less than 1 nm).\textsuperscript{38}

**Aim of this Thesis**

The aim of this thesis is two-fold:

1. to develop bacterial vibration spectroscopy as a new method to analyse the visco-elasticity of the bond between adhering bacteria and a substratum surface and to critically assess the virtues of vibration spectroscopy as compared with known methods, such as AFM or QCM-D.

2. to determine the influence of the general factors known to mediate specific- and non-specific bacterial binding to surfaces, on the vibration amplitudes of adhering bacteria.
REFERENCES


14. Meinders, J.; Van der Mei, H. C.; Busscher, H. J. Physicochemical aspects of deposition of *Streptococcus thermophilus* B to hydrophobic and hydrophilic


