Microfluidics in Biofouling and Life Sciences

Karlsruhe Institute of Technology
Institute of Functional Interfaces

UNIVERSITÄT HEIDELBERG

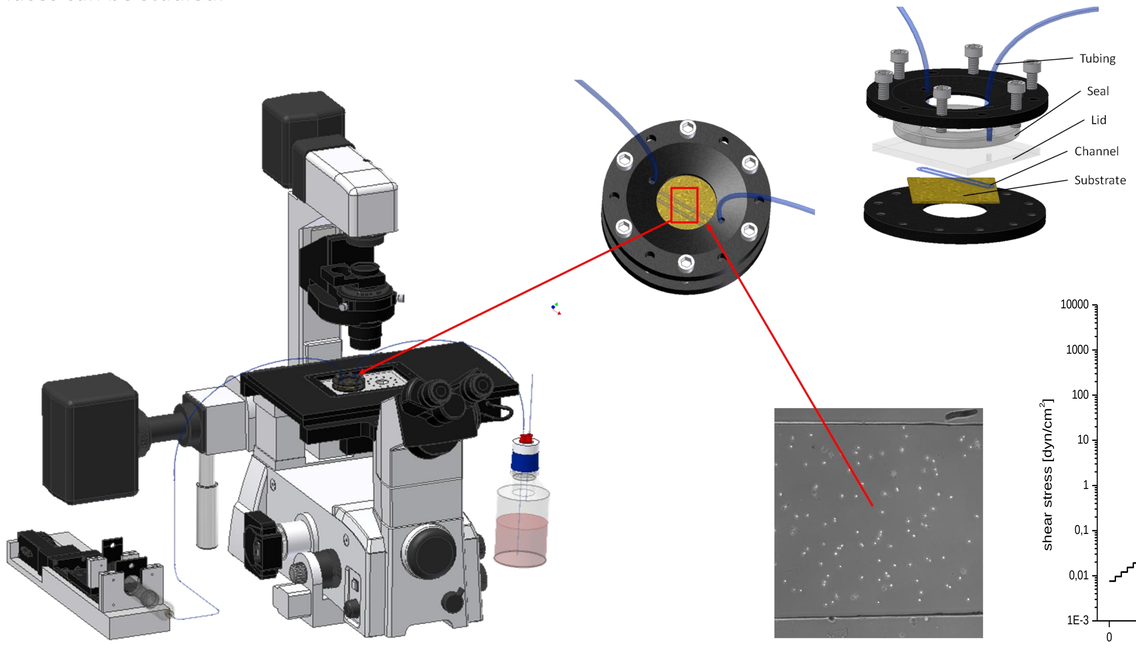
M. Hanke[1]*, M. Alles[1]*, M. P. Arpa Sancet[1]*, C. Christophis, M. Grunze[1,2], A. Rosenhahn[1,2]

[1] Applied Physical Chemistry, Ruprecht-Karls-University Heidelberg, Im Neuenheimer Feld 253, 69120 Heidelberg, Germany

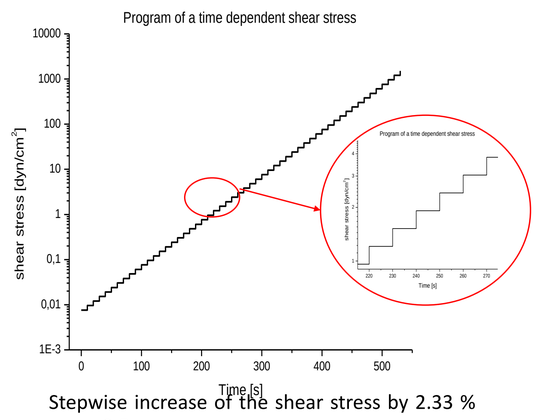
[2] Institute for Functional Interfaces, IFG, Karlsruhe Institute of Technology, PO Box 3640, 76021 Karlsruhe, Germany

MICROSCOPIC SHEAR FORCE SETUP

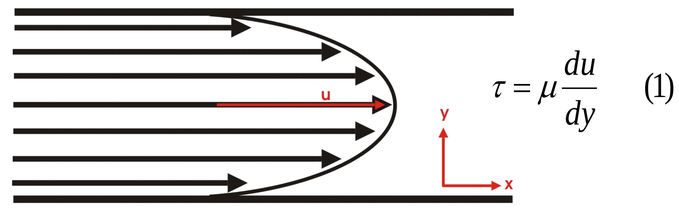
The microfluidic shear force setup is comprised of an inverted microscope housed in a self-built incubator allowing for measurements to be conducted a varying temperatures, e.g. 37 °C as in the human body. The microfluidic channels are made of polydimethoxysilane (PDMS) shapes placed between a glass lid and the substrate that presents the bottom of the channel meaning that the interaction of objects with a great variety of surfaces can be studied.[1]



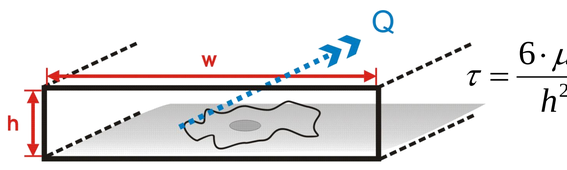
The setup: incubation microscope with syringe pump, microchannel and liquid reservoir.



Stepwise increase of the shear stress by 2.33 % every 0.5 s.



$$\tau = \mu \frac{du}{dy} \qquad (1)$$



$$\tau = \frac{6 \cdot \mu \cdot Q}{h^2 w} \qquad (2)$$

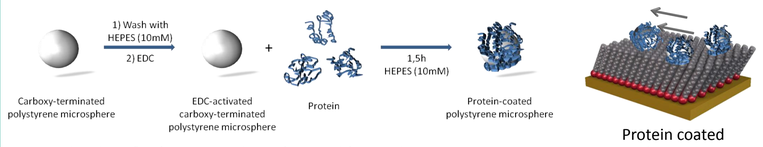
t = shear stress; μ = viscosity; Q = volumetric flow; h = channel height; w = channel width

Principle of hydrodynamic shear force in a parallel plate flow channel and flow program for the detachment experiment

BIOFOULING

Biofouling describes uncontrolled the adhesion and growth of organisms on structures both inside organisms and in see water.[2] The latter mentioned marine biofouling presents a great problem e.g. For the shipping industry where the fuel consumption is greatly increased.[3]

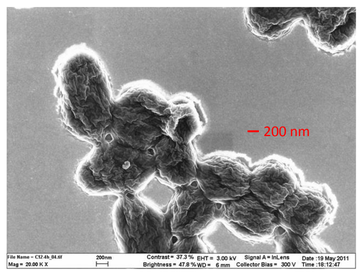
One first step in the study of adhesion to surfaces is to analyse the adhesion strength of proteins to a given surface. This may be done by either measureing the film thickness of aprotein layer after an incubation of a given surface into a protein solution[4] or in the mecrofluidic setup this may be achieved by coating carboxy terminated microspheres with proteins



Coating of polystyrene microspheres with proteins

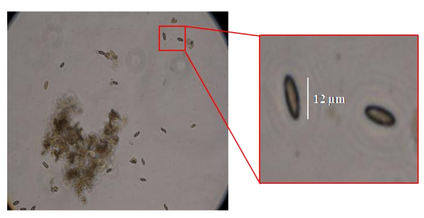
Protein coated microspheres on a SAM

The marine bacteria *Cobetia marina* an aerobic, gram-negative bacterium, is used as a model system for marine biofouling since its biofilms influence secondary colonization by invertebrates and algae[5]. Bacteria and microalgae as well produce extracellular polymeric substances (EPS), which mediate the initial adhesion to the material surface and constitute a biofilm matrix[6].



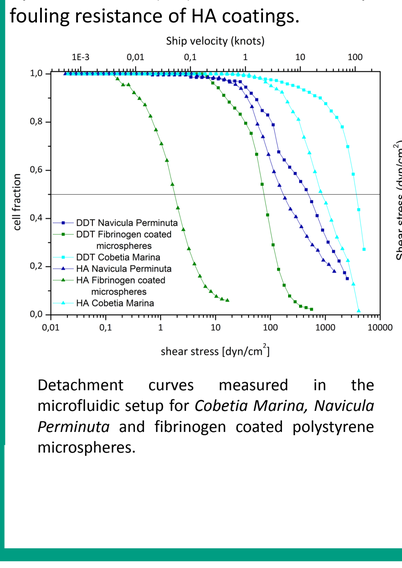
Cobetia Marina imaged by SEM.

The adhesion strength of the marine diatom *Navicula perminuta* was also studied as it is a good model for marine biofouling as it is found whenever biofouling is present. Another advantage is its ideal size for studies in our microfluidic setup.

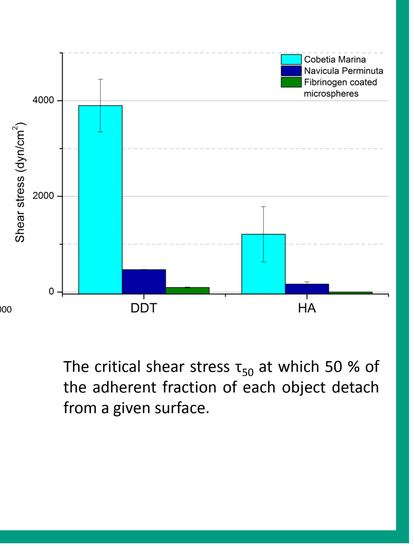


Navicula Perminuta imaged by optical microscopy.

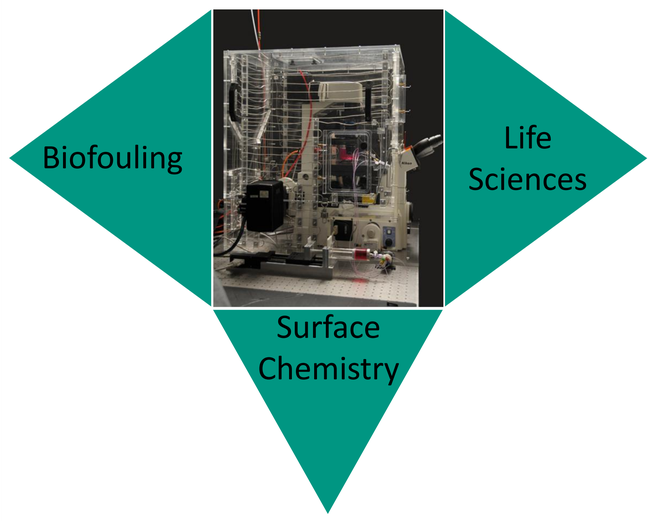
Comparison between the adhesion strength of the studied biofoulers:
An example of the adhesion strengths measured for different biofoulers is shown below. It demonstrates the great variety of adhesions strengths given by different organisms and structures. The graphs also present a comparison between a highly hydrophobic, "sticky" dodecanethiol SAM (DDT) and a hydrophilic, anti adhesive, hyaluronic acid (HA) surface. This comparison nicely demonstrates the pronounced fouling resistance of HA coatings.



Detachment curves measured in the microfluidic setup for *Cobetia Marina, Navicula Perminuta* and fibrinogen coated polystyrene microspheres.

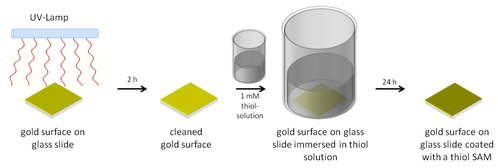


The critical shear stress τ_{50} at which 50 % of the adherent fraction of each object detach from a given surface.



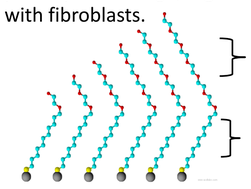
SURFACE CHEMISTRY

A great variety of surfaces may be studied in the microfluidic setup. As self-assembled monolayers present well defined sample surfaces these are a first choice as reference surfaces.



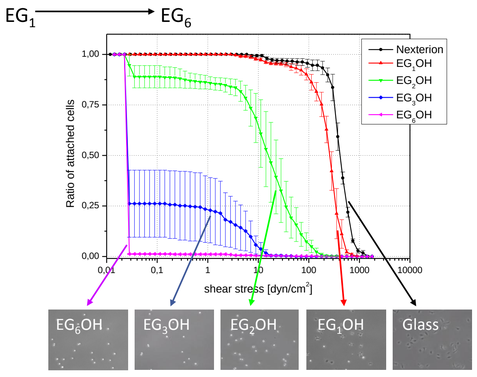
Preparation of SAMs on gold surfaces.

The preparation of SAMs allows the tuning of the surface polarity and charge, e.g when studying ethylenglycole SAMs with fibroblasts.

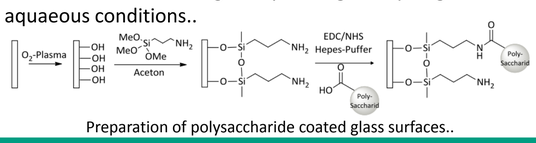


Hydration increases with ethylene glycol chain length while the surface energy (contact angle) stays constant

The aliphatic backbone is responsible for self assembly. The thiol head forms a bond to the flat gold substrate.



Surfaces coated with glycosaminoglycans (GAGs) such as hyaluronic acid (HA) or chondroitin sulfate (CS) have proven to have a highly anti adhesive character. This is attributed to the formation of negatively charged hydrogels under aquaeous conditions..

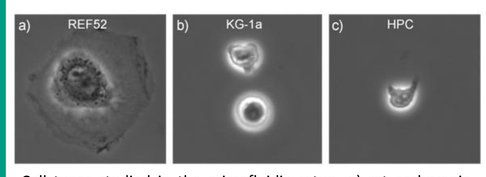


Preparation of polysaccharide coated glass surfaces..

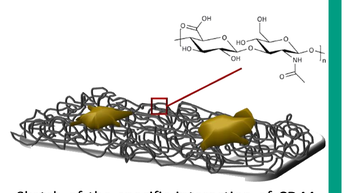
LIFE SCIENCES

Cell adhesion affects the cell cycle, proliferation and differentiation. In life sciences cell adhesion can also be an unwanted phenomenon, e.g. when implants are overgrown by tissue. Studying the interaction of cells with artificial surfaces can serve as models for biological systems.

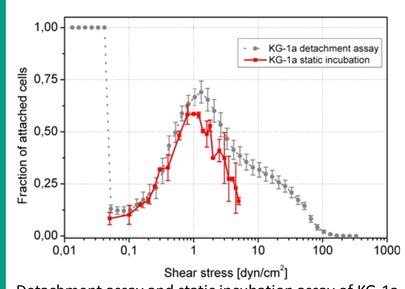
Cell types such as fibroblasts are capable of adhering to many surfaces by e.g. focal contacts, while other cell surface interactions are governed by a specific receptor-ligand interaction. An example is the specific flow induced binding of the receptor CD44 to hyaluronan.



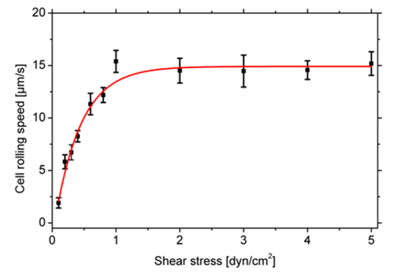
Cell types studied in the microfluidic setup. a) rat embryonic fibroblast, b) leukaemic suspension cell line KG-1a, c) haematopoietic progenitor cells from umbilical cord blood



Sketch of the specific interaction of CD44 expressing cells such as KG-1a with hyaluronic acid surfaces.

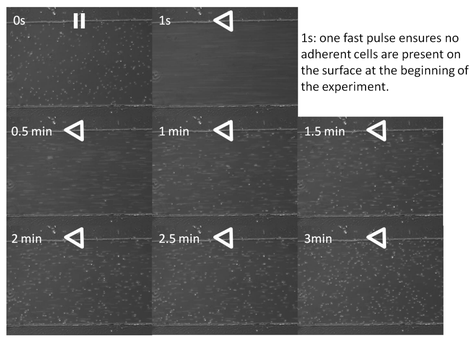


Detachment assay and static incubation assay of KG-1a on hyaluronic acid.



Rolling speed of KG-1a cells on hyaluronic acid.

Beside ramping through a spectrum of flow speeds it can also be of interest to examine the behaviour of cells under constant flow. CD44 expressing cells such as KG-1a for example accumulate when flushed over a hyaluronic acid surface.



1s: one fast pulse ensures no adherent cells are present on the surface at the beginning of the experiment.

DISCUSSION

General

- The microfluidic setup presents a good method of studying the adhesion strength of many different particles or organisms on a variety of surfaces.
- Incubator and CO_2 inlet enable the measurement of cells under physiologic conditions.

Biofouling

- Different marine biofoulers can successfully be studied.
- As demonstrated the adhesion strength of different organisms and proteins can vary greatly. The system is however adaptable so that it can also detach objects strongly adhered to a surface.
- Hyaluronic acid is strongly anti adhesive towards all studied organisms.

Life Sciences

- CD44 expressing cells can overcome the anti adhesiveness of hyaluronic acid surfaces and instead tightly adhere to these surfaces when under flow.
- Different types of measurements such as detachment assays and static incubation assays lead to the same results.

LITERATURE

[1] C. Christophis, M. Grunze, A. Rosenhahn, *Physical Chemistry Chemical Physics*, **2010**, *12*, 17, 4498-4504
[2] A. Rosenhahn, S. Schilp, H. J. Kreuzer, M. Grunze, *Physical Chemistry Chemical Physics*, **2010**, *12*, 17, 4275-4286
[3] J. J. Corbett, H. W. Koehler, *Journal of Geophysical Research*, **2003**, *108*, (D20), 4650
[4] K. L. Prime, G. M. Whitesides, *Science*, **1991**, *252*, 5009
[5]C. Shea, L. J. Lovelace, H. E. Smithsomerville, *Journal of Industrial Microbiology* **1995**, *15*, 290.
[6]I. B. Beech, J. A. Sunner, K. Hiraoka, *International Microbiology* **2005**, *8*, 157.

COLLABORATION

Callow group, Birmingham, UK (Navicula culture),
Lopez group, Duke, USA (Cobetia culture),
Ho group, University Hospital Heidelberg (haematopoietic cells)

ACKNOWLEDGMENTS

Spatz group for kindly providing access to cell culture facility.

KIT – University of the State of Baden-Wuerttemberg and National Research Center of the Helmholtz Association

Email: Max.Hanke@pci.uni-heidelberg.de
M.Alles@uni-heidelberg.de
Arpa@uni-heidelberg.de

www.kit.edu