Quantification of cell adhesion strength on self assembled monolayers

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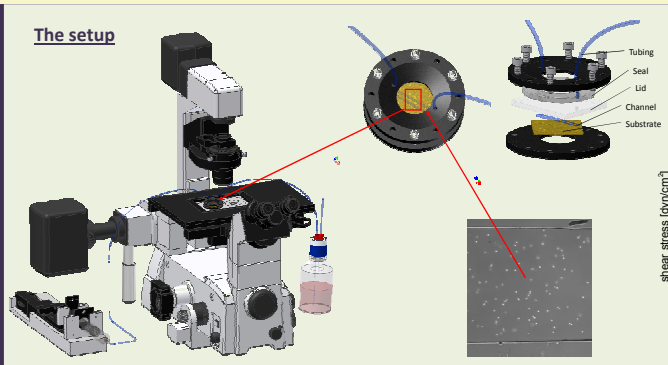
Introduction

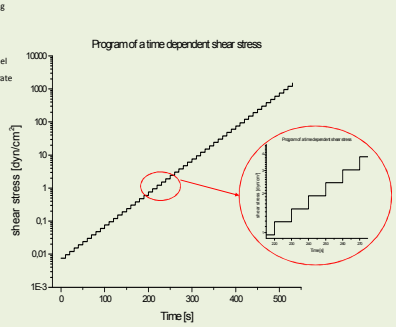
Marine Biofouling describes the undesired accumulation of microorganisms on artificial surfaces immersed in water.[1] Therefore the control of the interaction of cells with artificial surfaces is important to prevent these unwanted accumulations. One key parameter to characterize the interaction of cells with surfaces is the adhesion strength. We developed a microfluidic shear force assay which allows to simulate this situation and to study cell adhesion strength on different substrates quantitatively.[2] The adhesion strength of strongly bound cells can be measured by detaching cells from substrates using a stepwise increased flow along our microfluidic system. With this device we can determine the critical shear stress which is necessary to remove 50 % of the adherent cells. In the presented work we investigated the adhesion strength of microorganisms on five chemically different substrate, as well on a series of oligo ethylene glycol (OEG), both containing self-assembled monolayers (SAMs). Biofilms formed under real marine conditions were studied in field experiments at the Indian River Lagoon in Melbourne Beach Florida, in order to be compared to the laboratory experiments.

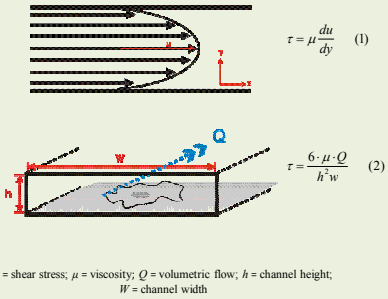
Experimental

The setup



Incubation microscope with syringe pump, microchannel and liquid reservoir





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

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

τ = 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

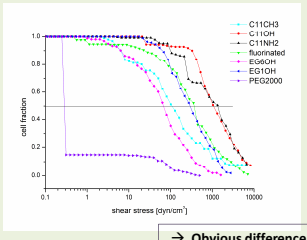
Results

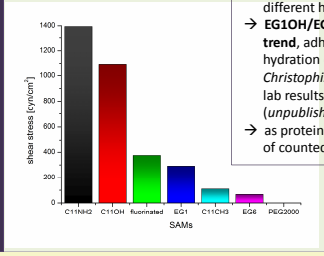
ATTACHMENT OF MICROORGANISMS *UNDER STATIC CONDITIONS*

Screening

of Self Assembled Monolayers (SAMs) with different chemistries and tunable hydration

SAMs Incubation time 4h	*thickness (ellipsometry) [Å]*	*contact angle [°]*	*shear stress (τ_{50}) [dyn/cm²]*
$C_{11}NH_2$	16	45-55	1394.30
$C_{11}OH$	13	30-40	1094.33
$C_{11}OC_2F_{10}CF_3$ (fluorinated)	17	115	375.09
EG_1OH	14	26	242.12
$C_{11}CH_3$	13	90-100	110.27
EG_6OH	25	30	60.90
PEG2000	34	30-40	0.28



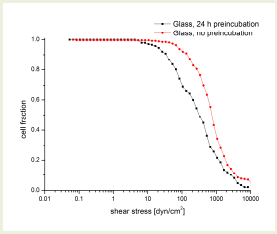


→ **Obvious difference** between surfaces with different hydrophilicity/hydrophobicity
→ **EG1OH/EG6OH**: attachment **follows the lab trend**, adhesion decreases with increasing hydration degree *[Schilp 2009, Fibroblast cells Christophis 2010]*[1,2]; trend also goes in with lab results of *Cobetia marina* bacteria (*unpublished results*)
→ as protein releasing known PEG2000: just 15 % of counted microorganisms were adhered cells

Influence of a conditioning film

on adhesion strength

(Glass surfaces, 24 h preincubation of the surfaces in 0.45 µm filtrated natural seawater)

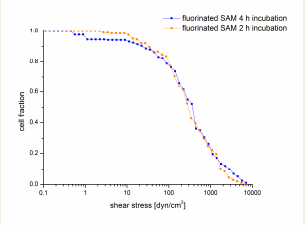


surface	*shear stress (τ_{50}) [dyn/cm²]*
Glass, 24 h Preincubation	316.05
Glass, no Preincubation	744.48

→ Conditioning film **reduces** adhesion strength (change of surface properties)

Variation of incubation time

on flourinated Self Assembled Monolayers



surface	*shear stress (τ_{50}) [dyn/cm²]*
fluorinated, 2 h incubation	322.67
fluorinated, 4 h incubation	375.09

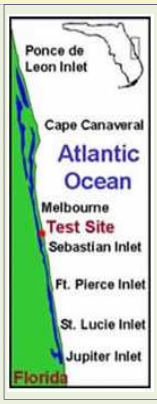
→ almost **no difference**, adhesion strength remains same between 2 h and 4 h

FIELD EXPERIMENTS

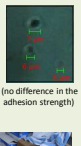
In collaboration with *Prof. G. Swain*, FIT

mobile laboratory (working in a van), electricity supply by car batteries, seawater taken out of tanks: microorganisms < 100 µm (prefiltrated natural seawater)

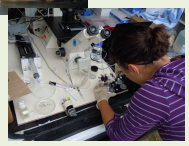
most commonly ***observed***



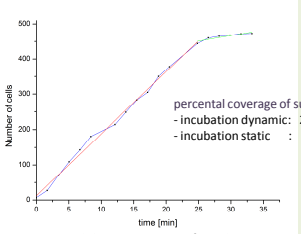
Test site:
EELS, Lagoon site



(no difference in the adhesion strength)



TIME DEPENDING ATTACHMENT OF MICROORGANISMS *UNDER DYNAMIC CONDITIONS*



percental coverage of surfaces after 2 h:
- incubation dynamic: 2.70 % ± 0.40 %
- incubation static : 1.00 % ± 0.01 %

approximately linear increasing of attaching microorganisms:
→ attachment rate in the beginning: ≃ 18 cells/min
→ attachment rate in the end: ≃ 3 cells/min (saturation)

→ Flow **animates** cells to settle

Summary

- Field Experiments were realized under difficult and often changing conditions to investigate real ocean biofoulers
- Surfaces with different wettability and hydration degrees were screened
- Parameter e.g. incubation time and surface natures were changed under static or dynamic conditions
- Results show significant difference depending on chemistries
- Most results follow the laboratory trend

Outlook

- Further Field Experiments and reproduction to prove the first theory
- Further correlation of field work with laboratory experiments
- Correlation of Microfluidics with other data obtained in the field (holographic data, settlement kinetics, Conditioning film formation, ect.)

Literature

[1] Luanne Hall-Stoodley, J.William Costerton, Paul Stoodley, *Nature Reviews* **2004**, *2*, 95-108.
[2] Christof Christophis, Michael Grunze, Axel Rosenhahn, *Physical Chemistry Chemical Physics* **2010**, *12*, 4498–4504.
[3] Sören Schilp, Axel Rosenhahn, Michala E. Pettitt, James Bowen, Maureen E. Callow, James A. Callow, Michael Grunze, *Langmuir* **2009**, *25(17)*, 10077–10082.

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