“Over 60% of bacterial infections (and up to 80% of chronic infections) are currently considered to involve microbial growth in biofilms”

Biofilm resistance
“Little or no industry standards, and a profound lack of awareness by industrial leaders and society in general”

J. Tyler, Tyler Advanced Corrosion Technologies. Interview - 5th of August 2015
We need a **reliable** and **reproducible** biofilm test.

With current technology this is **impossible**.
How can we tackle this with synthetic biology?

How can we control nanowire formation?
How does rhamnose relate to CsgA production?

CsgA

Rhamnose Promoter

RBS

csgA

Terminator

Fluorescence/OD$_{600}$

Time [h]

0% Rhamnose induction

0.2% Rhamnose induction

0.5% Rhamnose induction

3 molecules/sec per cell

2 molecules/sec per cell
How does rhamnose relate to CsgA production?

100-1000 CsgB units/cell

1 CsgB : 500 CsgA

“Gatekeeper residues in the major curlin subunit modulate bacterial amyloid fiber biogenesis”, Xuan Wang, Yizhou Zhou, Juan-Jie Ren, Neal D. Hammer, and Matthew R. Chapman, PNAS, (2010), vol. 107., no.1, 163-168
CsgA production is **limiting** in nanowire formation
Can we control biofilm formation?

pSB1C3 : Empty (Control)

pSB1C3 : CsgA

Rhamnose Promoter

RBS

csgA

Terminator
Crystal violet assay

![Crystal violet absorbance graph](image)

- **pSB1C3 (empty plasmid)**
- **CsgA**

* Note: Significant difference
Our engineered cells produce nanowires forming the adhesive network of a biofilm
Transmission Electron Microscopy

- Rhamnose

+ Rhamnose
How strong is our biofilm?

Estimate persistence length

0.2% Rhamnose

0.5% Rhamnose
Higher rhamnose induction levels lead to a **stronger biofilm**
Can we make the biofilm stick?


Hydroxyapatite affinity

HA-tag

Wash

23
Tooth affinity assay
Our affinity tag is able to **increase cell adhesion** of biofilms to teeth.
“The currently used biofilm models allow only for flat or simple curved surface areas.”

Dr. Marko de Jager, Principal Scientist Oral Healthcare, Philips Research
Can we actively control the shape and composition of a biofilm?
How do we add more control to printing?
KNEX - Building our own 3D printer
Building our own 3D printer - The BIOLINKER
The Biolinker
K’NEX: 291 €
Pump: 250 €
Total: 541 €

Cheapest 3D Bioprinter: 5000 €
We made a customizable **DIY** 3D-printer, that is **safe, inexpensive** and **easy** to build
How do we use our customized bioink?
Alginate thickness

![Graph showing the relationship between alginate layers and line thickness.](image)

- **One Layer**: 1.09 mm
- **Two Layers**: 1.35 mm
- **Three Layers**: 1.69 mm
Can we print separate layers?
Can we print separate layers?

+ Alginate/CaCl$_2$

- Alginate/CaCl$_2$
Can we print separate layers of bacteria?

Green Channel

Red Channel

1.1

1.2

2.1

2.2

3.1

3.2

4.1

4.2

10 μm

10 μm

10 μm

10 μm
We can print **structured layers** of different bacteria and control their **spatial distribution**
What about printing with other types of bacteria?

Groningen Collaboration

• *Bacillus subtilis*
• Platform technology
Cooperations for new applications

Berlin  KU Leuven  Amsterdam
What do experts think?

BUSINESS PLAN

concept → STRATEGY!
Discussion about ethics of synthetic biology

National Institute for Public Health and Environment
Ministry of Health, Welfare and Sport

Rathenau Instituut

Policy and Practice Tool
Youth
Tour to collect K’NEX donations

Students
Business Case

General Public
A Day of Wonder
Cheap, open source and user friendly. Promoting DIY in Synthetic Biology.

CsgA production is limiting in biofilm formation. Rhamnose induction levels control biofilm formation.

Our cells produce nanowires forming the adhesive network of a biofilm. Affinity tag increases cell adhesion to teeth.

Team Members

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