BIOLINK

From 3D printer to a Biological System

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INTRODUCTION

- **Biofilms** are communities of bacteria that live together on a surface, protected by an external layer.
- Bacteria inside the biofilm are far more **resistant** to antibiotics and industrial removal products.
- A better understanding of biofilms could accelerate drug **testing and research**.
- By engineering structured **artificial biofilms** testing could be improved, thereby saving time, money and lives.

HOW CAN WE MAKE **RELIABLE** AND **REPRODUCIBLE BIOFILMS** FOR TESTING?

HUMAN PRACTICES AND OUTREACH

Outreach

- **Involving students**: business case on biofilm innovation.
- Enriching interactions with society and academia.
- Appearances on radio and **national news.**
- We received K'NEX **donations**.

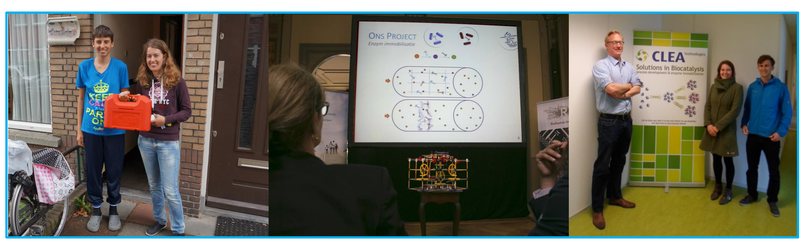
Societal impact

- Biolink may improve drug safety and understanding of biofilms.
- We analyzed how our project could be marketed by creating a **business plan**.
- We designed a tool that helps evaluate goals of a project.
- Ethics and safety!

Experts opinion

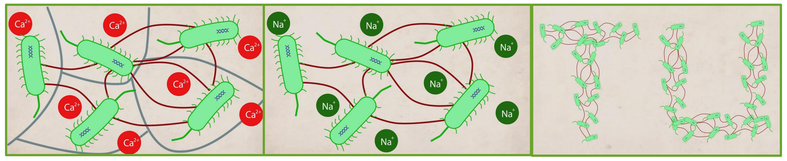
We received input and feedback from experts in the field!

- 3D printing companies
- Oral care
- Biofilm research
- Testing
- Industrial removal



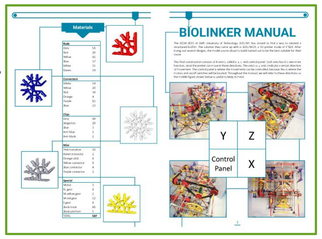
BIOLINKER

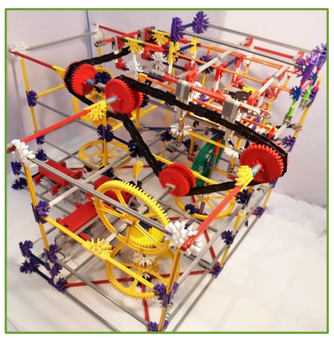
- Cells are printed within a **hydrogel scaffold** made of alginate and calcium chloride: our **bio-ink!**
- Induction with rhamnose enables the connection of neighboring cells by expressing **curli proteins** (CsgA) required for biofilm formation.
- The gel scaffold is dissolved with sodium chloride. The cells remain **connected in the printed disposition** due to the biofilm connections.



Cheap and safe

- Our printer is made of K'NEX pieces.
- Our printer is **safe** to use!
- Total price of Biolinker: **541 €**
- Our printer is a **DIY** product!





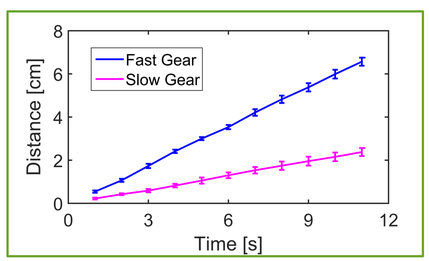
Check out our printer online!

Adaptable

- We are able to print **different types** of bacteria (collaboration Groningen).
- The printer is equipped with two gears.

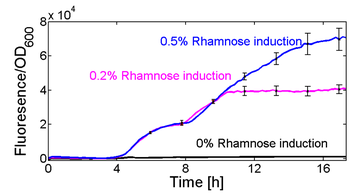
Accurate

- Both gears print with **consistent velocity**.

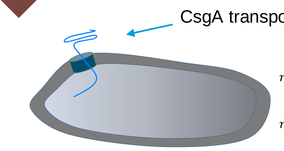


RESULTS

Inducible CsgA production



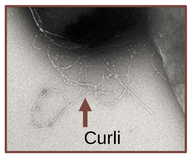
Modeling the CsgA production:

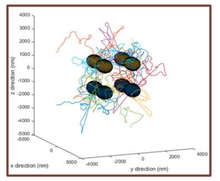


$$r_{csgA} = k_t \cdot P_{csgA,In}{}^{SS} = (1.8 \cdot 10^{-4}) \cdot (1.65 \cdot 10^{4}) \approx 3 \frac{\text{molecules}}{s \cdot \text{cel}}$$ 0.5% (w/v) rhamnose

$$r_{csgA} = k_t \cdot P_{csgA,In}{}^{SS} = (1.8 \cdot 10^{-4}) \cdot (0.95 \cdot 10^{4}) \approx 2 \frac{\text{molecules}}{s \cdot \text{cel}}$$ 0.2% (w/v) rhamnose

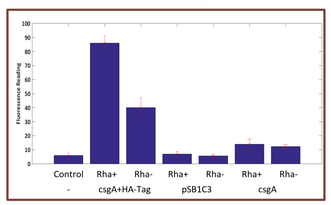
Curli characterization: TEM





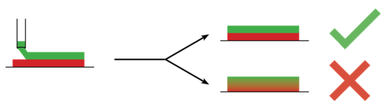
The persistence length was estimated from the TEM images and used as input for our model.

Affinity tag

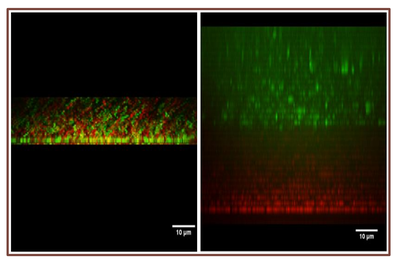


The hydroxy-apatite-tag results in increased adhesion to the teeth.

Biofilm 3D printing



After printing, the layered cells maintain their **defined structure** thanks to the bio-ink!



- alginate/ $CaCl_2$ + alginate/ $CaCl_2$

CONCLUSIONS

- We can control the **CsgA production** and therefore **the biofilm strength**.
- We incorporated affinity tags that show **increased adhesion**.
- We can print bacteria in **layers**.
- The **Biolinker** is a tool that contributes to the production of reliable and reproducible biofilms.

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REFERENCES

Roy, M. D., Stanley, S. K., Amis, E. J., & Becker, M. L. (2008). Identification of a Highly Specific Hydroxyapatite-binding Peptide using Phage Display. Advanced Materials, 20(10), 1830-1836.
Chen, A. Y., Deng, Z., Billings, A. N., Seker, U. O., Lu, M. Y., Citorik, R. J., ... & Lu, T. K. (2014). Synthesis and patterning of tunable multiscale materials with engineered cells. Nature materials, 13(5), 515-523.
Barnhart, M. M., & Chapman, M. R. (2006). Curli biogenesis and function. Annual review of microbiology, 60, 131.
Rowley, J. A., Madlambayan, G., & Mooney, D. J. (1999). Alginate hydrogels as synthetic extracellular matrix materials. Biomaterials, 20(1), 45-53.
Nguyen, P.Q., Botyanszki, Z., Tay, P.K., Joshi, N.S.. (2014). Programmable biofilm-based materials from engineered curli nanofibres. Nat Commun, 17(5), 4945.
Dueholm MS1, Nielsen SB, Hein KL, Nissen P, Chapman M, Christiansen G, Nielsen PH, Otzen DE. (2011). Fibrillation of the major curli subunit CsgA under a wide range of conditions implies a robust design of aggregation. Biochemistry. 4;50(39):8281-90.
Wegerer, A., Sun, T., & Altenbuchner, J. (2008). Optimization of an E. coli L-rhamnose-inducible expression vector: test of various genetic module combinations. BMC biotechnology, 8(1), 2.