The Coating GEMs 2013
The Team

The Problem

Costs of complications are estimated to be 30 billion dollars a year in the USA.
Goal Formulation

Reducing medical complications associated with bone, ligament and cartilage implants.
<table>
<thead>
<tr>
<th>Biomedical Material</th>
<th>Spider Silk</th>
<th>Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Inflammation</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>No immune response</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>High tensile strength</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Low bacterial adherence</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Stable, slow-degrading</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>No disease transmission</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Promotes cell adherence</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Promotes regeneration</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>
Project Plan

Heated implant (streptavidin coated)

Cooled vessel

25°C

37°C

25°C
Which Organism?

**Bacillus subtilis**

- Gram$^+$ bacterium
- Proteins only have to traverse a single membrane.
- Motile
- Generally Regarded As Safe.
Integrational Backbone

BBa_K1085014

Integrational plasmid for \textit{B. subtilis} \textit{amyE} locus with IPTG inducible promoter.
Validating our Backbone

WT  T0  T1
Validating our Backbone

**GFP\textsubscript{MG}**

![Graph showing the intensity of GFP\textsubscript{MG} with different IPTG induction times (0h, 1h, 2h, 3h).]

**GFP (BBa_E0840)**

![Graph showing the intensity of GFP (BBa_E0840) with different IPTG induction times (0h, 1h, 2h, 3h).]

Intensity (AU) vs. IPTG Induction Time (h)
MaSp2 \([\textit{Argiope aurantia}]\)
Major ampullate Spidroin 2

Subunits E and T are codon optimized for \textit{Bacillus subtilis}.
Silk Construct

- $E_{(n)}$, for the silk properties.
- T(ail), for the polymerisation properties.
- R, for the RBS
- SP, for the signal peptide sequence
- S, for the strep tag
Production & Secretion

IPTG

silk

Sec system

silk

SP cleavage
We successfully placed BBa_K1085018 into the *amyE* locus.

Unfortunately, we did not manage to test the expression and production yet.
Project Overview

- Coating GEM
- Movement to the implant
- Coat the implant
- Production & Secretion of Silk
Move to the Implant

*B. subtilis* needs to move when it’s cold and be immobilized when it’s warm.
Move to the Implant

- Native Chemotaxis and Motility

- CheY-P $\Rightarrow$ Swimming

- CheY-P $\Rightarrow$ Tumbling

Diagram:
- Attractant
- CheA
- CheY
- CheC
- Flagellum
DesK autophosphorylates when it is ‘cold’.

Sequentially, DesK-P phosphorylates DesR.

$(\text{DesR-P})_2$ activates $P\text{des}$.
In Silico

Cold

$P_{des}$

$amyE'$ → $cheY$ → 'amyE

$\Delta cheY$ $\Delta cheC$ $\Delta des$
First we modeled the native chemotaxis system.

Next, without CheC...
**In Silico**

**Exact Adaption CheC null mutant**

- Attractant: 2μM

![Diagram](image-url)
Modeling and Labwork
Modeling and Labwork

Motility Assay

<table>
<thead>
<tr>
<th></th>
<th>25</th>
<th>37</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔcheY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔcheY Δdes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Growth Temperature (°C)

Diameter (inch)
We also wanted to confirm the motility assay by using a phase-contrast microscope.

These results are still preliminary, however we saw some differences between the WT and CheY \textit{null} strain.
Microscopy
Microscopy
In Silico

Cold

P_{des}

amyE'

cheY

'amyE

ΔcheY  ΔcheC  Δdes
Starting in the cold area:
We then implemented the ‘cold inducible’ promoter.
Summary of Results

- We improved a *B. subtilis* integrational backbone with a functional IPTG inducible promoter (BBa_K1085014).

- We made a collection of various silk subunits (BBa_K1085000 - BBa_K1085009) that can be combined together.

- We modeled the chemotaxis system of *B. subtilis* and we successfully applied it for our system.
Replace the IPTG inducible promoter for an heat inducible promoter.

Combine all the created parts in one composite part and transform this to the ΔcheY, ΔcheC, Δdes strain.

Test the production and secretion of silk.
The Coating GEM

Cold

Heat

cheY

silk

ΔcheY
ΔcheC
Δdes

Yp

Y

Sec system

SP cleavage
Additional activities

Collaboration Log
- iGEM Netherlands
- Purdue
- Norwich
- Delft
- Chicago
- Trento
- Gottingen
- KU Leuven
- Munich
- Gottingen
Thanks Lyon
Our Sponsors

university of Groningen

MolGen

University of Groningen
Zernike Institute for Advanced Materials

centre for synthetic biology

DSM

MACROXGEN