Auto-adjustable Bio-drug
HOPE
Human Oral Protection for Everyone
Streptococcus mutans.

Biofilm

Acid
Auto-adjustable

3C
Relative Population

S. mutans

Other harmful microflora

Stage 1: normal population
Stage 2: excessive population
Stage 3: insufficient population

Control

Cleanse

Care
Relative Population

S. mutans

Control

Other harmful microflora

Stage 1: normal population
Stage 2: excessive population
Stage 3: insufficient population

Cleanse Care
Control  Cleanse  Care

![Graph showing the relative population size of S. mutans and other harmful microflora over time.](image)
Control

Sensor

Cleanse

Care

threshold
Control Sensor

CSP (competence stimulating peptide)

Cleanse Care
Control
Sensor

Cleanse Care

comD

comE

P

nlmC

Lysis

nlmC → Lysis
Control Sensor

Relationship between pH value & *S. mutans* population

![Graph showing the relationship between pH value and S. mutans population. The minimum pH point is indicated at 4.69 ng/ml with a 72% decrease.](image)

CSP = 4.69 ng/ml
lowest lethal concentration
= 1.5 U/mL
Control

Sensor

Input

Terminator

output

Leakage rate

Cleanse

Care
**Control**

Sensor

**Communication & Kill**

Lactobacillus casei

LuxR protein

pLuxR

Endolysin

S. mutans

Cell wall

AHL
Endolysin functional test

- Circuit without endolysin: negative control (white colonies around)
- Ampicilin (clear zone)
- BHI: negative control (white colonies around)
- Endolysin (clear zone)
Control

Sensor

Cleanse

Communication & Kill

E. coli

L. casei
Control

Sensor

Communication & Kill

Inhibitor

G proteins of *S. mutans*

Histidine kinase 11
Quantitation of biofilm formation

There is dramatic decrease of biofilm formation in *S. mutans* treated with sRNAs.
Biofilm Cleanse
Antibiofilm

**Biofilm**

**Control Cleanse Care**

**Asr promoter**

**Lysostaphn**

**Lactobacillus casei**

On

Biofilm
The biofilm formation drops dramatically as time passes by.
Higher efficiency

Biofilm

Cleanse

Care

Antibiofilm

Control

Asr promoter

Lysostaphin

Lactobacillus casei
Membrane anchor protein: INPNC
Membrane signaling peptide: C16
The fluorescent does increase apparently as time passes by, which means that our anchor protein (INPNC) is consistently expressed.
INPNC-C16-RFP expresses successfully
Relative Population

Stage 1: normal population
Stage 2: excessive population
Stage 3: insufficient population

Control Cleanse

S. mutans

Other harmful microflora

Care
Consititutive promoter

Banana1 → Banana2 → Banana3

Streptococcus sobrinus
Stage 1: normal population
Stage 2: excessive population
Stage 3: too small

S. mutans
Streptococcus sobrinus

Control  Cleanse  Care
Relative Population

Stage 1: normal population
Stage 2: excessive population
Stage 3: insufficient population

Care
Control
Cleanse

Sensor
Communication & Kill
Inhibitor

S. mutans

Other harmful microflora

Antibiofilm
Attachment
Ideally......
T. L. casei M102 phage
However,...
Our advisors thought that would be a bad idea...
A “Perfusion” machine.
HOPErfusion
1. Bacteria-free

2. Imitate our oral environments

3. Test three parts of our project
Biofilm formation assay by HOPErfusion

The great decrease of biofilm formation means that our antibiofilm module still work successfully in the imitated oral environments.
Biosafety?
Biosafety

L. casei

E. coli

M102 phage
Biosafety

Blue light activated

E. coli

Blue Light Promoter CcdB
Expose a dish of *E. coli* to light after 6 hr

Expose a dish of *E. coli* to light after 12 hr

Expose a dish of *E. coli* to light after 24 hr

Test the suicide circuit under white light
CFU counting assay
Colony-forming unit
**Escherichia coli** population after induced suicide

- Induced to death after 24 hr
- No exposure
- Induced to death after 12 hr
- Induced to death after 24 hr

Light-induced suicide efficiency

**population of E. coli**

**time of exposure to light**
After 8 hr exposed to light, almost all the *E. coli* was eliminated.
Application 3C Sustainable Auto-adjustable

L. casei M102 phage
Policy and Practices
Promotion
Legal
Ethical
Public outreach
Global view
Fostering discussion with the opposition

iGEM friendly

anti-GMO
Tien, Chiu-Chin

Legislator of the Democratic Progressive Party (DPP)

“No specific law for synthetic biology exists in Taiwan.”
Discussing ethical issues

Hung Shu De
Director of Chief Homemakers United Foundation

Warren Kuo
Renowned speaker and anti-GMO Advocate

“We are deeply concerned about the exchange of modified genome with the outside world.”

Enhance our biosafety mechanism
Visiting experts

President of the Association for Dental Sciences of Taiwan

Ming Lun Hsu

“Be aware of the difference of oral microflora between children and the elderly.”
Worldwide promotion

More than 1,000,000 dentists from 130 countries

“I favor your innovative idea of tackling the dental cavities crisis in a genetic way.”

FDI, World Dental Association
An Article for Scientific American Taiwan

Chia-Wei Li
Editor-in-Chief of Scientific American Taiwan
Public outreach

Oral Hygiene Service Team

Questionnaires

Speeches

Promotion Booklets

Dentists
Achievements

- Decrease risk of cavities
Achievements

• Improving 6 parts
• Sending 26 biobricks
• Discussed with anti-GMO supporters
• Write an Article for Scientific American Taiwan
• Consulted with World Dental Association
• Made HOPERfusion to simulate the oral cavity
• New modelling methods for realistic models
• Collaborated with NTU-Taida team
Human Oral Protection for Everyone