Communicating with and controlling biology via biofabrication, synthetic biology, and microelectronics

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Grand Challenges – building better medicines & new methods for discovery
Engineered Commensal Bacteria Reprogram Intestinal Cells Into Glucose-Responsive Insulin-Secreting Cells for the Treatment of Diabetes

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To investigate the possibility of using commensal bacteria as signal mediators for inhibiting the disease cholera, we orally administered a strain of E.coli carrying a cholera toxin (CT) gene and a reporter gene. We then monitored the expression of the reporter gene in the intestine of mice infected with CT. Our results show that the bacteria were able to inhibit the expression of the CT gene in the intestine, suggesting that they may be able to protect against cholera.

Engeneered bacterial communication prevents Vibrio cholerae virulence in an infant mouse model

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Cell Engineering
Synthetic Biology & Metabolic Engineering

Targeted mutations & transgene augmentation

Pathway & regulatory Engineering

Whole genome engineering
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Whole genome engineering

To find right cells, we employ methods that survey increasingly larger populations and ultimately select based on one or two traits:

e.g., productivity & tolerance

We bound phenotypic “noise” that stems from the heterogeneous cues
Spatiotemporally control stimulation to engineered (permissive) cells

...and while orthogonality is attractive, circuits & systems converge
Biofabrication

The use of biological components, biological means, or mimics of biological processes for synthesis or assembly.

- Self assembly
- Enzymatic assembly
- Stimuli-responsive assembly
Stimuli-responsive Assembly

pH, temperature, pressure, light, etc.
Biofabrication  Enzymatic Assembly

phenoloxidase, tyrosinase

L-tyrosine → oxidation → L-dopa → oxidation → dopaquinone
Enzymatic Assembly - tyrosinase to construct protein-chitosan conjugates

“Pro-tag” w/ 5 tyrosyl (Y₅) residues

protein + O₂ → “activated” protein

protein-chitosan conjugate

green fluorescent protein – chitosan conjugate

Biofabrication  Enzymatic Assembly

transglutaminase

\[ \text{O} \rightarrow \text{C} \rightarrow \text{N}^{\text{H}_{2}} \rightarrow \text{Gln} \rightarrow \text{NH}_{3} \]

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\[ \text{(CH}_{2}\text{)}_{2}\text{C} \rightarrow \text{NH} \rightarrow \text{CH}_{2}\text{)}_{4} \rightarrow \text{O} + \text{NH}_{3} \]

\[ \text{Glutamine} \rightarrow \text{Lysine} \]

\[ \text{Factor XIII} \rightarrow \text{Factor XIIIa} \]

\[ \text{Thrombin} \]

\[ \text{Tissue Damage} \rightarrow \text{Vessel Injury} \]

\[ \text{Surrounding Tissue} \]

\[ \text{Collagen} \rightarrow \text{Procollagen} \rightarrow \text{Fibrin} \rightarrow \text{Fibrinogen} \]

\[ \text{Calcium} \rightarrow \text{FV} \rightarrow \text{FVIIa} \rightarrow \text{FVIIIa} \rightarrow \text{Factor XIIIa} \]

\[ \text{Polymerization} \rightarrow \text{Stabilization} \]

W.E. Bentley, University of Maryland
Transglutaminase to construct tissue engineering scaffolds & protein-peptide structures

Facile assembly of cells and proteins...

spider silk

tyrosinase and transglutaminase

W.E. Bentley, University of Maryland

Creating “artificial cells” for guiding bacteria...

Apoorv Gupta


Apoorv Gupta

Can “artificial cells” modulate the microbiome?

“Artificial” cells with more capability....
“Artificial” cells with more capability....

1. Expression of αHL
2. Haemolysis
3. Release of IPTG

Lentini et al. (2014) Nature Communications
Organizing Structure - Biofabrication and Assembly....

Organizing Structure - Biofabrication and Assembly....

Clay core: Pseudo-nucleus – enables protein display and signaling
Guan et al., eLIFE, 2018

Electroassembly: Programmable

Integrated Hydrogel: Preserves bilayers
Weiss et al., Nat. Mat., 2017

Organizing Structure – Enabling New Function....

Relieving context

Embedding functions

Adding cell cycle

Ding, Tan, et al., ACS Appl. Mater. & Interfaces, 2018

Weiss et al., Nat. Mat., 2017

Guan et al., eLIFE, 2018
Organizing Structure – Enabling New Function....

Enabling Crosstalk: Connecting cells, Turing test

Lentini, Mansy, et al., Central Science

Grafting Enzymes: Quorum Quenching

Rhoads, Bentley, et. al., Biot. Bioeng., 2018
Rhoads, Bentley, et. al., Molecules, 2018
Grafting Enzymes & Embedding Substrates: Quorum Quenching

Rhoads, Bentley, et al., Biot. Bioeng., 2018
Rhoads, Bentley, et al., Molecules, 2018
Eukaryotic cells have internal compartments

Nucleus
Mitochondria
Amyloplasts

Should be able to control the contents of each type of compartment

All should be aqueous at normal pH, ionic strength

A
B
B
B
C

10 to 100 μm
Organizing Structure – Functional compartments?

Lysosome?  
Vacuoles?  
Mitochondria?

Feed Solution:  
Preformed capsules resuspended in alginate

Reservoir Solution:  
Chitosan + CaCl₂

MCCs with different numbers of compartments

1 compartment  2 compartments

MCCs with distinct contents in each compartment

1 Producer, 2 Reporters

2 Producers, 1 Reporter

Lu, Oh, Terrell, Bentley, Raghavan*, “A new design for an artificial cell.” Chemical Science, 2017, 8, 6893
Organizing Structure – Functional compartments?

Nuclear Membrane?  
Endoplasmic Reticulum?

Goal: controllable synthesis of lipid structures mimicking the function and morphology of the nuclear membrane and endoplasmic reticulum

Precise GUV formation in a microfluidic trap

Cell Biogenesis?

Modification of GUV morphology by chelation with charge-interacting lipids

DMPC:PI 9:1

+EDTA (0 min)

+EDTA (10 min)
Conclusions

We would like to recognize that signaling & perception can be context-dependent.

Biofabrication provides a means to create context that, in turn, facilitates meaningful communication.

Biofabrication, synthetic biology, and external stimuli can enable hierarchical assembly of biological components and in organized assemblies for advanced function.

We are developing means for organizing advanced functions such as energy generation, signaling, and replication.
Thanks for your attention!!

Questions?

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