

UNIVERSITY OF ILLINOIS AT CHICAGO

DOCTORAL THESIS

---

# Devices for Biological Systems: On-Chip Horizontal Gene Transfer and 3D-Printed Microfluidic Applications

---

*Author:*

Martin D. BRENNAN

*Committee:*

Dr. David T. EDDINGTON, Chair and Adviser

Dr. Donald A. Morrison, Dept. Biological Sciences

Person2

Person3

Person4

*A thesis submitted in fulfilment of the requirements  
for the degree of Doctor of Philosophy in Bioengineering*

*in the*

Eddington Lab  
Department of Bioengineering

April 2017

# Declaration of Authorship

I, Martin D. BRENNAN, declare that this thesis titled, 'Devices for Biological Systems: On-Chip Horizontal Gene Transfer and 3D-Printed Microfluidic Applications' and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University.
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed:

---

Date:

---

*“Thanks to my solid academic training, today I can write hundreds of words on virtually any topic without possessing a shred of information, which is how I got a good job in journalism.”*

Dave Barry

UNIVERSITY OF ILLINOIS AT CHICAGO

*Abstract*

Faculty Name

Department of Bioengineering

Doctor of Philosophy in Bioengineering

**Devices for Biological Systems: On-Chip Horizontal Gene Transfer and  
3D-Printed Microfluidic Applications**

by Martin D. BRENNAN

The Thesis Abstract is written here. The page is kept centered vertically so can expand into the blank space above the title too...

This is a test

# *Acknowledgements*

The acknowledgements and the people to thank go here, don't forget to include your project advisor...

# Contents

<b>Declaration of Authorship</b>	<b>i</b>
<b>Abstract</b>	<b>iii</b>
<b>Acknowledgements</b>	<b>iv</b>
<b>Contents</b>	<b>v</b>
<b>List of Figures</b>	<b>vii</b>
<b>List of Tables</b>	<b>viii</b>
<b>Abbreviations</b>	<b>ix</b>
<b>Physical Constants</b>	<b>x</b>
<b>Symbols</b>	<b>xi</b>
<b>1 Introduction and Background</b>	<b>1</b>
1.1 Main Section 1 . . . . .	1
1.1.1 Subsection 1 . . . . .	1
1.1.2 Subsection 2 . . . . .	1
1.2 Main Section 2 . . . . .	2
1.2.1 Subsection 1 . . . . .	2
1.3 Horizontal Gene Transfer . . . . .	2
1.3.1 History and discoveries . . . . .	2
1.3.2 Mechanisms . . . . .	3
1.3.3 Transformation . . . . .	3
<b>2 Materials and Methods</b>	<b>4</b>
2.1 3D Printed Microfluidic Devices . . . . .	4
2.1.1 Fabrication of the 3D-printed Part . . . . .	4
2.1.2 Attachment of Membranes . . . . .	4
2.1.3 Oxygen Characterization . . . . .	5
2.2 Microbiology . . . . .	5

---

2.2.1	<i>Streptococcus pneumoniae</i> strains . . . . .	5
2.2.2	Culturing . . . . .	5
2.2.3	Inducing Transformation . . . . .	6
2.2.4	Plating and assaying for Drug Resistance . . . . .	6
2.3	Droplet Generation and Cell Encapsulation . . . . .	6
2.3.1	Fabrication of Droplet Generating Device . . . . .	7
<b>3</b>	<b>Results</b>	<b>8</b>
3.1	Main Section 1 . . . . .	8
3.1.1	Subsection 1 . . . . .	8
3.1.2	Subsection 2 . . . . .	8
3.2	Main Section 2 . . . . .	9
<b>4</b>	<b>Discussion</b>	<b>10</b>
4.1	Main Section 1 . . . . .	10
4.1.1	Subsection 1 . . . . .	10
4.1.2	Subsection 2 . . . . .	10
4.2	Main Section 2 . . . . .	11
<b>A</b>	<b>Media Recipes</b>	<b>12</b>
	<b>Bibliography</b>	<b>13</b>

# List of Figures



# List of Tables

2.1	Summary of phenotypes of strains used for transformation studies . . . . .	5
A.1	Formula for inducer cocktail. *typically 0.04% BSA is used to prevent non-specific binding but was reduced in these experiments to prevent fouling of they hydrophobic droplet generator . . . . .	12
A.2	Concentration for drug agar overlay. This concentration assumes a 3 mL overlay layer with a 12 mL total volume per plate. . . . .	12

# Abbreviations

**LAH** List Abbreviations **Here**

# Physical Constants

Speed of Light  $c = 2.997\,924\,58 \times 10^8 \text{ ms}^{-\text{s}}$  (exact)

# Symbols

$a$	distance	m
$P$	power	W ( $\text{Js}^{-1}$ )
$\omega$	angular frequency	$\text{rads}^{-1}$

*For/Dedicated to/To my...*

# Chapter 1

## Introduction and Background

### 1.1 Main Section 1

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Aliquam ultricies lacinia euismod. Nam tempus risus in dolor rhoncus in interdum enim tincidunt. Donec vel nunc neque. In condimentum ullamcorper quam non consequat. Fusce sagittis tempor feugiat. Fusce magna erat, molestie eu convallis ut, tempus sed arcu. Quisque molestie, ante a tincidunt ullamcorper, sapien enim dignissim lacus, in semper nibh erat lobortis purus. Integer dapibus ligula ac risus convallis pellentesque.

#### 1.1.1 Subsection 1

Nunc posuere quam at lectus tristique eu ultrices augue venenatis. Vestibulum ante ipsum primis in faucibus orci luctus et ultrices posuere cubilia Curae; Aliquam erat volutpat. Vivamus sodales tortor eget quam adipiscing in vulputate ante ullamcorper. Sed eros ante, lacinia et sollicitudin et, aliquam sit amet augue. In hac habitasse platea dictumst.

#### 1.1.2 Subsection 2

Morbi rutrum odio eget arcu adipiscing sodales. Aenean et purus a est pulvinar pellentesque. Cras in elit neque, quis varius elit. Phasellus fringilla, nibh eu tempus venenatis, dolor elit posuere quam, quis adipiscing urna leo nec orci. Sed nec nulla auctor odio aliquet consequat. Ut nec nulla in ante ullamcorper aliquam at sed dolor. Phasellus fermentum magna in augue gravida cursus. Cras sed pretium lorem. Pellentesque eget

ornare odio. Proin accumsan, massa viverra cursus pharetra, ipsum nisi lobortis velit, a malesuada dolor lorem eu neque.

## 1.2 Main Section 2

### 1.2.1 Subsection 1

Sed ullamcorper quam eu nisl interdum at interdum enim egestas. Aliquam placerat justo sed lectus lobortis ut porta nisl porttitor. Vestibulum mi dolor, lacinia molestie gravida at, tempus vitae ligula. Donec eget quam sapien, in viverra eros. Donec pellentesque justo a massa fringilla non vestibulum metus vestibulum. Vestibulum in orci quis felis tempor lacinia. Vivamus ornare ultrices facilisis. Ut hendrerit volutpat vulputate. Morbi condimentum venenatis augue, id porta ipsum vulputate in. Curabitur luctus tempus justo. Vestibulum risus lectus, adipiscing nec condimentum quis, condimentum nec nisl. Aliquam dictum sagittis velit sed iaculis. Morbi tristique augue sit amet nulla pulvinar id facilisis ligula mollis. Nam elit libero, tincidunt ut aliquam at, molestie in quam. Aenean rhoncus vehicula hendrerit.

## 1.3 Horizontal Gene Transfer

### 1.3.1 History and discoveries

In 1928 Griffith reported on the discovery of the ‘transforming principal’ where mice that were injected with a non-virulent strain resulted in a lethal infection when injected along with a heat killed virulent strain[? , Griffith1928] Both virulent and non-virulent strains could then be isolated from the blood of the dead mouse. Somehow the presence of the virulent strain, although dead, passed information that allowed the non-virulent strain to transform and become virulent. A subsequent study by Avery, McLeod and McCarty demonstrated that the presence of an overlooked substance, DNA, was responsible for re-programing strains[? , Avery1944] At the time it was believed that some yet to be discovered protein complex was the carrier of genetic information, but after this result scientists began looking more closely at DNA. In 1951 Freeman demonstrated that a phage could re-program *Corynebacterium diphtheriae* from non-virulent to virulent[? ]. Hershey and Chase found that the DNA is incorporated into the cell rather than the protein from the phage[? ]. They confirmed this by producing two groups of phages, one with a radio labeled protein and one with radio labeled DNA. Watson, one of the scientist that discovered the structure of DNA was a phage scientist as well.

### 1.3.2 Mechanisms

Horizontal gene transfer (HGT) or (sometimes lateral gene transfer) describes the introduction of genes from an outside source which is distinct from vertical gene transfer where genes are passed from mother to daughter cells. The mechanisms of HGT are transformation, transduction and conjugation.

**Transduction** involves a phage which carries and infects the host cell by injecting the DNA or RNA into the cytoplasm.

In **conjugation** genetic material is passed directly during cell-to-cell contact.

### 1.3.3 Transformation

**Transformation** is the uptake of exogenous DNA from the environment by a cell[? ]. Many species naturally transformable species of bacteria have been reported such as *Streptococcus pneumoniae*, Species such as *Streptococcus pneumoniae* undergo transformation naturally. Transformation was first described in *Streptococcus pneumoniae* [? ]. In transformation the recipient cell controls the process and expresses all the proteins required



## Chapter 2

# Materials and Methods

### 2.1 3D Printed Microfluidic Devices

#### 2.1.1 Fabrication of the 3D-printed Part

The fluidic distribution networks were designed in Autocad in 2D and then extruded to 3D with Blender. Because the channel path length to each well varied the flow resistance in each channel was balanced by adjusting the width. Each distribution network services six pillars that reach into the well of the plate leaving a 500  $\mu\text{m}$  gap to the culture surface. A pipe within a pipe design was used to allow a forward and return path in each pillar that also created uniform flow across the diffusion membrane. The design was repeated to create four sets that service six wells. Hose barbs were added to each inlet and outlet port. The resulting STL file was printed by fineline prototyping with a Viper SLA system in WaterShed XC, an ABS or PBT-like proprietary material.

#### 2.1.2 Attachment of Membranes

Gas permeable membranes were made by compressing 10:1 mixed and degassed PDMS between two glass plates that were spaced 100  $\mu\text{m}$  apart with scotch tape. Molds were baked at 50°C on a hot plate to avoid bubble formation. Membranes were transferred to a transparency and placed over a cutting template and cut to size for the pillar bottoms. To attach the membranes to the 3D printed part, a small amount of PDMS was applied to the membranes and spread thin to act as a mortar. The membranes were left in place to cure overnight.

TABLE 2.1: Summary of phenotypes of strains used for transformation studies

Strain	Competence	Rifampin	Spectinomycin	Novobiocin	Label
CP2204	CSP inducible	Resistant	Sensitive	Sensitive	RFP
CP2215	non-competent	Sensitive	Resistant	Resistant	GFP

### 2.1.3 Oxygen Characterization

PtOEPK (Pt(II) Octaethylporphine ketone) sensors were created by spinning thin films from a PtOEPK in polystyrene-toluene solution. Thin film sensors were cut and fixed to the bottom of each well with PDMS.

to each well in the network we One central input branches to central input that equalizes the flow along each path length by varying the channel width to the proximal, intermediate, and distal wells (Fig 1).

## 2.2 Microbiology

### 2.2.1 *Streptococcus pneumoniae* strains

Two strains of *Streptococcus pneumoniae* were produced by Dr. Morrison of the Biological Sciences Department at UIC: CP2204 and CP2215. The strains were made to be complementary in antibiotic resistances to aid in assaying transformation events. The CP2204 strain has inducible competence so is the designated recipient where CP2215 can not become competent so it is the natural donor of DNA. Traits of these strains are listed in table 2.1

### 2.2.2 Culturing

*Streptococcus pneumoniae* strains CP2204 and CP2215 were grown separately in 12 mL of CDM with 1% CAT medium at 37°C to the desired OD (media formulas can be found in Appendix A). When the desired OD was reached suspended cultures were spun down at 8000 RCF for 8 minutes in a 4°C centrifuge. The supernatant was poured off and the cultures were re-suspended in M9 or a ratio of up to 20% CDM in M9 medium depending on the application and held at 4°C.

### 2.2.3 Inducing Transformation

Strain CP2204 was made competent with and inducer cocktail containing CSP, BSA, and  $\text{CaCl}_2$  (The specific formula can be found in Appendix A Table A.1). The inducer cocktail is introduced to the chilled mixture of CP2204 and CP2215. The cells can be held in at  $4^\circ\text{C}$  in the presence of the inducer cocktail and will remain inactivated. This suspension is then brought up to  $37^\circ\text{C}$  to initiate cell-cell attack and transformation. Typically these reaction suspensions are transferred to a cryogenic vials and placed in a heater block at  $37^\circ\text{C}$  for 30 minutes. After 30 minutes suspension are diluted ten-fold into CAT media and incubated at  $37^\circ\text{C}$  for an hour. This hour incubation step dilutes the inducer inactivating it and allows the generation of recombinants to emerge.

### 2.2.4 Plating and assaying for Drug Resistance

Suspensions were diluted in more CAT media for anticipated survivor counts. Typically suspensions were diluted by 100,000 for single drug, 100 for two drug agar, and 10 for triple drug agar. Agar was made from either CAT medium or THY medium by adding 4.5 g of agar per 300 mL of medium and autoclaving. 50 mm plates are filled with the following layers in order:

1. 3 mL Agar
2. 1.5 mL Agar + 75  $\mu\text{m}$  to 1.5 mL cell suspension quickly mixed
3. 3 mL Agar
4. 3 mL drug agar

Layers are added at least a few minutes apart to allow them to set before the next layer is added. Plates are incubated at in a  $37^\circ\text{C}$  room for 48 hours before counting. Plates are incubated upside down to slow the diffusion of drug to the cell layer. Concentrations in the drug layer are listed in Appendix A Table A.2.

## 2.3 Droplet Generation and Cell Encapsulation

A microfluidic droplet generating device was used to encapsulate cells during transformation. A flow focusing device with two aqueous inlets was used to keep the cells and inducer separate until right before droplet generation.

### 2.3.1 Fabrication of Droplet Generating Device

**Master mold fabrication.** A 2D design was made in AutoCad and exported as a DFX format. Small features such as the filter arrays were saved in a separate file and then subtracted via boolean operation in LinkCAD. SU-8 2015 (MicroChem Corp.) was spun at 4500 RPM on a 100 mm silicon wafer to a thickness of 15  $\mu$ . The wafer was baked at 95°C for 10 minutes shielded from light. The wafer was loaded into the  $\mu$ PG 101 (Heilderberg Instruments). The converted file from LinkCAD was imported into the  $\mu$ PG 101 software. The wafer was exposed at 18 MW, 100% intensity and 4x exposure time. After exposure the wafer was baked at 95°C for 10 minutes and then at 120°C for 30 minutes. I found that this additional bake at 120°C prevented de-lamination that is typical for SU-8 heights less than 50  $\mu$ m. The uncured SU-8 was then washed away with SU-8 developer (MicroChem Corp.) on a shaker for 30 minutes. The wafer was then rinsed with acetone followed by IPA and blown dry with nitrogen.

**Casting, assembly and treatment of device.** About 30 grams of 10:1 monomer to curing agent PDMS was mixed with a Thinky mixer for 2 minutes. About 10 grams of mixed PDMS was poured onto the master mold and spun at 2000 RPM. A 22 x 50 mm glass cover slip was layed on the uncured PDMS. The mold was placed in a vacuum desiccator to pull air bubbles from under the cover slip. The cover slip was then adjusted to be over the incubation chamber of the design and baked at 65°C to set in place. The remaining PDMS, 20 grams, is poured on the mold and degassed. The mold is then baked at 65°C for 2 hours. The PDMS is then carefully peeled from the mold containing the embedded cover slip. Holes are punched with a 1 mm biopsy punch and the PDMS is placed in contact with a glass slide after 45 seconds of oxygen plasma exposure (Plasma Etch, Inc.) to permanently bond. The completed chip is then baked at 135°C for at least 1 hour to strengthen plasma bonding. The chip is then removed and allowed to cool before treating with hydrophobic coating. Novec 1720 (3M) is flowed through the device and then left to stand for 10 minutes. The Novec is then flushed from the device with air and returned to a 135°C hot plate to bake in the coating.

## Chapter 3

# Results

### 3.1 Main Section 1

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Aliquam ultricies lacinia euismod. Nam tempus risus in dolor rhoncus in interdum enim tincidunt. Donec vel nunc neque. In condimentum ullamcorper quam non consequat. Fusce sagittis tempor feugiat. Fusce magna erat, molestie eu convallis ut, tempus sed arcu. Quisque molestie, ante a tincidunt ullamcorper, sapien enim dignissim lacus, in semper nibh erat lobortis purus. Integer dapibus ligula ac risus convallis pellentesque.

#### 3.1.1 Subsection 1

Nunc posuere quam at lectus tristique eu ultrices augue venenatis. Vestibulum ante ipsum primis in faucibus orci luctus et ultrices posuere cubilia Curae; Aliquam erat volutpat. Vivamus sodales tortor eget quam adipiscing in vulputate ante ullamcorper. Sed eros ante, lacinia et sollicitudin et, aliquam sit amet augue. In hac habitasse platea dictumst.

#### 3.1.2 Subsection 2

Morbi rutrum odio eget arcu adipiscing sodales. Aenean et purus a est pulvinar pellentesque. Cras in elit neque, quis varius elit. Phasellus fringilla, nibh eu tempus venenatis, dolor elit posuere quam, quis adipiscing urna leo nec orci. Sed nec nulla auctor odio aliquet consequat. Ut nec nulla in ante ullamcorper aliquam at sed dolor. Phasellus fermentum magna in augue gravida cursus. Cras sed pretium lorem. Pellentesque eget

ornare odio. Proin accumsan, massa viverra cursus pharetra, ipsum nisi lobortis velit, a malesuada dolor lorem eu neque.

## 3.2 Main Section 2

Sed ullamcorper quam eu nisl interdum at interdum enim egestas. Aliquam placerat justo sed lectus lobortis ut porta nisl porttitor. Vestibulum mi dolor, lacinia molestie gravida at, tempus vitae ligula. Donec eget quam sapien, in viverra eros. Donec pellen-tesque justo a massa fringilla non vestibulum metus vestibulum. Vestibulum in orci quis felis tempor lacinia. Vivamus ornare ultrices facilisis. Ut hendrerit volutpat vulputate. Morbi condimentum venenatis augue, id porta ipsum vulputate in. Curabitur luctus tempus justo. Vestibulum risus lectus, adipiscing nec condimentum quis, condimentum nec nisl. Aliquam dictum sagittis velit sed iaculis. Morbi tristique augue sit amet nulla pulvinar id facilisis ligula mollis. Nam elit libero, tincidunt ut aliquam at, molestie in quam. Aenean rhoncus vehicula hendrerit.

## Chapter 4

# Discussion

### 4.1 Main Section 1

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Aliquam ultricies lacinia euismod. Nam tempus risus in dolor rhoncus in interdum enim tincidunt. Donec vel nunc neque. In condimentum ullamcorper quam non consequat. Fusce sagittis tempor feugiat. Fusce magna erat, molestie eu convallis ut, tempus sed arcu. Quisque molestie, ante a tincidunt ullamcorper, sapien enim dignissim lacus, in semper nibh erat lobortis purus. Integer dapibus ligula ac risus convallis pellentesque.

#### 4.1.1 Subsection 1

Nunc posuere quam at lectus tristique eu ultrices augue venenatis. Vestibulum ante ipsum primis in faucibus orci luctus et ultrices posuere cubilia Curae; Aliquam erat volutpat. Vivamus sodales tortor eget quam adipiscing in vulputate ante ullamcorper. Sed eros ante, lacinia et sollicitudin et, aliquam sit amet augue. In hac habitasse platea dictumst.

#### 4.1.2 Subsection 2

Morbi rutrum odio eget arcu adipiscing sodales. Aenean et purus a est pulvinar pellentesque. Cras in elit neque, quis varius elit. Phasellus fringilla, nibh eu tempus venenatis, dolor elit posuere quam, quis adipiscing urna leo nec orci. Sed nec nulla auctor odio aliquet consequat. Ut nec nulla in ante ullamcorper aliquam at sed dolor. Phasellus fermentum magna in augue gravida cursus. Cras sed pretium lorem. Pellentesque eget

ornare odio. Proin accumsan, massa viverra cursus pharetra, ipsum nisi lobortis velit, a malesuada dolor lorem eu neque.

## 4.2 Main Section 2

Sed ullamcorper quam eu nisl interdum at interdum enim egestas. Aliquam placerat justo sed lectus lobortis ut porta nisl porttitor. Vestibulum mi dolor, lacinia molestie gravida at, tempus vitae ligula. Donec eget quam sapien, in viverra eros. Donec pellen-tesque justo a massa fringilla non vestibulum metus vestibulum. Vestibulum in orci quis felis tempor lacinia. Vivamus ornare ultrices facilisis. Ut hendrerit volutpat vulputate. Morbi condimentum venenatis augue, id porta ipsum vulputate in. Curabitur luctus tempus justo. Vestibulum risus lectus, adipiscing nec condimentum quis, condimentum nec nisl. Aliquam dictum sagittis velit sed iaculis. Morbi tristique augue sit amet nulla pulvinar id facilisis ligula mollis. Nam elit libero, tincidunt ut aliquam at, molestie in quam. Aenean rhoncus vehicula hendrerit.



# Appendix A

## Media Recipes

CDM

CAT

M9

TABLE A.1: Formula for inducer cocktail. \*typically 0.04% BSA is used to prevent non-specific binding but was reduced in these experiments to prevent fouling of the hydrophobic droplet generator

Component	Stock	unit	Inducer
CSP	250	$\mu\text{g/mL}$	0.1
BSA	4	%	0.004*
CaCl <sub>2</sub>	1	M	0.005

TABLE A.2: Concentration for drug agar overlay. This concentration assumes a 3 mL overlay layer with a 12 mL total volume per plate.

Drug	Overlay	Stock
Rifampin	40 $\mu\text{g/mL}$	20 mg/mL
Novobiocin	10 $\mu\text{g/mL}$	10 mg/mL
Spectinomycin	160 $\mu\text{g/mL}$	100 mg/mL

# Bibliography