Microcosm for Plant Roots and Bacteria

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Abstract

The Handelsman Lab needs a microcosm apparatus to investigate the interaction of plant roots and bacteria. This would be done by growing the plant roots and bacteria in the chamber then imaging from below. The current prototype lacks the ability to test the effects of competing types of bacteria on the plant root, ease of use, and volume adjustability because it only has one layer of PDMS on the glass slide and a single port for loading. In order to improve the functionality of the apparatus and the efficiency of the research procedure, a microcosm apparatus with inlet and outlet ports, separated ports for different bacteria and detachable structures need to be developed.

Impact

This apparatus is designed to study the interaction of bacteria and early plant roots. This research can be used to better understand why bacteria can help plants grow better in its presence.

Background

Our device is mainly made of PDMS material due to the below properties:

- Oxygen permeable[3]
  - To allow for proper plant root growth
- Biologically inert[3]
  - Prevents bacteria from interacting with chamber
- Hydrophobic[2]
  - To keep the PDMS separated from the culturing liquid
  - The oval shape is also due to this property (prevent bubbles during loading of liquid and bacteria)

Design Specifications

- Include two inlets for bacteria 2 mm in diameter and one plant seed hole 3mm in diameter.
- Detachable structure to simplify the loading and extraction process
- Reusability is favorable to lower experiment costs
- Bottom layer clear to allow microscopy, above 90% transmittance[1].
- Can hold liquid media, or a mix of liquid media and sand.

Final Design

Manufacturing: Two polystyrene molds for PDMS chamber layer and PDMS lid are milled out at first. Then siloxane elastomer and curing agent are mixed in the mold and baked for 3 hours to form the PDMS layers.[4] At last, the PDMS chamber layer is plasma bonded to glass slide.

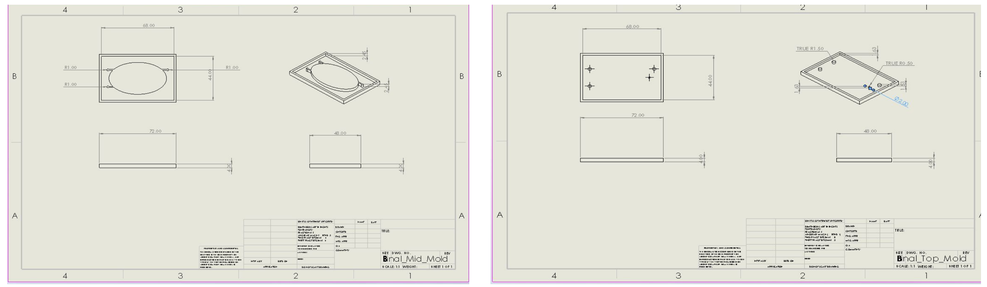

Figure 1: Solidworks for molds

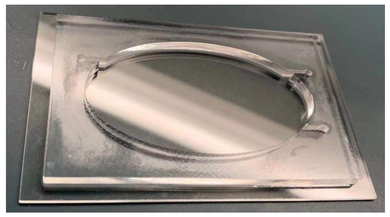

Figure 2.1: PDMS chamber layer and glass slide

Figure 2.2:PDMS lid

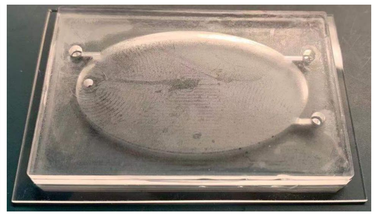

Figure 2.3:assembled prototype

The bacteria microcosm has three layers: PDMS lid(Fig2.2), PDMS chamber and bottom glass slide(Fig2.1).The 2 mm diameter countersink hole is for the positioning of the plant seed and three 1.5mm holes on the sides are for the pipette(Fig2.3)

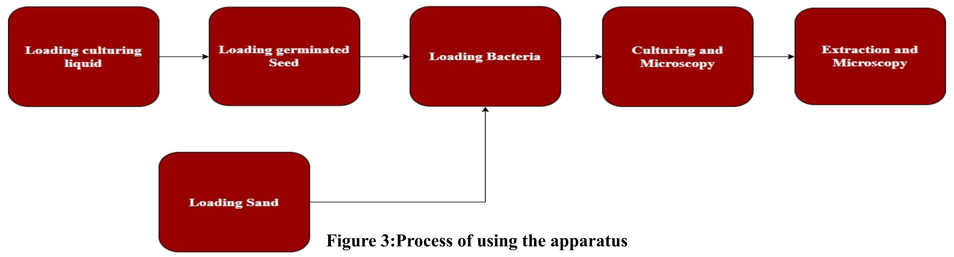

Figure 3:Process of using the apparatus

Testing

I. Beads test for microscopy resolution
   - 1 micrometer size fluorescent beads are loaded into the apparatus and the beads are imaged under Nikon TE2000-E microscope to test the imaging condition in the PDMS chamber.

II. Colored test for leak proof ability
   - Colored DI water is loaded and put into the incubator for three days to simulate the bacteria culturing process.

III. Multiple colors beads test for bacteria spreading
   - Two colors of beads are loaded through two neighboring ports to simulate the bacteria spreading during and shortly after loading process.

Results

I. Fluorescent Beads Under Microscope

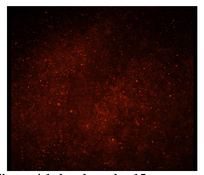

Figure 4.1: beads under 15x

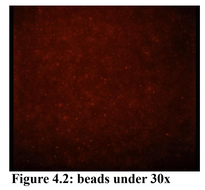

Figure 4.2: beads under 30x

Clear beads(light spots in Fig 4) can be seen under microscope.

II. Leak proof test under simulated culturing process

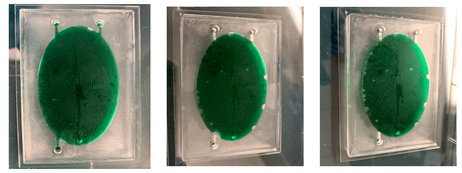

Figure 5:leakage testing day 1-3 (from left to right)

There's no leakage during the 3 days period. (bubbles caused by evaporation)

III. Simulated spreading test

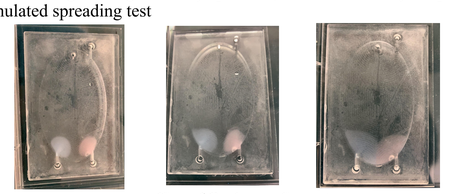

Figure 6:Beads spreading patterns

The beads spread slowly in a controlled manner during and after loading.

Future work

- Add an elastic membrane between top and mid layers to secure seeds in place, while adapting to various seed sizes
- Use plastic polishing components to make the top lid mold surface smoother to increase clarity of PDMS layer
- Test if device can be reused after being autoclaved
  - current method including 1hr UV sterilization is proven reliable

References

[1]"Transparency Meter - Haze Gard-i | Qualitest", Worldoftest.com, 2019.
[2]T. Trantidou, Y. Elani, E. Parsons, and O. Ces, "Hydrophilic surface modification of PDMS for droplet microfluidics using a simple, quick, and robust method via PVA deposition," *Nature News*, 24-Apr-2017.
[3]D. A. Markov, E. M. Lillie, S. P. Garbett, and L. J. McCawley, "Variation in diffusion of gases through PDMS due to plasma surface treatment and storage conditions," *Biomedical microdevices*, Feb-2014.
[4]J. Gao, J. Sasse, K. M. Lewald, K. Zhalnina, L. T. Cornmesser, T. A. Duncombe, Y. Yoshikuni, J. P. Vogel, M. K. Firestone, and T. R. Northen, "Ecosystem Fabrication (EcoFAB) Protocols for The Construction of Laboratory Ecosystems Designed to Study Plant-microbe Interactions: Protocol," *JoVE (Journal of Visualized Experiments)*, 10-Apr-2018

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