Microcosm for Bacteria and Plant Roots

BME Design

7 October 2019

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Abstract

Professor Handelman's lab asked for a device that acts as a microcosm of a plant in nature. They will be studying how bacteria interact with plant roots, and study how they inhabit it and also how/if they help the plant grow. They also want to study the structures that bacteria will create on sand. They have a prototype of the design that is not actually functional in the lab. We are to make a device that can hole culture media, sand, bacteria and a plant seed that will eventually grow roots and at the end of the growing period be placed on a microscope plate, and image the roots from the bottom of the device. There are a few designs that act as a microcosm for plants and bacteria, however, they are much larger than is required for the Handelmans lab. Our current designs is a modified version of the current prototype they have. The height will now be adjustable by stacking pieces, more holes for the loading of bacteria, and a rectangular chamber space instead of an oval. After the prototype is made we will be testing transparency, adjustability, ease of loading, and how well the bacteria can spread using fluorescent bacteria. Lastly, we will have the Handelsman lab try to run their experiment with our final design and make further adjustments if needed.

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Introduction

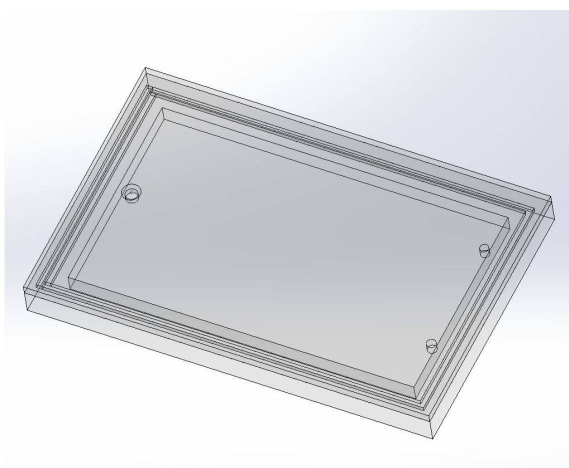
In the current biological research community, study of the interaction between plant growth and bacteria is still a popular topic. Within this topic, the study of interaction between the root growth and bacteria is relatively novel because how bacteria can act as a stimulus to the root growth still needs more investigation. In Handelsman's Lab, researchers intend to investigate how to manipulate and engineer the microbiome[5] including how bacteria can impact root growth and how bacteria competes under the plant root condition. The thing currently bothers the researchers is that a device which can satisfy all requirements for their investigation is not commercially available. Due to unavailability, an apparatus that is being designed specifically for the client to use in her lab is necessary. To meet the research needs, the device should be a transparent box that will hold culture media, bacteria, and a plant or some sand so the substances can interact and researchers can culture the bacteria and observe them. This could reach a broader need and allow researchers to use microscopy on a growing plant without intervening the growing environment. There are several devices that also investigate the interaction of microbiome communities such as the microfluidic co-cultivation platform to investigate microbial interactions at defined microenvironments[6], which merges different species of bacteria at microtubes to observe their interactions, and the microcosm for raising plants under biotic and abiotic conditioning which investigates how the microbiomes intervene plant growth and plant functions[7]. Researchers developed an apparatus which can allow observation and study of the interaction between bacteria and plant root or sand and possible structures formed by bacteria under those conditions. However, the current prototype lacks the ability to test the effects of competing types of bacteria on the plant root, the ability to be reused and is not friendly to users. In order to improve the functionality of the apparatus and the efficiency of the research procedure, a microcosm apparatus with inlet and outlet pores, separated ports for different bacteria, detachable structure and adjustable volume needs to be developed by either modifying the prototype or designing a new one.

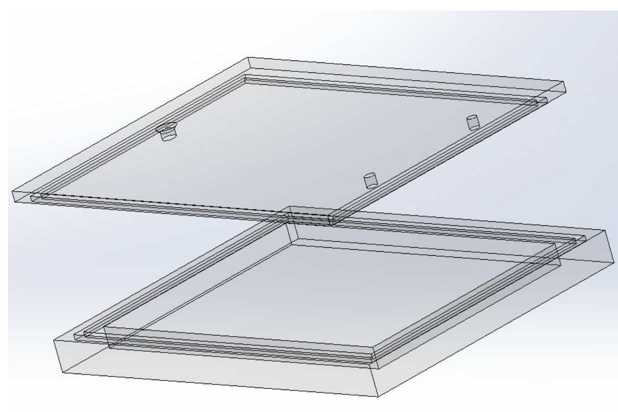
Background

In order to do this experiment the apparatus must be able to hold the liquid media and support plant life. The devices bottom needs to have at least 90% transmittance [4] to be able to be imaged through, having either a glass slide, polystyrene or PDMS as the base. The inner chamber should be able to hold sand, liquid media, plant roots and bacteria [5]. The material it is made out of should be oxygen permeable to allow the bacteria to grow inside the chamber. Logistically and functionally PDMS is likely to work the best due to its permeability to oxygen and CO2 [1][8]. The client needs to the device to be able to come apart so they are able to obtain what is inside the device without disturbing any structures that may have formed while opening it. Also, that it is leak proof so the bacteria and culture media doesn't spill out, and that it is easily loadable with a pipette. The apparatus size should be within 5-7cm x 6-9cm x 1-2cm. The inner chamber is required to have a depth of less than 3mm to start, but has the ability to adjust the height larger if needed. Two inlet/outlet holes (1mm diameter) for bacteria and one larger hole (2mm diameter) for the seed are also necessary. The whole device should be made out of biologically inert materials such as PDMS, glass or polystyrene [2] [3].

Preliminary Designs

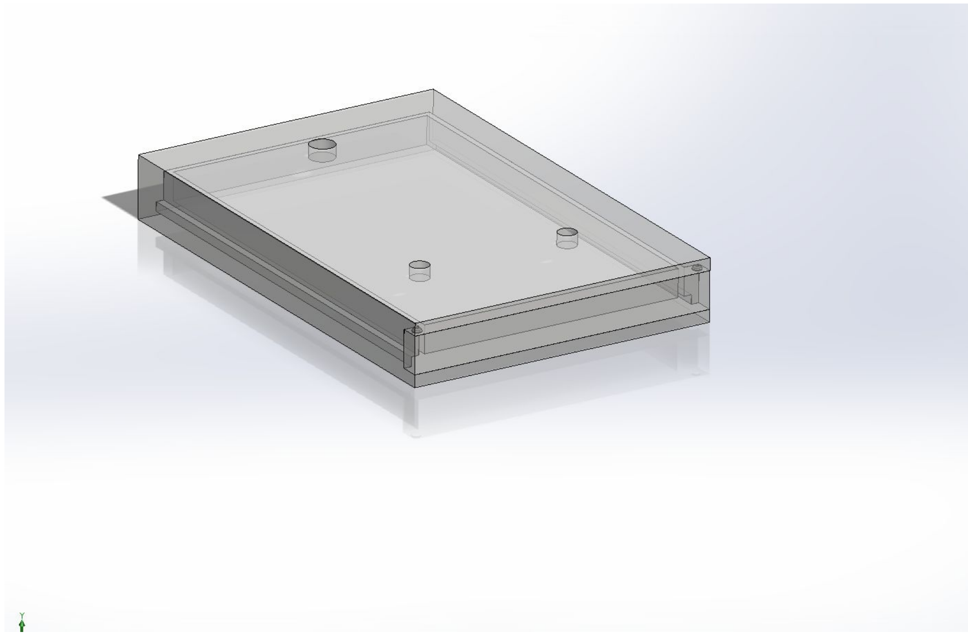
Design 1: Lego

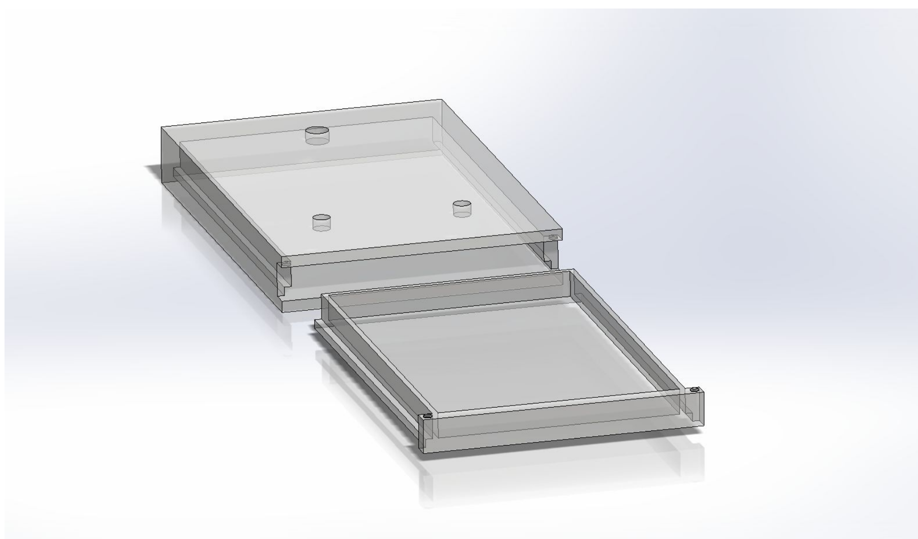




This design contains a lid and a base part with an overall dimension of 68mm*44mm*6mm. The base part has a 3mm deep camber inside the base and 0.75 mm deep continuous grooves on the sides. The lid part has 1mm continuous extrusions that can fit into the groove, sealing the chamber while leaving space for open it. Three pores are located at the lid: the larger one is for the input of the plant seed and two smaller ones are inlets for different bacteria. All the structures are made of PDMS, which is gas permeable and transparent enough to grow the plant and observe the plant roots.

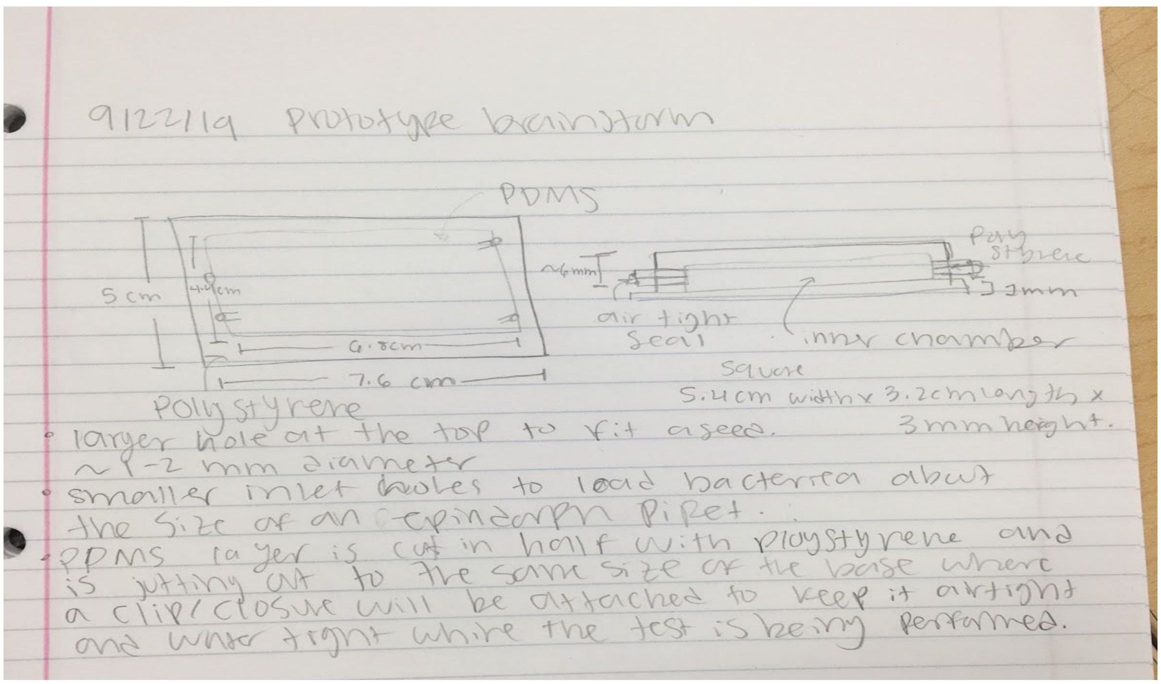
Design 2: Match Box:





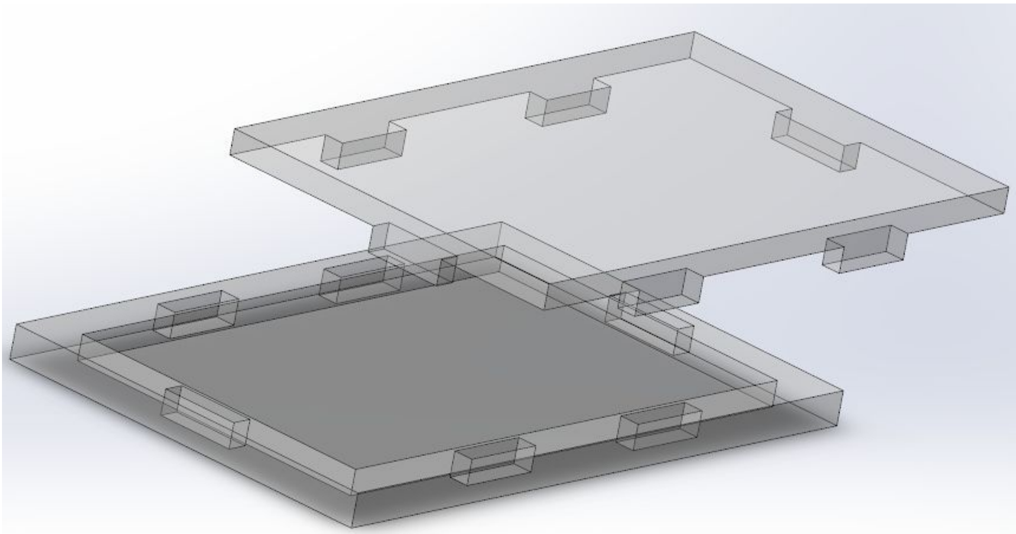
This design contains two parts. The first part is the main frame, and the second part is the platform. The material for the main frame will be PDMS, which is biocompatible, oxygen preamble, and highly transparent. Its size is 76mm x 50mm x 10mm. The size of the device allows easy observation under microscope. On the top surface of the main frame there are three holes. The large hole has a diameter of 4mm, which is the inlet hole for seed settling and seed growth. The two small holes each has a diameter of 2mm. These two holes are the outlet holes for air flow and loading of bacteria into the device. The fixed positions of the outlet holes allow the researcher to load bacteria at fixed locations, which help keep the positions of bacteria for each experiment constantly and eliminate potential unexpected and uncertain variables. In the middle of the main frame, a chamber is designed to make sure that the platform can slide into the mainframe and assemble to become the complete form of Match Box. The chamber is 70mm in depth. The height of the cavity is 6mm, which enables sufficient room for the growth of seed root, media, bacteria and sand. For the platform, the material for the slide in the middle will be 1.5mm glass slide. The material for other structure of polystyrene. The purposes of using different materials is that the glass slide has high transparency to enable clear microscopy and that the polystyrene can be more easily fabricated into detailed and complicated structures via milling than glass. The surrounding walls ensure leakproof and containment. The upper surface of the glass slide will be treated with a thin layer of PDMS for better biocompatibility. The Match Box can ease the loading process, culturing and microscopy to a large extent.

Design 3: Sealed Jar:



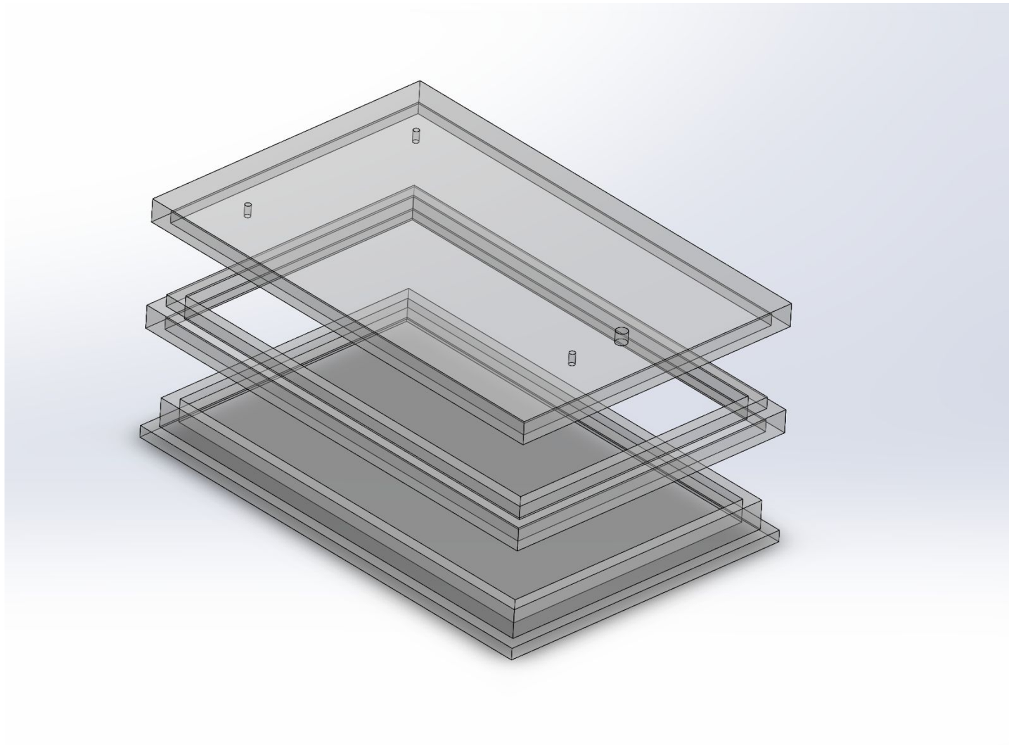
The design has a glass slide like bottom to allow for imaging from a microscope, the layer is about 1mm thick. Then there is a PDMS layer on top of the class slide that is about 2-3mm thick with a rectangular cutout making the chamber to where the experiment will take place, then there are two glass slides that match the cutout shape, but are flush with bottom glass slide to allow for clips to seal the device together. On the top there is another layer of PDMS that will enclose the whole chamber. There is one big hole about 2mm in diameter to allow for the plantation of a seed. Then there is 1mm diameter holes at the bottom and at the top to allow for the addition of bacteria, it is important that the bacteria be loaded through the smaller holes so that the location is standardized through all tests.

Design 4: Puzzle Piece



This design contains two parts to make it easy to load and unload material as well as image the bacteria and sterilize after use. The bottom piece is made of glass to allow for microscopy and is 5cm by 7.6cm, the same measurements as the prototype already created by the Handelsman Lab. This allows it to easily fit on the microscope platform. The glass piece is 4mm thick and the chamber is cut out into the glass 3mm deep, there is only 1mm between the chamber and the microscope which allows for high quality imaging. On the walls of the chamber are cutouts, two on each side and one on each end, which will be roughly 2.5 mm in width and 1 cm in length.The lid or top of the device will be made from PDMS that is 6.8cm long, 4 cm wide and 3 mm tall. On the bottom on the PDMS will be a very thin layer of glass with teeth that exactly fit the grooves in the sides of the chamber. The idea is that the lid will slide into the chamber and have a very tight fit, therefore closing and sealing the chamber. This design scored well because of simplicity and ease to manufacture. There is one inlet hole at the top for adding the plant seed and two smaller inlet holes at the bottom for adding bacteria.

Design 5: Modular



This Modular design is composed of three parts, including a top cover with holes for loading, a middle optional layer for increasing the chamber volume, and a bottom layer for holding bacteria, root, liquid medium, and sand. The top cover has one hole for holding seed and three smaller holes for either loading bacteria or assisting airflow. The bottom layer is designed to be shallower so that root of the plant can be pushed against the bottom piece as close as possible and the middle optional layer can be put in between the other two layers to increase the chamber capacity by increasing the height if required. The bottom piece is designed to be made of very transparent material, either glass or PDMS. The top cover and the optional layer can be made of polystyrene due to its biologically inert attribute.

Preliminary Design Evaluation

Criteria	Weight	Design 1: TShawn Lego		Design 2: Yanbo Match Box		Design 3: Courtney Sealed Jar		Design 4: Salina Puzzle Piece		Design 5: Xavier Modular	
Transparency	25	5/5	25	2/5	10	5/5	25	5/5	25	5/5	25
Ease of manufacturing	25	3/5	15	2/5	10	2/5	10	5/5	25	3/5	15
Ease of Loading	15	5/5	15	5/5	15	5/5	15	5/5	15	5/5	15
Leakproof	15	5/5	15	5/5	15	4/5	12	5/5	15	4/5	12
Adjustability	10	0/5	0	0/5	0	3/5	6	0/5	0	5/5	10
Safety/Contamination	5	5/5	5	5/5	5	5/5	5	5/5	5	5/5	5
Cost	5	5/5	5	5/5	5	5/5	5	5/5	5	5/5	5
Total	100		80		60		78		90		87

Ease of loading: The top surface of the device needs to have holes to fix a location to load the bacteria in each experiment. The structure of the device needs to ease loading of culture media and sand before loading the bacteria.

Transparency: The distance from the root to the microscope has to be as minimal as possible, which means that the bottom of the device needs to be as thin as possible for more quality microscopy. The material has high transparency as well.

Ease of manufacturing: Increased modules and cutouts means that more time needs to be spent on manufacturing due to high difficulty and complexity. The difficulty of injection molding of PDMS depends on the shape of the specific design.

Leakproof: Changing the position of the device will cause leakage. Whether the chamber for holding liquid can be completely sealed is important for this design.

Adjustability: The height of the chamber can be adjusted to control the size of the chamber.

Safety/ Contamination: The potential of breaking glass can cause safety issues. Also the possible contamination of bacteria is also part of the safety concerns.

Cost: The material and fabrication process need to be both monetary and environmental economical.

Fabrication/Development Process

- Materials
    - PDMS(Polydimethylsiloxane) used to form the chamber part of the device. This is a gas permeable material that has a consistency a bit harder than jelly. This is fairly cheap to manufacture for the small device that is being designed.
    - Glass slide. This will be used as the bottom of the design that is attached to the PDMS. This is used because it is very transparent and we know that is can be imaged through. It is also biologically inert and is low in cost for small pieces.
    - Polystyrene: This is also an option for the bottom of the device that will sit on the microscope. It depends on what will work best for imaging.
- Methods
    - First the PDMS will have to be poured into a rectangular mold and cured to be hardened, then a milling machine will be used to cut the indentations of the design. The slide and the PDMS will be cleaned and then placed together and cured again and through this process they will securely attach together. (specific curing guidelines in appendix)

- Final Prototype
    - The final prototype will mainly follow the design of the Modular with some further changes to improve the efficiency.
    - Advantages from other alternative designs will be added into the Modular.
- Testing/ Results: When the first prototype is made we will perform the following tests.
    - Transparency test with a transparency meter to determine how clear the bottom of the device is [9]
    - The loading will be tested by using a pipette and fluorescent bacteria to see if the bacteria is able to penetrate where it is supposed to. This will be a pass/fail test.
    - We will be testing adjustability by the number of different heights that can be accommodated up to a 6mm inner chamber height.

Discussion

Overall, based on the design matrix, the Puzzle Piece and the Modular earn the highest points among all alternative designs. However, because adjustability of the chamber size is a necessary element for the final design, the finalized prototype will be mainly based on the structure of the Modular but merged with the advantages from other designs, such as the tooth feature from the Puzzle Piece and the protrusion feature from the Lego. As a result, the final prototype will be a design with the top cover layer with protrusion, the middle optional layer with cavity for the protrusion on the top surface and protrusion on the bottom surface, and the bottom layer with chamber and cavity along the sides for closure with the middle layer or upper layer. In the future, if the testing experiments suggest large chamber volume, then more optional layers can be added between the top and bottom pieces. The design allow easy manufacture and simple usage. In the later manufacture and testing stage, this final design will be created with Solidworks, fabricated using milling, injecting modeling and other fabrication methods, and eventually tested by the team and researchers in Handelsman's Lab to see if the design needs some further modifications to improve its efficiency. Because there are not many competing microcosm designs similar to this one, this device can make a good contribution to this research field.

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Appendix

Appendix A: Product Design Specifications

Microcosm for Bacteria and Plant Roots

Date:	18 September 2019
Team Members:	Yanbo Feng, Xavier Fan, TShawn Zhu, Salina Loer, Courtney Mohs
Advisor:	Prof. Melissa Kinney
Client:	Prof. Jo Handelsman

Function:

The microcosm apparatus should consist of large seed pore for settlement of plant seed, a trapezoid chamber for root growth, an outlet pore for extracting material and assisting airflow, one or two inlet pores for media and bacteria insertion or settlement of two different bacteria. The material for the apparatus should be crystal clear for microscopy, oxygen permeable for bacteria growth and root growth, biologically inert for plants and bacteria and resistive to possible deformation caused autoclave. The apparatus should be able to be disassembled easily for studying the interaction between bacteria and plant roots under microscopy.

Problem Statement:

Researchers developed an apparatus which can allow observation and study of the interaction between bacteria and plant root and possible structures formed by bacteria under the condition of culturing media and sand. However, the current prototype lacks the ability to test the effects of competing types of bacteria on the plant root, the ability to be reused and is not friendly to users. In order to improve the functionality of the apparatus and the efficiency of the research procedure, a microcosm apparatus with inlet and outlet pores, separated ports for different bacteria and detachable structure needs to be developed by either modifying the prototype or designing a new one.

Client requirements:

- Apparatus size can fit into the microscope platform
- Upper layer material permeable to oxygen
- Material transparent to observe the bacteria under the microscope
- Inner chamber for root growth and interaction between roots and bacteria
- Include an inlet for culturing liquid, bacteria, and sand/plant seed
- Include an outlet for drawing out the liquid and preventing spills while loading
- Include pores for different bacteria
- Detachable structure to simplify the extraction process
- Better be reusable to lower the experiment cost

Design requirements:

1. Physical and Operational Characteristics

a. Performance requirements:

- The transmittance of the bottom part needs to be over 90%[5]
- Culturing media and sand can be loaded into the chamber without spills
    - 50% of the chamber can be loaded with sand and the other 50% culture media
- The substance can be extracted during interaction period
    - At least one pipet tip can be extracted during the experiment
- The substances can be extracted easily from the chamber after observations
- Apparatus can be autoclaved and reused for at least 10 times without deformation and color change.

b. Safety:

- Bacteria should not leak from the chamber
- The material used should not contain any possible toxin to human

c. Accuracy and Reliability:

- The apparatus must provide a clear view of the inner chamber
- The sight should still be clear after multiple reuses
- The size and dimensions should be constant after multiple autoclaves and reuses
- The dimensions of the inner structure of the device should be precise in micrometers.

d. Life in Service:

- The chamber layer should maintain the structure within 10 reuses
- The design protocol must be easily repeatable for future fabrication of the device.
- The device should remain reliable during normal use during the experiment

e. Operating Environment:

- The top part and the bottom part should be sealed together to avoid leakage of media and experiment subjects.

f. Ergonomics:

- The upper part must not be too thick to reduce the oxygen permeability and cause significant refraction of light.
- The device must be able to be assembled to the platform of the microscope easily and stably.
- The opening-and-closing mechanism must not cause inconvenience to the users.

g. Size:

- The current device size that works well for them is 68mmx 44mm x 7mm
- Each chamber layer should not be deeper than 3mm

h. Weight:

- The device does not have a weight restriction

i. Materials:

- The chamber layer, used for inspection and later microscopy, will be made out of PDMS, since it is one of the most used materials for molding of microfluidics devices due to its high transparency for microscopy, easily bonding ability with a simple plasma treatment, leak-proof ability, gas permeability, inexpensiveness, and easiness for molding[1]
- The lid compartment, used as a chamber for holding roots, bacteria, sand, and media can be made out of polystyrene, since it is widely used for medical applications, like tissue culture trays,[3] and can be easily injection molded[4]

j. Aesthetics, Appearance, and Finish:

- The apparatus should be manufactured smoothly without blurring on layers

2. Production Characteristics

a. Quantity: 1 (prototype).

b. The total cost of the device should be less than $250.

3. Miscellaneous

a. Standards and Specifications:

- No international or national standards need to be met while the device is in the prototype phase of the design process.

b. Customer:

- The customer expects a device that can be seen through into the chamber that is holding liquid medium/sand and plant roots to observe the interactions of bacteria and plant roots by using a microscope. The customer would like it to be detachable so the customer can easily access the inside of the chamber without cutting into it and having to make a new prototype every test.

c. Patient-related concerns:

- The device must be sterilized between uses. The top and bottom parts of the device must also be leak proof to prevent bacteria or anything spilling out and coming into contact with humans.

Reference:

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