On Monday, 22nd May, Binish, Ziyi, Zhijun and myself gathered at JIC for a mini-workshop to discuss and share fabrication protocols for the Low-Cost Root Micro-environment Device. The fabrication steps that were shared include:

1. Mould design on Inkscape and Import into Silhouette Studio (the vinyl cutter's native print package)
    1. Silhouette studio can import .png files (from CAD as well)
    2. Need to verify pattern dimensions after cutting - Studio Trace feature is not perfect but maybe good enough
2. Vinyl mould preparation through cutting and transfer
    1. Use appropriate blade height settings (blade value units roughly correspond to 100 microns)
    2. Use 3M Magic tape, and only press tape onto cut vinyl pattern
    3. Vinyl adhesive is strong enough to hold 3-4 vinyl layers together with tape transfer
3. PDMS mixing ratios with cross-linker (by volume) and pouring over mould
    1. Decided ratio of 10:1 (PDMS:crosslinker) by volume is better, perhaps use more crosslinker if no baking will be used
    2. Need to test additional baking on hot plate after overnight or multi-day cure on counter-top
4. PDMS setting and curing procedures - overnight setting vs. baking
    1. Vinyl (Ora-Cal 345 .75 micron, yellow-gold color ?? -- double-check vinyl sample) appears to interact poorly with PDMS, preventing a full cure in channels; try mylar or another vinyl instead
5. Plasma bonding using the Corona Plasma Treater
    1. Tried a 20-second treatment holding the plasma treater wire a few centimeters from exposed PDMS surface -- mixed bonding results
    2. Determined later a longer exposure at higher voltage (by turning knob on back of treater) yielded a stronger PDMS-PDMS bond

We also practiced seed preparation:

1. Seeds are sterilized with 5% bleach by agitation for 10-min
2. One seed is placed in a cut-off 200uL pipette tip filled with phytogel by capillary action transfer
3. Seed-filled tips are stored at a 45 (+) -degree angle on an agar plate in a cold room (Temp = 4 C ??) for 2 days to germinate
4. Agar plate is transferred to a growth chamber (22-23 C ??) with ample fluorescent light on a long day (16hr on - 8 hr off) cycle for 7 days
5. When primary roots have nearly reached end of pipette tip, each tip is transferred to the middle inlet of a prepared microfluidic device

Binish and I separately worked through the details of setting up the fluidics system:

1. A single microfluidic device can be set up using these component
    1. Microfluidic device (currently devices from Cambridge work; need to test devices made as above)
    2. Fine tubing (need to determine gauge or dimensions of this smallest tubing) to connect needle to device inlets and outlets
    3. Appropriate size syringe for amount of liquid (1, 10, 20 or more mL) (with standard Luer head size)
    4. Needles (locking-luer size)
    5. Syringe Pump (Harvard Apparatus dual syringe pump)
    6. Luer sized tubing (1/4 inch?)
2. Multiple devices can be connected in parallel using the following additional items:
    1. Luer-connections with male and female ends in various combinations (male-male, male-female, male locking-male locking, etc.)
    2. Three-way valve with one female and two male locking connections
    3. Additional tubing and needles
3. The components are connected in the following order:
    1. Syringe pump
    2. Syringe filled with a appropriate media
    3. Luer-sized tubing
    4. Male-end of a luer connector (appropriate to connect to next item in line)
    5. *Optional: a series of valves and connectors as needed to branch or control flow to one or multiple devices
    6. Needle
    7. Smallest tubing
    8. Microfluidic device (using upstream inlet)
    9. Smallest tubing (connected to device at downstream outlet)
    10. Needle (used as a weight at end of waste line – this end should hang into waste container)
4. Before a plant is inserted into the device, the entire connected fluid line should be sterilized, flushed, and primed (air-bubbles removed) using an appropriate flow rate from the pump, i.e. < 40 uL/min for a single device

    Do not use a flow rate that is too high, or the pressure with cause the device to fail, i.e. the plasma bond will not hold or the PDMS may crack

    1. Use a sterilization media, i.e. 25-75% ethanol or 5% bleach
        1. Note: It may be easier to sterilize all components separately in a bleach bath rather than using the pump
    2. Flush using either sterile milli-Q water or excess growth media
    3. Prime using growth media

    ***These steps should be performed in order to ensure bleach is diluted enough to not interfere with growth and no bubbles remain in the line. To ensure no bubbles

remain in the device, it may be necessary to use clear tape to temporarily cover seed inlets and additional outlets while bubbles are escaping.***

5. The seed tips may be inserted into the appropriate inlets. Before inserting, it is recommended that the pump is run while the tape covering the inlet is removed, causing media to flow out of the inlet. This will prevent bubbles from entering when the tip is inserted.
6. Turn on the pump at an appropriate flow rate to keep the channels filled, prevent bubbles from entering through the phytogel in the seed tip, and prevent too great of a pressure that causes the tip to flood with media.
7. The plant-filled devices should be placed in a growth chamber fitted with an outlet so the pump can be run continuously. Media should flow at an appropriate rate (?? See above, still need to find this rate), and be checked regularly to ensure supply does not run out or leaks do not occur.