Project Title

Development of a Low-Cost Micro-Environment Device for Root-Nutrient Interaction

Report Title

Progress on the Development of a Low-Cost Micro-Environment Device for Root-Nutrient Interaction

Summary

The plant root system is adapted to forage for nutrients in heterogeneous, dynamic soil conditions. Even crop plants encounter substantial nutrient variability in tilled fields. The Grieneisen Lab at the John Innes Centre is investigating root foraging behaviour in the model system *Arabidopsis thaliana*, but standard lab techniques are limited to homogeneous, static nutrient media. With OpenPlant funding, the Grieneisen (JIC) and Abell (Cambridge) groups are collaborating to develop a low-cost microfluidic device that can finely control rapid changes in the micro-environment surrounding *Arabidopsis* root structure. This project has three specific aims: produce a proof-of-concept microfluidic device using standard soft-lithography techniques, replicate this device using low-cost methods, and use the device in preliminary studies to systematically alter the environment around a growing root. To date, we have grown roots in working prototypes built using soft lithography, and we have established a preliminary protocol to fabricate low-cost versions of these devices. In the coming months we will test root growth in the low-cost devices and study root growth in a dynamic or heterogeneous nutrient environment. Accomplishing these remaining objectives is only a matter of time, as there does not appear to be insurmountable technical difficulties.

Report and outcomes

We provide an update for each specific aim as listed in our project proposal:

1) Producing a proof-of-concept microfluidic device that is capable of creating heterogeneous and dynamic nutrient environments for a developing Arabidopsis root:

Z. Yu and Z. Meng have designed and fabricated a device using soft lithography techniques (Fig 1 – Cross Channel Design). W. T. McCleery has developed a small growth chamber for individual seedlings using a phytogel-filled pipette tip, which maintains humidity and encourages root growth into the microfluidic channel (Fig 2 – Plant In Device). Additionally, a network of devices can be combined in parallel to allow multiple plants to grow simultaneously (Fig 3 – Parallel Device Setup). However, testing of this device (individually and multiple in parallel) has shown that the original plan to use a very cheap method of gravity-drip control of flow rates is inadequate for creating a sustained flow of nutrients for the 7 or more days necessary for root growth. To this end, we decided to purchase a duel syringe pump. This device has been used to successfully grow a root, marking a major milestone as a proof of concept (Fig 4 – Root Growth In Device). One further difficulty with the device is being investigated – the pipette tip chamber that holds the seedling is packed with phytogel to encourage sprouting and growth into the device. The phytogel serves a second purpose to plug the positive pressure of the media flow from flooding the pipette tip and seedling. Over several days of the growth, the phytogel in many (but not all) devices tends to lose its rigidity and the media fills the pipette tip (visible in Fig 2). We are looking at modifications to the design to prevent such flooding. More time is necessary to complete testing of this soft lithography

device and its ability to precisely control the flow rates necessary for optimal root growth and for creating a heterogeneous micronutrient environment.

Figure 1. Cross channel design

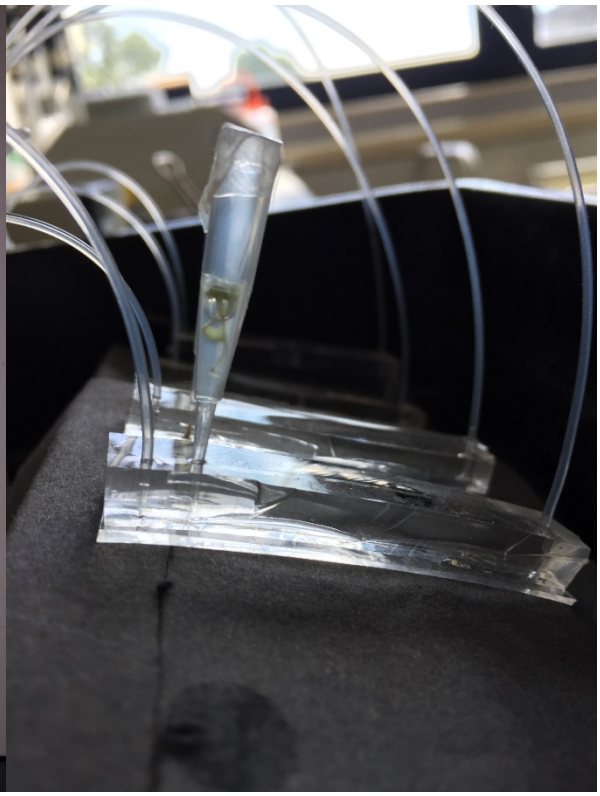

Figure 2. Plant in device

Figure 3. Parallel device setup

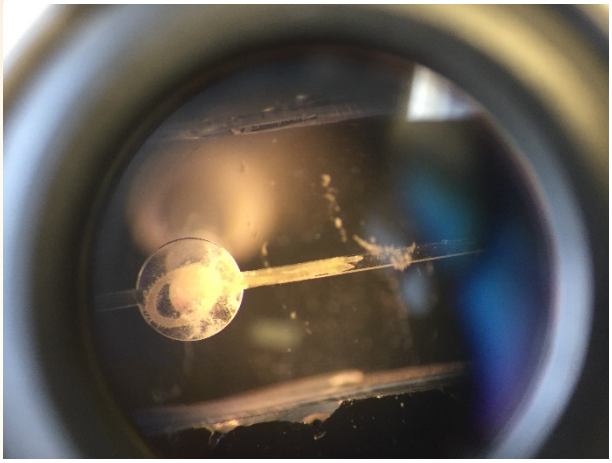

Figure 4. Root growth in device

2) Fabricating a viable proof-of-concept device in a low-cost manner:

The low-cost fabrication process is being tested and refined. Xurography methods work well for cutting the vinyl (Fig 5 – Vinyl Mould Cutting). The vinyl thickness is roughly 75 microns. To produce channels of the appropriate size (150-225 microns in height) we have successfully adhered multiple vinyl layers prior to cutting and used these layered moulds in fabrication (Fig 6 – Vinyl Mould Thickness). The PDMS is then measured by volume, mixed with its crosslinker with a plastic fork and spoon and poured over the vinyl mould. We have created a protocol based on these methods, which will need continued updating as better methods are found (Fig. 7 – Low Cost Fabrication, see below). Unfortunately, this vinyl material interacts with the surface chemistry of the PDMS elastomer and crosslinker, preventing a completely solidified device. Z. Yu and Z. Meng are testing different low-cost materials, such as mylar, to allow for solidification. Additionally, microfluidic devices are typically bonded to a substrate such as a glass coverslip of a thin layer of PDMS to seal the channels. Standard soft lithography techniques treat the two surfaces with plasma to allow adhesion. This treatment requires a plasma chamber, which is expensive and unlikely to be found in a standard biology wet lab. Rapid prototyping in soft lithography sometimes relies on clamping methods rather than surface plasma treatment to seal the device from leaks. Due to the expected intricacies of the device channels, we decided against relying on a clamping method. Rather we found a lower cost method of generating plasma. Electo-Tecnic Products manufactures a handheld Corona Plasma Treater Leak Detector. This device costs around 600 GBP and has been shown to work well specifically for bonding PDMS to itself or to glass (Fig 8 – Plasma Bonding). Our preliminary results suggest this bonding technique is adequate.

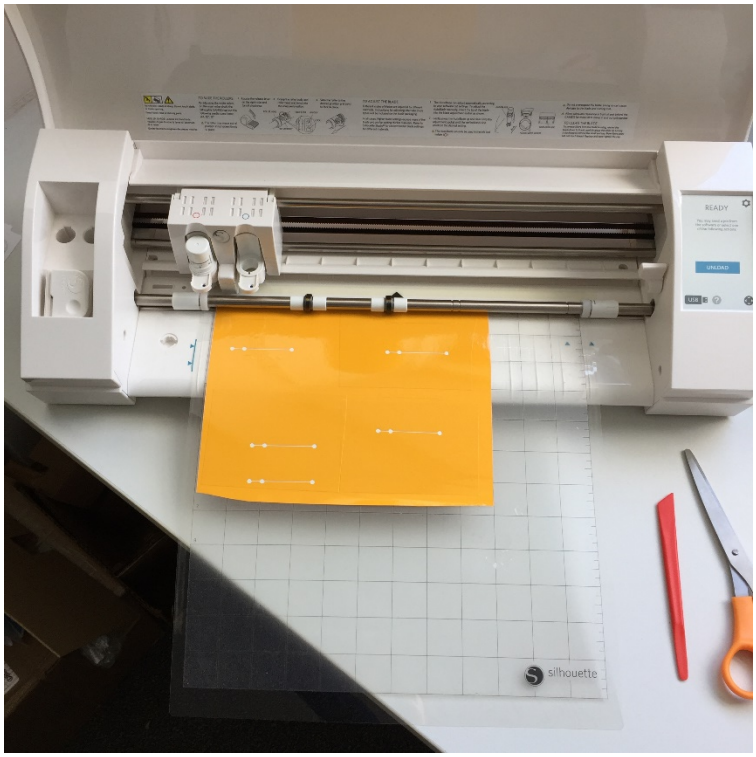

Figure 5. Vinyl mould cutting

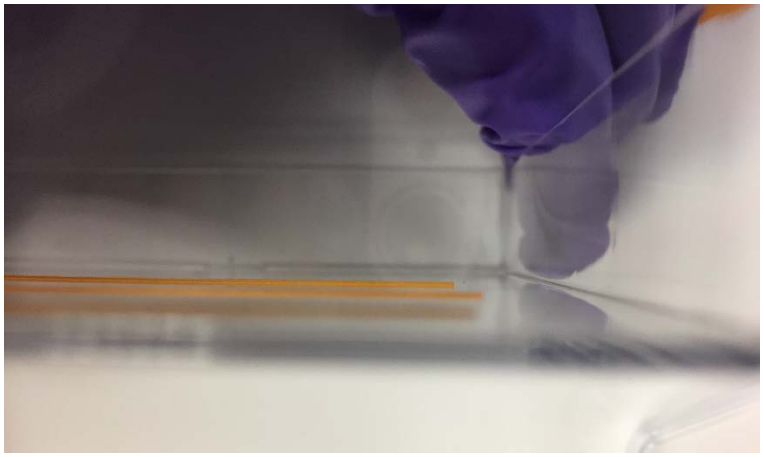

Figure 6. Vinyl mould thickness

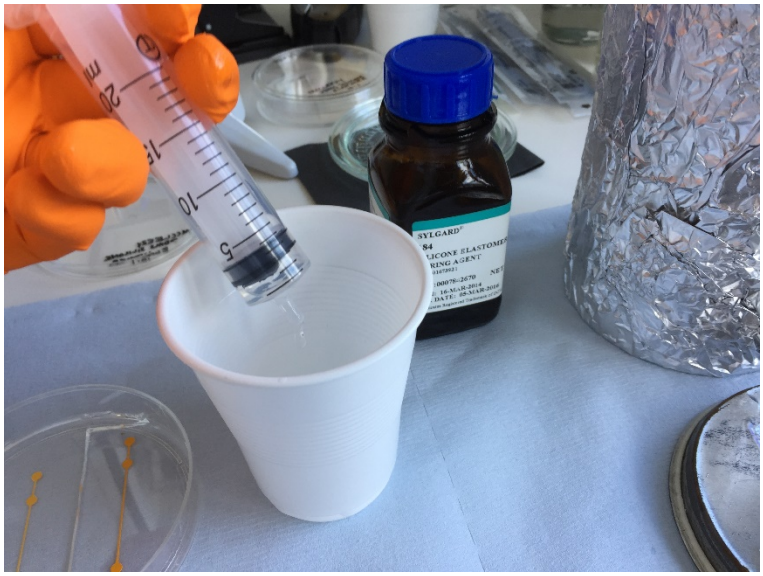

Figure 7. Low cost fabrication

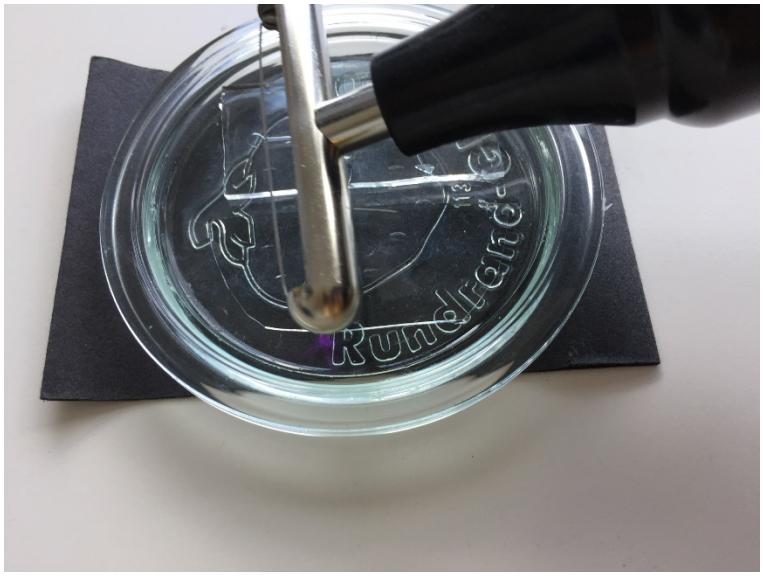

Figure 8. Plasma bonding

3) Preliminary testing on a fluorescently-tagged root system to measure the effect of heterogeneous and dynamic nutrient conditions on growth:

We have achieved root growth in a channel. Separately, we have prepared the fluorescently-tagged *Arabidopsis* lines, specifically D2-Venus (auxin response) or TCS-Venus (cytokinin response) as proposed. We have not yet tested these lines in the device as we are still fine-tuning optimal growth conditions in homogeneous nutrient media. We will continue this investigation in the coming months.

In addition to the proposed aims, we have held two internal workshops to share techniques and ensure continuity of ideas. Our first workshop was at Cambridge, where Z. Meng and W. T. McCleery participated in fabricating simple low-cost designs. Later, W. T. McCleery hosted Z. Meng and Z. Yu at JIC along with Grieneisen lab member Binish Mohammad to walk through the entire low-cost fabrication pipeline (Fig 9 – Workshop at JIC). Techniques discussed included design of the device with Inkscape vector graphics software, implementation of the design into Silhouette's cutting software; vinyl cutting; mould preparation; PDMS pouring, setting and bonding; and fluidics control. We also discussed plant preparation including seed sowing, germination, and growth as well as preparation of the pipette tip growth chamber. An outline of these techniques was compiled in a Workshop Report to aid in creating a detailed protocol of the low-cost device fabrication once refined (WorkshopProtocol.pdf). We continued these workshops with discussions on how to refine and improve the designs.

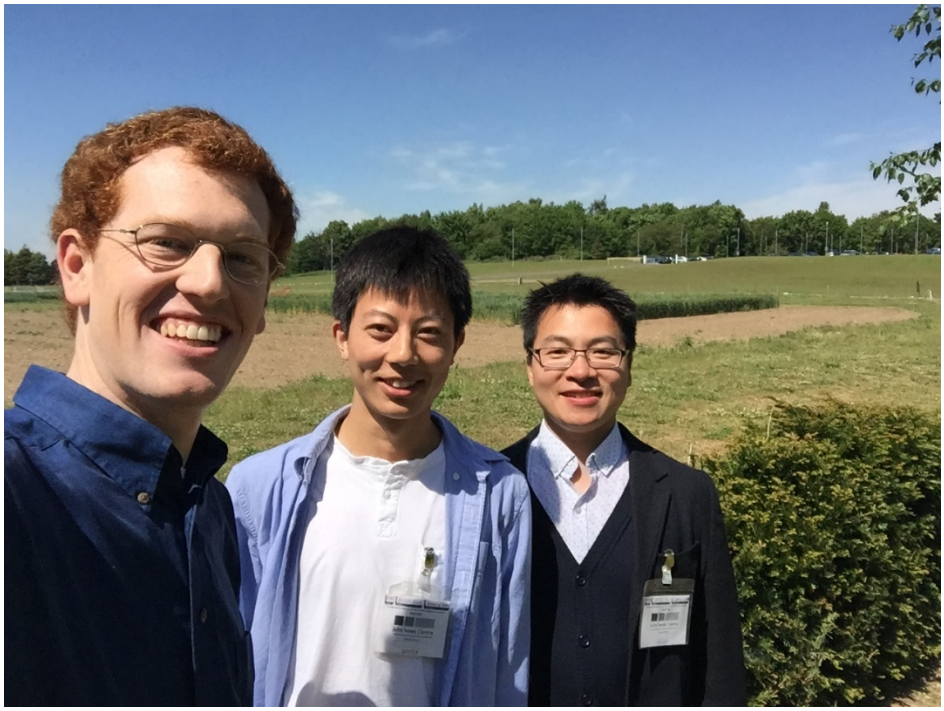

Figure 9. Workshop at JIC

Expenditure

Item Description	Amount
Syringe Pump	£ 506.40
2 Corona Plasma Treaters	£ 1,217.18
2 Silhouette Cameo 3 Vinyl Cutters	£ 636.00
Chemicals for Device Prototyping	£ 618.37
Consumable Supplies for Device Prototyping	£ 1,020.65
6-month Makespace membership (Z. Yu)	£ 240.00
Courier and Post	£ 184.35
Travel Expenses between JIC and Cambridge	£ 120.55
Workshop Expenses	£ 13.75
TOTAL	£ 4,557.25

Follow on Plans

We plan to continue development of both the soft-lithograpy and low-cost devices. Our immediate goals will be to solve the problems of pipette tip flooding and solidification of the low-cost PDMS device. We expect to make significant progress on these goals by mid-October. Solutions to these problems will require a few additional consumables such as mylar instead of vinyl for moulds. Additionally, to aid in rapidly prototyping and testing and to reduce delays due to shipping time, we have purchased duplicates of the Silhouette vinyl cutter and the corona plasma treater. Having these tools in both locations will allow us to electronically share designs and create the low-cost prototypes on site. Once these immediate problems are solved, we will direct our focus to Aim 3 where we characterize the device and begin testing roots in heterogeneous micro-environments. We will file our final report at the end of December as per the follow-on agreement.

Item Description	Estimated Cost
2nd Corona Plasma Treaters (expense above initial £4000)	£ 557.25
Consumables for Device Prototyping i.e. mylar	£ 50.00
Consumables for Testing as per Aim 3, i.e. plant nutrients, phytogel media, etc.	£ 392.75
TOTAL	£ 1000.00