Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Figures

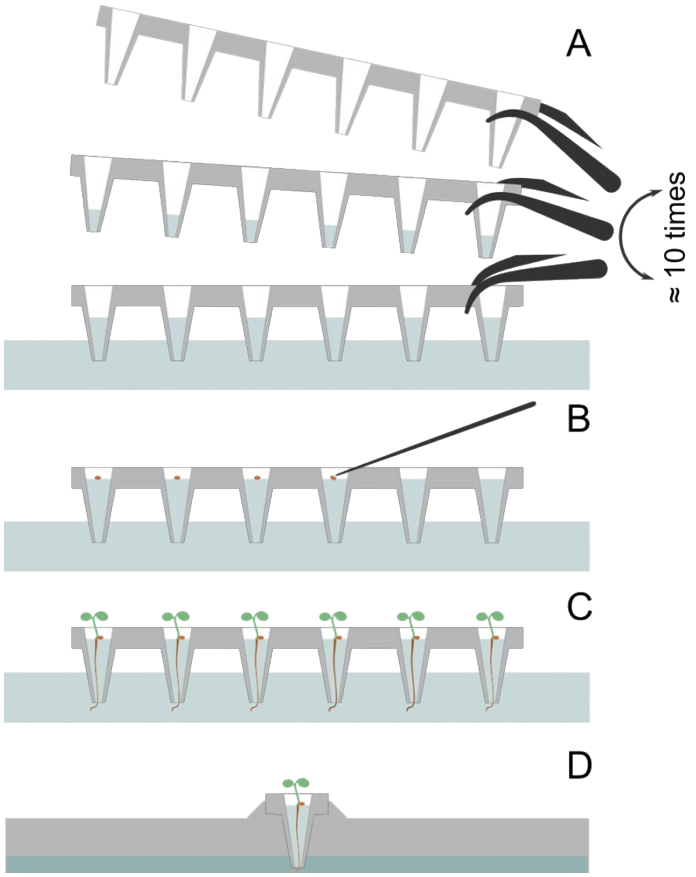

Figure S1: Plant cultivation on the RootChip

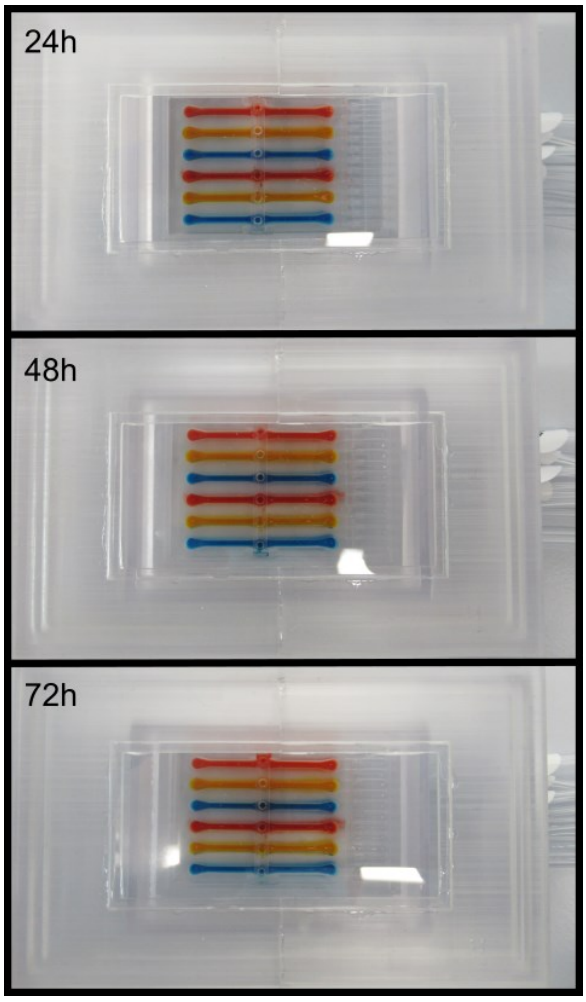

Figure S2: Cross contamination test between channels. Different food dyes were injected into each channel and change of color was monitored during 3 days.

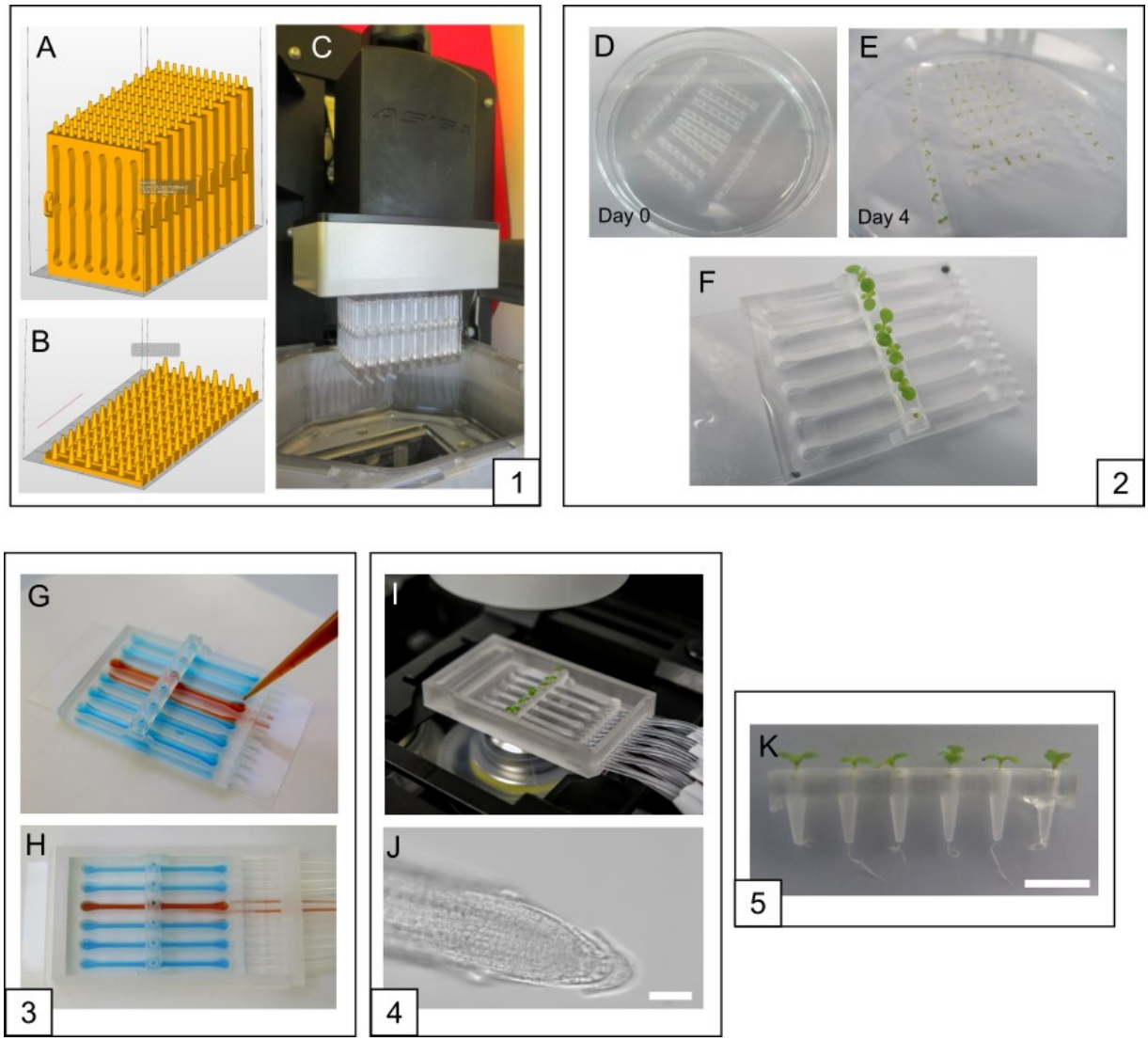

Figure S3: Illustration of the key steps for the fabrication and use of the RootChip. 1) 3D printing of the RootChip components: Screenshots of the *Asiga Composer* software (A): RootChip Base and (B) germination array are printed directly on the printing platform with conical features facing upwards. (C) Batch of 10 RootChip bases printed on a Pico Plus 27 (Asiga, Australia) DLP-SLA printer. 2) On chip cultivation of *A. thaliana*. Germination arrays are placed on top of solidified plant medium in a sealed petri dish (D) One seed is placed on top of each growth cone. (E) Plants after four days of germination in a plant growth chamber. (F) Germination array mounted on a RootChip base. 3) Media is exchanged either by pipetting directly in the growth channels (G) or by aspiration (H) through the integrated inlets and outlets. 4) (I) The RootChip mounted on the stage holder of an inverted microscope. (J) *A. thaliana* root tip imaged in a RootChip growth channel with a 20x objective. Bar: 50µm 5) (K) The germination array can be removed from the RootChip base for downstream analysis. Bar: 5mm.

Supplementary Movies:

Movie 1:

On chip observation of the evolution of the primary root development of *A.thaliana* following a three day treatment with 0, 10% and 20% PEG-6000 concentration. Scale bar = 500um.

Movie 2:

Example of use of the integrated inlets/outlets to rapidly exchange liquid media in the growth channels.

Supplementary Table:

Table 1: Comparison of *A. thaliana* root phenotype in response to the different PEG-6000 concentrations over time. (data= mean ± std)

	CTRL	**PEG 10%**	**PEG 20%**
Root length (mm)			
Day 1	2,71 ± 0,78	2,18 ± 0,95	2,07 ± 0,5
Day2	4,54 ± 0,74	3,33 ± 1,45	2,79 ± 0,62
Day3	6,03 ± 1,22	4,53 ± 1,79	3,42 ± 1,17
Root tip width (mm)			
Day 1	71,21 ± 2,95	71,21 ± 3,49	69,59 ± 4,26
Day2	76,63 ± 3,49	73,44 ± 3,29	69,87 ± 3,08
Day3	79,35 ± 4,18	76,63 ± 4,64	69,66 ± 2,27
Root EZ width (mm)			
Day 1	71,21 ± 2,95	71,21 ± 3,49	69,59 ± 4,26
Day2	76,63 ± 3,49	73,44 ± 3,29	69,87 ± 3,08
Day3	79,33 ± 4,18	76,63 ± 4,64	69,66 ± 2,27
Root hair			
Day 1	19,44 ± 16,75	3 ± 3,2	0,33 ± 1
Day2	42,11 ± 33,99	3,66 ± 4,21	0,78 ± 1,56
Day3	60,67 ± 44,43	6,22 ± 6,94	1,44 ± 3,36

RootChip step by step fabrication guide

1. 3D printing of the RootChip components :

Material note: Brands, suppliers and order numbers are only provided where using specific materials or instruments are recommended. If no such information is indicated, any model or brand of the stated materials or equipment will work for the purpose intended.

Material

• SLA 3D printer

• STL files

• Tweezers

• 100 mL vessel for washing

• Scraper

• Resin: Pro3dure GR-10 Clear (Pro3dure Medical GmbH, Germany)

• Isopropanol

Procedure

1) Fill the 3D Printer VAT with Pro3dure GR-10 Clear resin.
2) Load the desired STL file(s) onto the 3D Printer through its software interface (Figure S3 A & B).
   Caution: A higher resolution and decreased bending of the printed parts is achieved when printing the parts directly on the building platform, instead of using support pillars.
   Critical: The RootChip base has to be printed inlet/outlet ports facing upwards, in order to resolve all features.
3) Set printing resolution. A z-resolution of 100 µm is sufficient to resolve all features of the different components of the RootChip.
4) Set printing parameters for the resin. We used default parameters of the vendor.
5) After the print is completed use a scraper to carefully remove parts from the building platform.
6) **Caution**: At that stage the germination array is particularly fragile and need to be manipulated with care.
7) Using tweezers, transfer printed parts in a vessel filled with isporpopanol.

Note: Depending on the dimensions of your building platform, you can directly print the water tank and lid at the same time. Otherwise split the print into two separate parts and glue the parts afterwards together (see step 23). We included STL files corresponding to both situations, with files named either “full version” or “half version”.

2. Post-processing of the RootChip components :

Material

• Ultrasonic cleaner

• Compressed air

• Compressed nitrogen

• Drying oven

• UV Curing Chamber : Otoflash G171 Post Curing Light Pulsing Unit (NK-Optik, Germany)

• 100 mL vessel for washing

• Tweezers

• Isopropanol

• 99% ethanol

Procedure

8) Transfer the 3D printed parts to a vessel filled with isopropanol. Place the vessel into an ultrasonic cleaner for 5 minutes to remove uncured resin.
9) Repeat the step with fresh isopropanol. Dry the 3D printed part using a compressed.
   Critical: Inspect the 3D printed parts for potential uncured resin that could clog small features, e.g. inlets/outlets or growth cones. If you notice shining areas on the material, repeat step 8.
10) Dry printed parts at 80 °C for 1 hour.
    Caution: At this stage the germination array and inlet/outlet features are fragile and need to be manipulated with care.
11) Transfer printed parts to the UV Curing Chamber. Expose parts to 4000 flashes/side under nitrogen environment.
12) Immerse printed parts in two successive 99% ethanol solutions for 12 hours.
13) Dry printed parts at 80 °C for 30 min.
14) Immerse printed parts in deionized water for 12 hours.
15) Air dry using a compressed air source.

3. *Assembling* of the RootChip

Material

• Cutting plotter (e.g. ScanNCut SDX1200, Brother, Japan)

• SVG file (Double_sided_adhesive_tape_pattern.svg)

• Double-sided adhesive tape (ARCare 90445Q, Adhesives Research, USA)

• Foil squeegee

• Scissors

• Tweezers

• Glass microscope slides (26.0 mm x 76.0 mm)

For the humidity chamber only:
• UV Curing Chamber

• UV curable glue (Norland Optical Adhesive 68, USA)

Procedure

RootChip base:

16) Load the SVG file onto the cutting plotter through its software interface.
17) Cut the desired quantity of double-sided adhesive tape corresponding to the number of patterns to be simultaneously produced.
18) Load the double-sided adhesive tape into the cutting plotter and proceed with the cut.
Note: The blade depth has to be adopted for optimal cutting.
19) Use tweezers to peel off one side of the double-sided adhesive tape and glue the sticky side to the bottom of the RootChip base.
20) Homogeneously press the tape against the RootChip base using a foil squeegee.
21) Remove the second part of the double-sided adhesive tape and align it onto the glass substrate.
22) Homogeneously press the RootChip base against the glass substrate for approximately 1 minute.

For the custom humidity chamber:

As noted in the “3D printing of the RootChip” procedure, you might need to print the water tank and lid as 2 separate parts and glue them together.
In that case:

23) Add UV curable glue on both sides of the 3D printed parts and join them together.
24) Transfer the joined parts to the UV Curing Chamber. Expose parts to 2000 flashes under nitrogen environment.

For the lid:

25) Add UV curable glue on the internal edges of the lid and carefully press a microscope glass slide against it.
26) Transfer the lid into the UV curing chamber. Expose parts to 2000 flashes under nitrogen environment

4. Plant cultivation on the RootChip

Material

• UV-sterilisator

• RootChip components

• Sterile bench

• Sterile petri dishes, 10 cm diameter

• Sterile solid culture medium in a petri dish

• Sterile liquid culture medium

• Stratified surface-sterilized seeds

• Sterilized toothpick or pipette

•Plant growth chamber

•Parafilm®.

•Sterile water

Procedure

27) Sterilize the RootChip components for 15 minutes in a UV-sterilizer and store parts in sterile petri dishes.
28) Prepare the solid culture medium of your choice in a petri dish. The final medium thickness should be at least 3-5 mm.
29) Using tweezers, pick a germination array and tap the conical parts against the solid medium (Figure S1 A). Repeat until the cones are completely filled with medium. After completion, transfer the germination array in a new petri dish containing solid culture medium (Figure S3 D).
    Note: Depending on the height and composition of the solid culture medium, you might need to tap the cones 5 to 20 times in order to fill them completely.
30) Place one stratified surface-sterilized seed on top of each cone.
    Note: You can either use a sterilized toothpick if you use dry seeds or a pipette if your seeds are stored in liquid medium.
31) Seal the petri dish with Parafilm®.
32) Place the petri dish into a plant growth chamber.
33) Regularly check the growth of the roots with an inverted microscope. *A. thaliana* roots usually grown out of the cone tips 4 or 5 days after germination (Figure S3 E).
34) Fill the root growth channels of the RootChip base with sterile liquid culture media.
35) When approx.1 mm roots have emerged out of the tip of the cones, mount the germination array on the bridge of the RootChip base (Figure S3 F).
    Note: We observed a higher success rate for roots growing into the channels, when roots had emerged out of the tip of the cones before being transferred on chip.
36) Transfer RootChips in parafilm® sealed petri dishes filled with 2ml sterile water to keep moisture.

5. Media exchange on the RootChip

The RootChip is designed to enable media exchange around the roots either by pipetting (Figure S3 G) or by aspiration (Figure S3 H), through inlets and outlets integrated at the end of each observation channel.

The aspiration system is desirable for experiments where the RootChip is immobilized several days on the microscope with the need to exchange media in sterile conditions. It is also an advantage for unperturbed live microscopy under varying culture conditions.

6. Using the aspiration system:

Material

- Tygon® flexible plastic tubing (0.51 mm inner diameter)
- Disposable Syringes
- Stainless dispensing tips, 0.63mm OD
- Tweezers
- RootChip base
- RootChip adapter

Procedure

37) Cut the desired length and number of Tygon® tubing.
38) Autoclave Tygon® tubing and dispensing tips.
39) Using tweezers, connect the tubing directly to the inlets and outlets of the RootChip Base. The RootChip adapter is required to encase the 3D printed humidity chamber on the base.

7. Imaging on the RootChip

For long working distance objectives (usually up to 10x magnification), roots can be imaged with the RootChip settled in a sealed and humidified petri dish. For short working distance objectives (usually above 10x magnification) imaging needs to be performed directly against the glass on top of which roots are growing. Consequently, you can either use your own or our custom 3D printed humidity chamber. For sterile media exchange and unperturbed live microscopy it is advisable to use the aspiration system (Figure S3 I). Roots can be observed from several days to several weeks depending on their growth rate. When the roots have reached the end of the observation channel, they usually follow the rounded path of the extremity and continue to grow in the opposite direction.

After finishing an experiment, you can easily remove the germination array and proceed to downstream analysis with the roots (Figure S3 K). Unless a very stiff solid medium was used in the growth cones, it is possible to remove the whole plant from the germination array without any damage. Further, the RootChip base can be reused several times. Note, for this do not clean the chip with an organic solvent as this might alter the bonding strength of the double adhesive tape. Channels can be cleaned with DI water and subsequently the whole chip UV sterilized.