3-D Printing of Meristem and Differentiated Root Cells in Arabidopsis

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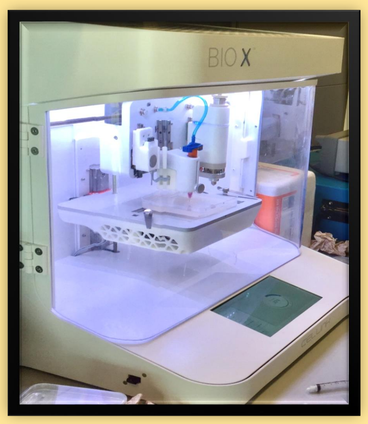
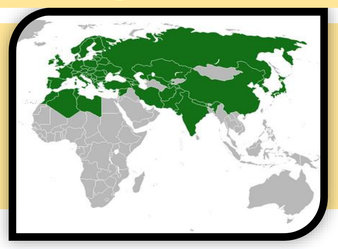


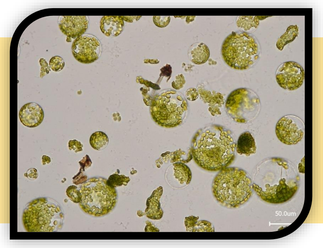
Background

As the human population grows and land use changes over time, the need for scientific research to prepare for, and solve, future health and environmental problems also grows. We know how to genetically engineer plants and other species to serve our purposes. With the ever increasing ability to 3D print custom objects, researchers are beginning to “bioprint” living cells, usually in the biomedical field. This project aims to build plants that better serve our need for biological sustainability, without genetic modification.



Arabidopsis is a plant in the mustard family that is **native** to most of Eurasia. It is studied extensively in plant biology genetic research as a ‘model organism’ due to its ease of use in the laboratory.

Plant cells have cell walls which can be removed using enzymes. Without them, the cells are referred to as protoplasts. As this figure shows, protoplasts are circular.



Research Questions

Can we take plant cells from multiple germinated seeds, protoplast them (remove cell walls), and print them using a 3-D bioprinter?

Will plant cells regenerate after being extruded from a 3-D bioprinter onto a medium containing antibiotic (A) and growth hormone (GH)?

Methods

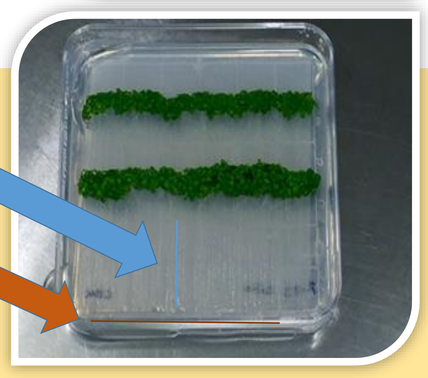
Day 1

Prepare plates using MS plant growth medium and sterilized mesh.
Sterilize seeds.
Plate rows of seeds under sterile hood.
Grow in Percival growth chamber for 7-10 days.

Day 2

Under a sterile hood, cut differentiated (D) root matter and meristematic (M) root tips separately.

Protoplast M & D separately to remove cell walls. Add gelatin to M &D protoplast solutions.



3-D print Control plates: M and D without antibiotic (A) & growth hormone (GH).
3-D print Experimental plates: M and D with A & GH.

Grow in Percival growth chamber for 22 days.

Day 3

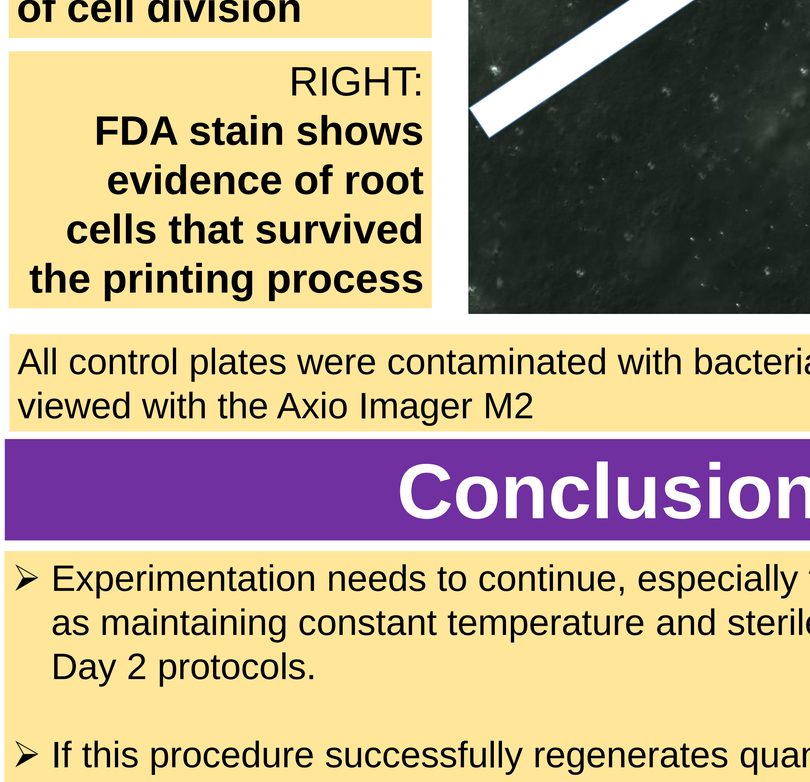
Prepare Calcofluor-white (CFW) and fluorescein diacetate (FDA) stains.

Transfer experimental M & D cells to Microscope slides. Add CFW stain.

View slides using Axio Imager M2 microscope.

Repeat the process using FDA stain.

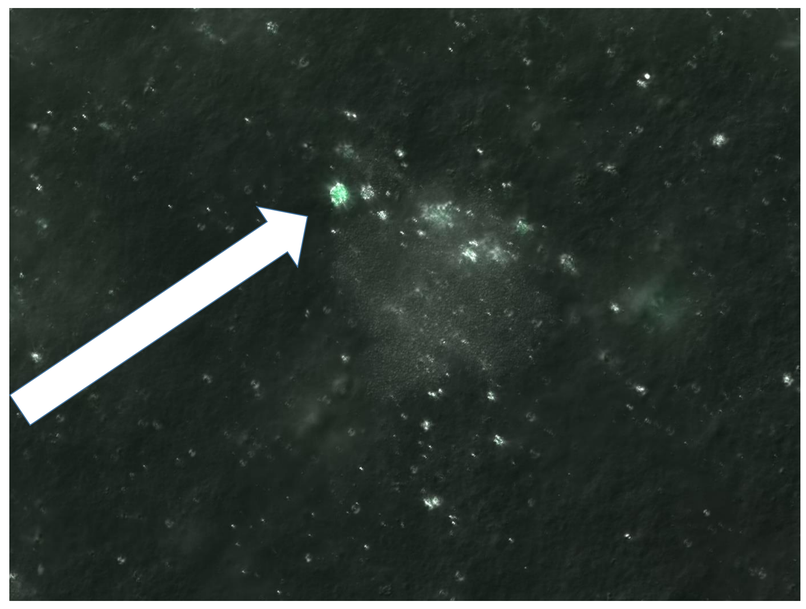
Results



22 Days Post 3-D Printing

- At 20x magnification
- A & H medium
- Whether the cells are M or D has not been determined as of August 12, 2019

ABOVE:
CFW stain shows cell walls regenerated, and suggests evidence of cell division



RIGHT:
FDA stain shows evidence of root cells that survived the printing process

All control plates were contaminated with bacteria or fungi, and were not viewed with the Axio Imager M2

Conclusions

- Experimentation needs to continue, especially to control variables such as maintaining constant temperature and sterile conditions throughout Day 2 protocols.
- If this procedure successfully regenerates quantifiable meristematic and differentiated Arabidopsis cells, the next step is to 3-D print specific root cells, such as quiescent center cells, from different plants to grow one new plant.
- Once a new Arabidopsis plant has been grown successfully, the 3-D printing procedure could potentially be replicated in many other plant species.
- While the procedure is in its infancy, the future potential impacts on biodiversity and agronomy could be tremendous. 3-D printing could provide an alternative to current crop engineering techniques.

Acknowledgements

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