

Full Plant Regeneration From Protoplasts Using 3D Bioprinting

Aitch Hunt¹, Stephanie M. Stewart², Zach Hetzler¹, Zach Mangan³, Imani Madison¹, Rosangela Sozzani¹

1 NC State University, Department of Plant and Microbial Biology
 1 NC State University, Department of Biomathematics
 2 NC State University, Department of Bioinformatics

NC STATE UNIVERSITY

Abstract

Breeding efforts face challenges in meeting the global demand for food due to a lack of efficient systems for genetic manipulation and crop improvement. 3D bioprinting of plant cells and protoplasts offers several advantages over conventional plant cell culture including ability to more accurately mimic the native plant environment, precisely observe cell-to-cell interactions, and provide a stable infrastructure from which plant regeneration can initiate.

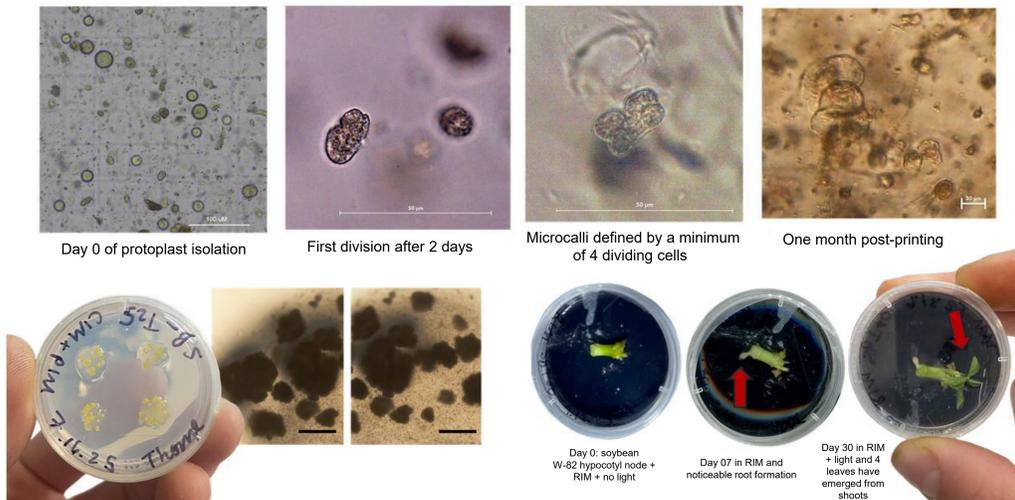
We begin by optimizing various stages of plant regeneration through protoplasts and 3D bioprinting. This involves first optimizing protoplast isolation from specific tissues, then preparing them for 3D bioprinting. We have three experimental pipelines that validate the effectiveness of each tested variable.

The first pipeline tests the effectiveness of the printing process itself and how quickly we can see regeneration, as defined by viability and division of cells over time. The second pipeline considers the growth media into which we print multicellular constructs. We use advanced imaging and analysis processes to analyze division rates over time and validate developmental succession from single-cell to multi-cell to dividing callus and ultimately to the initial stages of a full plant, which can be initiated by shooting or rooting. The third pipeline integrates the validated variables from the first two. It involves printing the isolated protoplasts in the optimized media onto optimized media, then monitoring the developmental success of the single cell structure to a full plant over the course of a few months. This will be applied using various species.

Goal: Regenerate full plants from protoplasts using 3D bioprinting

Regenerative Capabilities

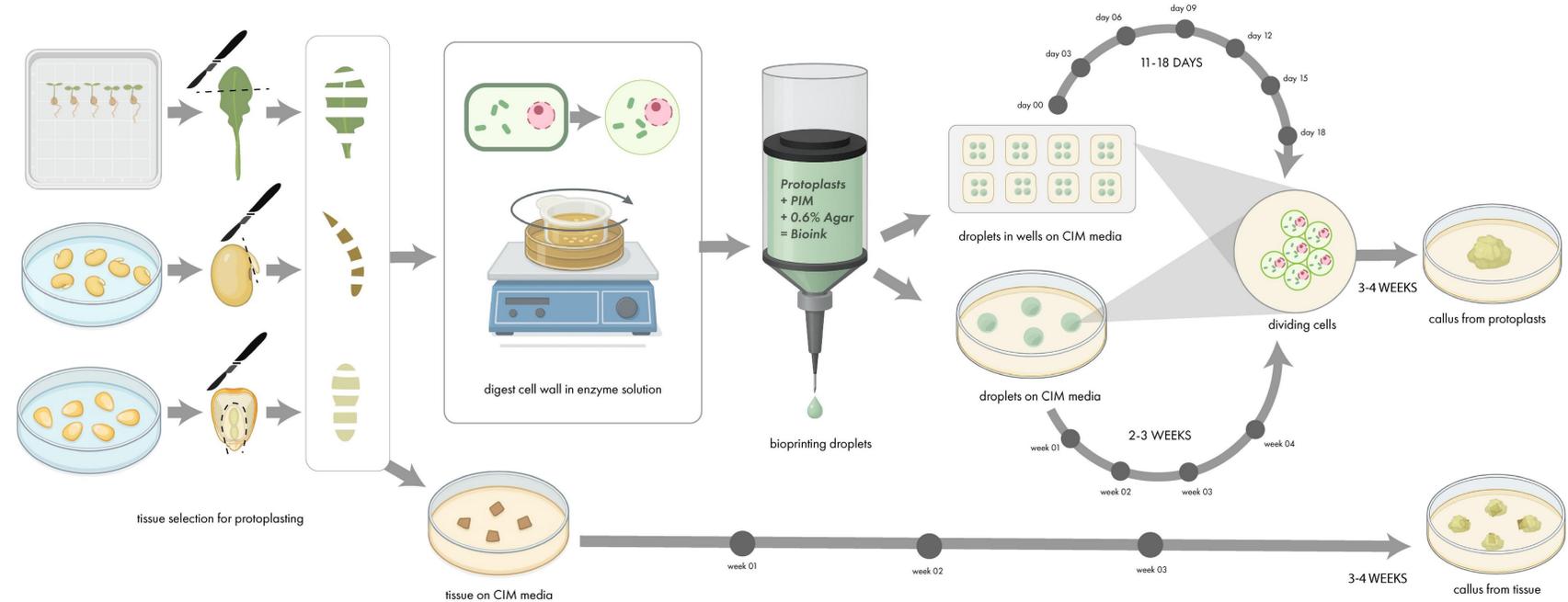
Protoplast Regeneration: Soybean



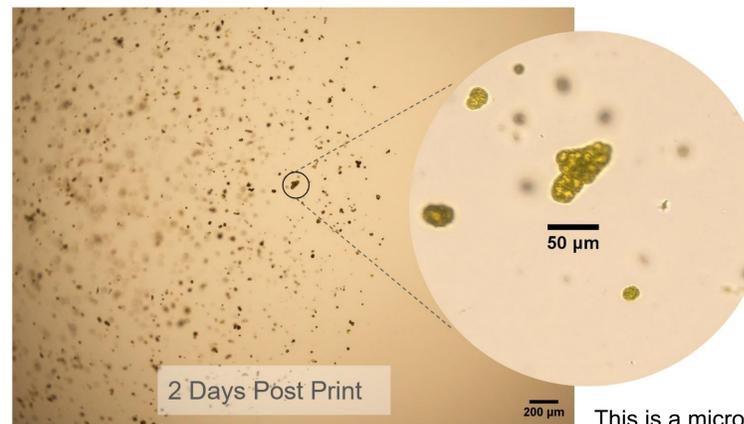
Next Steps

The spatiotemporal information of plant cells will be harnessed by 3D bioprinting to program specific tissue fates by precisely organizing cells within the 3D construct. Controlling the spatial deposition of protoplasts, such as their density, layering, and proximity to different cell types or supportive matrices, allows the bioprinter to replicate the positional cues found in a developing plant. These engineered spatial arrangements essentially "code" the instructions, directing the cells to differentiate into target tissues, which guides the overall structure and function of the regenerating plant.

Experimental Pipeline



Preliminary Results + Key Findings



Recent Bioprinting

Protoplasting and Bioprinting Parameters:

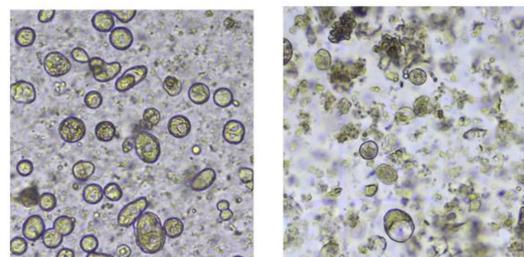
- 4 hour CPW incubation
- Printing Density: 375,000 cells/mL (3:1 bioink:scaffold)
- Printed on PIM agar with PIM media

This is a microcalli seen after two days post print and is defined by a cluster of four or more dividing cells.

Enzymatic Effects on Protoplasting Efficiency

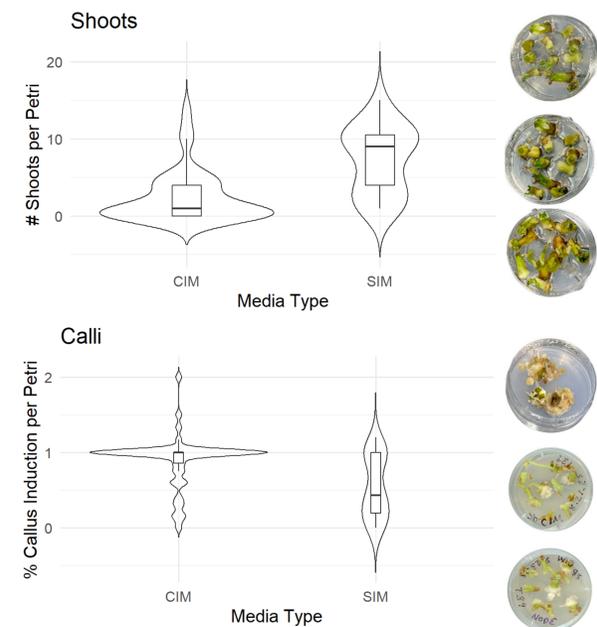
1.5% Cellulase
 0.5% Macerozyme
 0.1% Pectolyase

1.5% Cellulase
 0.1% Pectolyase



We want to use specifically embryonic tissue from the seed which is highly regenerative but very challenging to protoplast. These images compare the results of different enzyme solutions used for breaking down the cell wall of specific explant tissues to obtain a minimum density of isolated protoplasts. This comparison is critical for identifying the most effective enzymatic formulation for the subsequent 3D bioprinting and regeneration pipeline.

Tissue Regeneration by Media Type



Specific compositions of growth media are curated to induce specific stages of development of regeneration from the protoplasts. The four medias are protoplast, callus, shoot, and root induction media (PIM, CIM, SIM, RIM), and are each designed to induce plant regeneration at different stages.